OPTIMISING NITROGEN STORAGE IN WHEAT CANOPIES FOR GENETIC REDUCTION IN FERTILISER NITROGEN INPUTS

BY

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Agricultural Development and Advisory Service	ADAS
Apparent fertiliser recovery	AFR
Above ground dry matter	AGDM
Above-ground N	AGN
Analysis of Variance	ANOVA
Biomass production efficiency	BPE
Complete canopy senescence	CCS
Canopy N requirement	CNR
Days after anthesis	DAA
Days after sowing	DAS
Department of Environment, Food and Rural Affairs	DEFRA
Degrees of freedom	DF
Dry matter	DM
Food and Agriculture Organisation of the UN	FAO
Fresh weight	FW
Green area	GA
Green area index	GAI
Grains per ear	GPE
Growth stage	GS
Home Growth Cereal Authority	HGCA
Harvest index	HI
International Fertiliser Industry Association	IFA
Individual grain weight	IGW
Intercepted radiation	IR
Light extinction coefficient	K
Leaf area index	LAI
Lincoln 2006/07 experiment	LC07
Linear plus exponential function	LEXP
Long term mean	LTM
Manipulation (defoliated, F or degrained, G)	M
Megajoules	MJ
Megatonnes	Mt
Nitrogen	N
Maximum N treatment	N max-trt
Optimum N treatment	N opt-trt
Zero N treatment	N zero-trt

Economic optimum amount of fertiliser N	N opt
N contribution to the grain	NC
Critical N concentration	N_{crit}
N harvest index	NHI
N nutrition index	NNI
N per grain	NPG
N remobilisation	NR
N remobilisation efficiency	NRE
N-use efficiency	NUE
Thermal days	^{o}Cd
Oil seed rape (*Brassica napus* L.)	OSR
Probability	P
Post-anthesis N uptake	PANU
Photosynthetically active radiation	PAR
Photosynthetic N	PN
Photosynthetic photon flux density	PPFD
Quadrat	Q
Quantitative trait loci	QTL
Reserve N	RN
Ribulose 1,5-bisphosphate carboxylase/oxygenase	RUBISCO
Radiation-use efficiency	RUE
Standard error of means	SE
SE of differences of means	SED
'Wheat calculator'	SIRIUS
Specific leaf N	SLN
Soil mineral N	SMN
Structural N	SN
Solar radiation	SR
Sub-sample	SS
Tonnes per hectare	t ha^{-1}
Thousand grain weight	TGW
Terrington 2005/06 experiment	TT06
Terrington 2006/07 experiment	TT07
N-uptake efficiency	UPE
N-utilisation efficiency	UTE
Variety (Atlanta, At; Claire, Cl; Istabraq, Is; and Savannah, Sa)	V
Vegetative storage protein	VSP
Grain yield	Y
Economic optimum grain yield	Y opt

ABSTRACT

Wheat (*Triticum aestivum* L.) is the major UK arable crop with a total annual production of about 15 Mt. Intensive cultivation of wheat requires large inputs of fertiliser nitrogen (N), and of the total annual UK use of 1.1 Mt of N fertiliser, 0.36 Mt are applied to wheat crops. However, current cultivars are only able to take up a limited proportion of this applied N (50-60% in NW Europe; Bloom *et al*., 1998), and large amounts of N are lost to the environment. These fertilisers represent a cost to the grower, and have negative environmental impacts through nitrate, ammonia and greenhouse-gas emissions. There is therefore an increasing need to reduce fertiliser N inputs whilst maintaining or increasing yields. Developing new N-efficient genotypes is an important approach, and could be achieved by increasing the crop N-uptake efficiency (UPE; above-ground N uptake / N available) and/or the N-utilisation efficiency (UTE; grain DM yield / above-ground N uptake). Since only around 50% of the total canopy N is in the leaf lamina at anthesis, there may be scope to reduce the remainder, in particular the significant quantities of N contained in the true stem (up to 25% of canopy N). The overall aim of the present study was to investigate the physiological basis of yield responses to N supply in winter wheat and how cultivars differ in their responses, and to identify breeding targets for new cultivars with lower fertiliser requirements.

Three field experiments were carried out: the first (sown October 2005) and third (sown October 2006) were ADAS Terrington, near King's Lynn, UK, and the second (sown June 2006) was at the Institute for Crop and Food Research, Lincoln, New Zealand. At Terrington, six N fertiliser treatments were randomised on main plots and four cultivars of winter wheat (Istabraq, Atlanta, Claire, and Savannah) were randomized on sub-plots in a split-plot design with three replicates. The cultivars were chosen to contrast for N partitioning amongst plant organs at anthesis according to previous data sets. At Lincoln, six N fertiliser treatments were randomised on plots with six replicates for one cultivar (Istabraq). Plots were sampled at five developmental stages, with particular emphasis on anthesis and harvest. At each sampling, crop growth (above-ground N uptake, green

canopy area, above-ground dry matter, and DM and N partitioning) was assessed, as well as fractional interception of photosynthetically active radiation (PAR).

Data for N uptake and crop DM growth were related to the canopy N requirement (Sylvester-Bradley *et al.*, 1990a) and critical N concentration (Justes et al., 1994) models for winter wheat, and the crop N status at anthesis was quantified according the N nutrition index (Lemaire *et al.*, 1989). The crop N content at anthesis was allocated to three conceptual N pools: structural (SN), photosynthetic (PN) and reserve (RN); the reserve N pool was sub-divided into '*storage*' (remobilised post-anthesis) and '*accumulation*' N (not remobilised post-anthesis) pools (Staswick, 1994). Two N source-sink manipulation treatments were imposed in the experiments approximately two weeks after flowering: defoliation (removal of leaf 3 and below on each shoot) and degraining (removal of all the spikelets from one side of the ear), to test responses of remobilisation of canopy N to changes in grain N source-sink balance.

Results showed that NUE (grain dry matter yield / N available) decreased with N supply. Between the unfertilised and optimally fertilised N treatments the decrease was approximately equally associated with declining UPE and UTE. However, above the optimally fertilised N treatments only UPE continued to decline. The main driver of lower UTE was the biomass production efficiency (BPE; above-ground DM / above-ground N), and varietal differences in BPE at Terrington in 2006/7 indicated the potential to breed for superior UTE. The amount of fertiliser N required to maximise above-ground DM at anthesis was considerably less than that required to optimise yields at harvest (N opt-trt), and reserve N was observed to accumulate within the canopy at anthesis in all N treatments. This reserve N accounted for 41 and 44% of above-ground N (AGN) at the optimal and supra-optimal N rates, respectively.

Reserve N was particularly located in the leaf lamina and true stem. The leaf lamina showed the highest PN content. However, the relationship between radiation-use efficiency (RUE; above-ground DM / PAR) during stem elongation and specific leaf N content (all culm leaves) at anthesis showed that the concentrations of N at the optimal

and supra-optimal N treatments exceeded that required for effective photosynthesis, which was *ca*. 2 g N m^{-2}, and indicated that the crop may be using these tissues as RN capacity, most likely in the photosynthetic enzyme 'Rubisco'. Results showed that a large quantity of N is loaded in the true stem at anthesis (ca. 25% of AGN at the N opt-trt). The true stem had the highest SN content, but also contained considerable quantities of RN at all N treatments, particularly at the optimal and supra-optimal N treatments (averaged across experiments at 45 and 45 kg N ha^{-1}, respectively; representing 42 and 38% of crop RN, respectively). The large physical capacity, central position and vascular role of the true stem makes this organ particularly suited to a RN function. Overall there was little genetic variation in N partitioning to the SN, PN and RN pools at anthesis (at the N opt-trt in the ranges 0.21-0.22, 0.42-0.44 and 0.35-0.37, respectively). This may have reflected the relatively narrow genetic basis of the germplasm used in this study (i.e. four elite UK cultivars with similar dates of release and end-use).

Large quantities of N were remobilised post-anthesis (overall in the range 90-153 kg N ha^{-1} across the three experiments). Most N was from the leaf lamina - contributing 29-35% to the grain N at harvest, with leaf sheath and true stem also providing 10-14% and 9-17%, respectively. This was relatively consistent across varieties. The N remobilised in the post-anthesis period (NR) appeared to be drawn mostly from RN pool in the first half of the grain-filling and then from PN pool in the second half of the phase. The timing and rate of canopy senescence was associated with canopy RN accumulation at anthesis, with senescence occurring predominantly after mid-grain filling in the well fertilised treatments when canopy RN capacity had declined. Senescence was also faster or slower where post-anthesis N remobilisation was increased or decreased in response to defoliation or degraining treatments, respectively. Present results showed that proportionally less true stem N at anthesis was remobilised during the grain filling period (i.e. lower N remobilisation efficiency; NRE) compared to the leaf lamina and leaf sheath, with little genetic variation observed in the Terrington experiments. Therefore the true stem contained considerable quantities of *accumulation* N at harvest at the optimal and supra-optimal N treatments (overall 12 and 17 kg N ha^{-1}), and would appear to provide a realistic breeding target for reducing canopy N requirement. However,

responses in the defoliation treatments demonstrated that true stem NRE could be significantly increased (overall by 20%) compared to the control, whilst the degraining treatments showed that grain N was mainly source limited, up to the upper limit of 1.1-1.2 mg N grain^{-1} when it became sink limited.

Overall observed genetic variation in UTE and underlying traits related to canopy N loading in the pre-anthesis phase and canopy N unloading in the post-anthesis phase was small in the present study. Nevertheless, several candidate traits were identified with potential to reduce fertiliser requirements in feed varieties. Firstly, increasing true stem RN capacity as means to increase the maximum rate of N uptake (kg N per day) during stem elongation may be feasible through optimisation of traits such as stem length and wall thickness. Secondly, modifying true stem RN unloading by increasing *storage* N in relation to *accumulation* N may offer a realistic mechanism for improving crop BPE and thus UTE. Such an increase in true stem NRE might be achieved through manipulation of key N assimilation enzymes. Thirdly, it may be possible to select for 'stay-green' traits associated with lower leaf lamina NRE and lower grain N% to boost UTE. However, in each case further phenotyping studies are required to characterise genetic variability, identify the most appropriate germplasm resources for genetic studies, and to identify appropriate genetic sources of variation for breeding.

1 INTRODUCTION

1.1 INTRODUCTION : THE CONTEXT

Three crops: wheat (*Triticum aestivum*, L.), rice (*Oryza sativa*) and maize (*Zea mays*, L.), supply nearly 90% of world cereal production and feed over half the world's population (FAOSTAT database, 2008). Current global wheat production is split almost equally between the developed and the developing countries, and the intensive and extensive agricultural systems, respectively. The high yields of intensive agricultural systems are underpinned by an understanding of the physiological basis of yield determination (both quantity and quality) and the use of appropriate technologies to maximise potential yields.

The most essential mineral element in determining the yield potential of wheat crops is nitrogen (N). Intensive cultivation of wheat requires large quantities of N to maximise yields, and modern varieties, particularly in NW Europe, have been selected to respond to high levels of N availability (Foulkes *et al.*, 1998). As a result, there has been a dramatic increase in the amount of fertiliser N applied to crops during the agricultural intensification of the past 50 years. However, wheat crops are currently only able to take up a limited proportion of this applied N; the worldwide average is 30-40% (Raun and Johnson, 1999) and the European average is 50-60% (Bloom *et al.*, 1988). So a significant amount of applied N is lost to the environment with deleterious economic and environmental effects.

Therefore, there is an increasing need to reduce fertiliser N inputs in current cereal systems whilst maintaining acceptable grain yields and adequate grain protein contents. Breeding to develop new wheat varieties which can use N more efficiently through improved uptake and utilisation would lower crop fertiliser N requirements without compromising yields. The optimisation of fertiliser N use through the identification and selection of traits favouring more efficient use of N is now a high priority in wheat production systems. This chapter introduces wheat as a major global crop, explains how

intensive cultivation depends on the requirement for N fertilisation, and considers how this has led to potentially detrimental environmental impacts. The concept of N-use efficiency (NUE; grain dry matter yield / N available) is introduced in the context of improving NUE through breeding strategies in wheat to maintain yield potential with lower N fertiliser inputs.

1.2 WHEAT AS A GLOBAL CROP

Wheat was one of the first grains domesticated by humans in Southwest Asia about ten thousand years ago and has been a staple food of the major civilisations of Europe, West Asia and North Africa for the past eight thousand years (Curtis, 2002). Wheat is presently consumed by a third of the world's population, providing the main source of calories for more than 1.5 billion people, and on average providing one third of the total human calorific input (FAO, 2003). The wheat crop is widely adapted and grown in most regions across the globe; from almost 60^{o}N in Northern Europe (Norway) to 40^{o}S in South America (Chile) (Satorre and Slafer, 1999). It survives extremes of temperature, from below -35^{o}C in the vegetative phase in Ontario (Haji and Hunt, 1999) to over 40^{o}C during grain filling in the Sudan (El-Ahmadi, 1994). Yields can range from less than 1 t ha^{-1} in severe drought-stress environments to over 15 t ha^{-1} in agronomically optimum conditions in some countries, e.g. New Zealand.

Bread wheat belongs to the *Triticeae* tribe in *Poaceae*, the grass family; hybridizations between species in the same genus or related genus have occurred. It is an annual grass with a main shoot and a variable number of tillers (lateral stems) arising from the base of the plant or the axils of the lower leaves. The inflorescence ('ear' or 'spike') typically consists of 10-25 spikelets, each of which produces up to six florets which give rise to grain. Bread wheat is an allohexaploid (2n = 6x = 42) with a genome size of 16 billion base pairs of DNA organized into 21 pairs of chromosomes, seven pairs belonging to each of the genomes A, B and D (Sears, 1954). Wheat possesses one of largest and most complex genomes but because of its hexaploid nature and economic importance as a food source, it is the most cytogenetically studied of the crop species.

Hexaploid (6n) bread wheat resulted from the in-field hybridisation of an emmer or durum wheat with a diploid grass (*Aegilops tauschii*; Figure 1.1). Cultivated wheat is hexaploid with genomes AABBDD coming from three progenitors (wild einkorn *T. uratu*; wild emmer *T. dicoccoides*, and *Aegilops tauschii* Coss.) (Salamini *et al*., 2002).

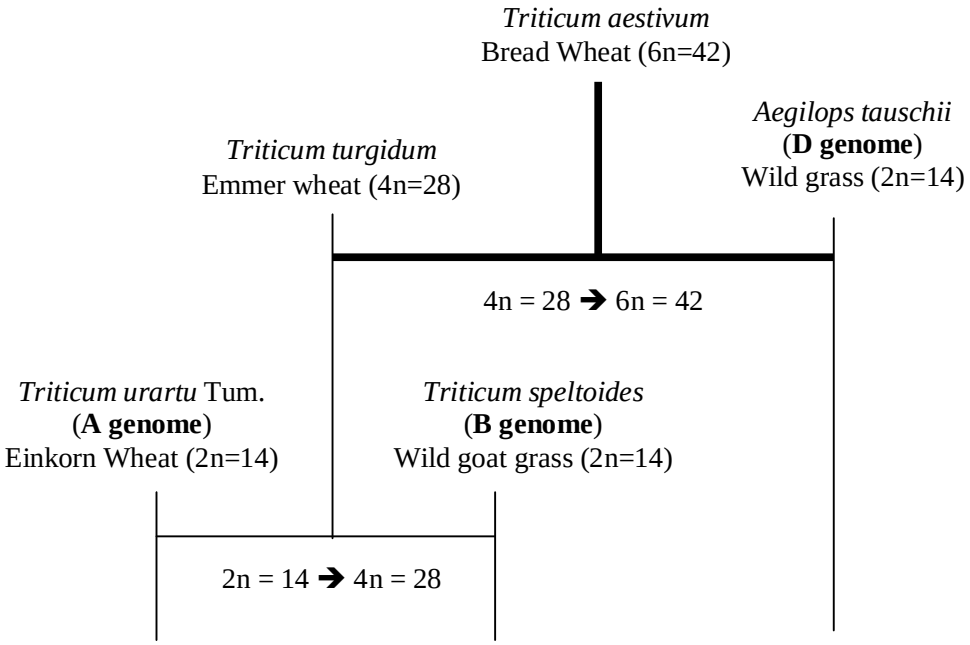


Figure 1.1 The origin of hexaploid bread wheat from natural hybridizations between three different species or genera of wild grasses (adapted from Gaju, 2003).

Within hexaploid bread wheat, cultivars are further classified by breeders and farmers, by: (i) length of growing season, e.g. winter wheat (with a requirement for cool vernalizing days before floral development) vs. spring wheat (no vernalization requirement); (ii) end-use quality, e.g. high vs low gluten content and/or hard vs soft grain texture and (iii) grain colour (red, white or amber). A wide variety of uses are based on these grain characteristics. High gluten is required for dough elasticity for bread-making. Hard wheats are used for bread and pasta (durum) making, whilst the soft wheats are used for the manufacture of biscuits and breakfast foods, fermentation to make whisky, beer or bio-ethanol, or feeds for livestock (mainly cattle). In the UK, winter wheat is used for bread-making (42%), biscuit-making (11%), animal feed (45%), and

other uses (3%; including distilling and bio-ethanol manufacture) (data for 2007-08; source DEFRA).

1.3 GLOBAL WHEAT PRODUCTION AND DEMAND

Wheat is a major arable crop. It is currently grown on about 220 million hectares worldwide and provides 606 Mt of grain per annum (27% of the total cereal output FAOSTAT database, 2008; maize 31% and rice 29%) at an average yield of 2.8 t ha^{-1} (FAOSTAT, 2006). Average regional yields can range from less than 1 to more than 7 t ha^{-1}, depending on the level of inputs and management as well as the edaphatic-climatic environmental conditions. Typical of the high-output regions, the UK annually produces about 15 Mt of wheat at an average yield of 8.0 t ha^{-1} (FAOSTAT database, 2006).

Throughout the first half of the twentieth century on-farm yields increased only slowly. However, between the 1950s and the late 1980s, yields increased (ca. 10 times) faster (Slafer *et al*., 1994). Yield increases in the UK were attributed approximately equally to agronomic and breeding advances (Austin *et al*., 1989). The 'Green Revolution' in the 1960s and 1970s was driven by the development of semi-dwarf varieties, with greater lodging resistance and increased harvest index (HI; proportion of above-ground biomass as grain at harvest), allowing the use of higher rates of N fertiliser. The 1980s and 1990s saw yields continuing to increase at ca. 1% per year in optimal environments worldwide. This was assisted by the introduction of yield-enhancing alien chromatin into wheat germplasm, such as the 1BL/1RS chromosome translocation from rye (*Secale cereale* L.) (Foulkes *et al*., 2007), and more recently the LR19 segment from tall wheat grass [*Agropyron elongatum* (Host) P.Beauv.] (Reynolds *et al*., 1999).

In the last two decades, however, despite continued genetic gains in yield potential in the most productive environments (Reynolds *et al*., 1999), on-farm yield gains have begun to level off at less than a 0.5% average worldwide increase per year (Reynolds *et al*., 2007b) (Figure 1.2). Studies suggest that on-farm yields have reached a plateau in some countries (Calderini and Slafer, 1998) and this is thought to be partly due to economic reasons,

such as the reduction in use of expensive agro-chemical inputs. It may also be in part due to the fact that harvest index is approaching a theoretical maximum value estimated to be 0.62 (Austin, 1980) in some counties.

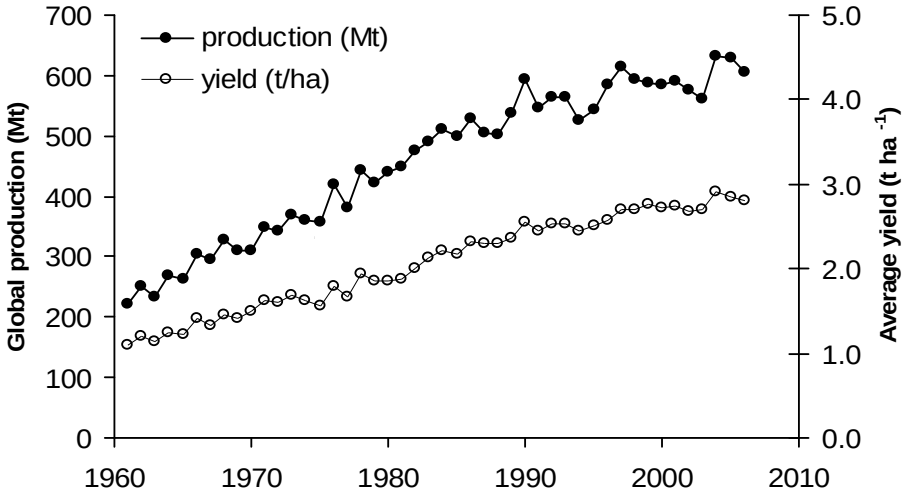


Figure 1.2 Trends in global wheat production and average grain yield; 1961 to 2006. Source: Food and Agriculture Organisation of the UN, 2008 (http://faostat.fao.org)

There are additional challenges to increasing production in the twenty first century. These include the increasing cost and scarcity of resources involved in intensive agricultural systems; in particular, fossil fuels for agro-chemical manufacture and application (including nitrate fertilisers), and the requirement to reduce the 'environmental footprint' of crops. There are also the issues such as more unpredictable climates leading to drought in wheat growing regions, e.g. Australia (Araus *et al.*, 2002), and increasing urbanisation and industrialisation causing the loss of land from agriculture (Hobbs, 2007).

By 2050 the global grain demand is projected to double (Semenov *et al.*, 2007), associated with an increase in world population from 6.1 billion in 2000 to 9.4 billion (US Census, 2008). The worldwide demand for wheat is expected to grow from 2072 Mt in 2000 to 2860 Mt in 2025 (Rosengrant *et al.*, 2002), and it is estimated that global demand for cereals will double by 2050 (Tilman *et al.*, 2002). In particular, human dietary changes in Asia are creating a shift from maize to wheat, and to diets with a

higher proportion of meat and dairy products (much of it grain-fed) (Tilman *et al*., 2002). Global meat demand is expected to increase by more than 50% by 2030 (FAO, 2007).

The market for wheat is also changing with increasing non-food uses. The emerging 'bio-fuels' market in Europe and North America is creating a demand for low protein grain (8-10%) to produce 'bio-ethanol' as a petrol additive or substitute. Demand for 'bio-fuels' is expected to grow through policy initiatives, such as the EU target for 10% of road transport fuels to come from 'bio-fuels' by 2020. Although it is difficult to predict the rise of the demand of grain for 'bio-fuels' due to the influence of an array of governmental and industrial policies, it is estimated that bio-energy crops will account for 2-16% of fertiliser consumption in 2030 (Smeets and Faaji, 2006).

1.4 DEPENDENCE OF WHEAT PRODUCTION ON N FERTILISATION

Nitrogen is the leading plant macro-nutrient (with phosphorous (P) and potassium (K)), and dominates crop nutrition (Grindlay, 1997). N is the mineral element required in the greatest quantities by wheat and comprises 1 to 2% of the total wheat plant dry weight at anthesis (Justes *et al*., 1994). However, despite being the most abundant element in the atmosphere, it is also one of the most limiting mineral nutrients to crop growth in natural ecosystems worldwide (Vitousek *et al*., 2002). The majority of N present in nature is held either in primary rocks (about 98%) or in the atmosphere (about 2%) and only a very small fraction is available for plants to take up as nitrate (NO_3^-) or ammonium (NH_4^+) ions.

Since 1962 annual global production of N fertiliser has increased from 13.5 Mt to 86.4 Mt N in 2001 (FAO, 2004), and N fertiliser use is expected to continue to increase three-fold by 2050 (Tilman *et al*., 2001). Until recently, the total N fertiliser use was almost equally divided between developed and developing countries (FAO, 1990). However, it is estimated that two-thirds of global N fertiliser use with be in developing countries by 2025 (Galloway *et al*, 1995) where the immediate goal is economic survival, and not the

preservation of the environment (Campbell *et al*., 1995). Cereals account for about 56%, and wheat 19%, of total N fertiliser usage worldwide (IFA, 2008). The amount of N fertiliser applied to UK wheat in 1951 was only 28 kg N ha^{-1}, by 1975 rates had increased to 73 kg N ha^{-1}, but between 1975 and 1985 rates increased dramatically to around 185 kg N ha^{-1} (Austin, 1999). Since 1985 the amount of N applied has remained stable at about 190 kg N ha^{-1} (DEFRA, 2005). Farmers have latterly conserved the amount of N applied due to the significant price rise of N fertilisers relative to the price of grain.

The use of high amounts of N fertiliser may have detrimental environmental impacts and has an economic cost to the grower. Firstly, the manufacture of N fertilisers in the Haber-Bosch process requires a high fossil-energy input, typically from natural gas, and the distribution of fertilisers uses considerable amounts of diesel oil. This contributes to the release of significant quantities of the 'green house gases', particularly carbon dioxide, into the atmosphere. Once applied, significant losses of fertiliser N into the environment then occur attributed to the combined effects of volatilization, leaching and denitrification.

Volatilization of fertiliser N creates gaseous emissions of ammonia and nitrogen oxides which have critical roles in tropospheric and stratospheric chemistry and cause air pollution problems, including radiative forcing (Khalil *et al.*, 2002). These losses for applied urea can exceed 40% (Fowler and Brydon, 1989). Nitrate ions within the soil system are very mobile and are easily leached into groundwater and surface waters. This can cause over enrichment of freshwater and drinking water supplies which can cause human health problems (London, 2005), and into marine ecosystems causing eutrophication and hypoxia (low-oxygen) conditions, endangering fisheries, changing species composition, and/or reducing biodiversity in non-agricultural systems (Burt and Haycock, 1991; Malakoff, 1998). Finally denitrification losses due to the activity of soil bacteria can be considerable, especially in anaerobic conditions (2-18% of applied N; Owen and Jurgens-Gschwind, 1986). In addition, loss of fertiliser N also has a direct economic cost to the grower, and the excessive use of N fertilisers in poorly managed systems can have a negative, sometimes 'catastrophic', effect on yield due to an

increased risk of lodging (Berry *et al*., 2004), and weed, pest and disease infestations (Davies and Sylvester-Bradley, 1995).

1.5 FACTORS INFLUENCING THE EFFICIENCY OF N FERTILISER USE

To increase crop NUE, two complementary strategies can be employed: improvement through crop management or breeding.

1.5.1 Management strategies to increase N-use efficiency

Fertiliser N losses have been reduced and yield has been increased by agronomic (or crop management) strategies. These include optimising N inputs according to Raun and Johnson (1999), by:

(i) consideration of the growing environment (i.e. soil type and climate)

(ii) fine tuning regimes for N fertilisation (quantity, timing, and rate of applications) taking into account sources of N (soil, previous crops, animal manures)

(iii) optimising soil management practices (tillage and organic matter) and irrigation practices

Further examples of optimising N management to improve NUE include: (a) splitting the amount of N fertiliser applied into two or three doses (Addiscott *et al*., 1991), and placing the fertiliser below the soil surface layer to decrease immobilisation and increase plant N uptake (Sharpe *et al*., 1988); (b) correctly timing application to achieve a synchrony between N supply and crop demand in time and space (Shanahan *et al*., 2008), the highest demand is during leaf expansion and the 'optimal' timing for a single N application should coincide with the appearance of the first large leaf on the mainstem (Sylvester-Bradley *et al*., 1998); and (c) appropriate crop rotations.

1.5.2 Breeding strategies to increase N-use efficiency

The alternative approach to improving NUE is through the breeding of more N-efficient wheat cultivars. Breeding progress can be accelerated through the identification and selection of desirable traits which increase the uptake and/or utilisation efficiency of crop N to complement empirical selection for yield under low N availability (Araus *et al*., 2002). Candidate traits may exist at the canopy, leaf, or biochemical level, and can be identified through detailed physiological and modelling studies. Further genetic studies applying molecular marker mapping approaches using appropriate segregating populations can identify Quantitative Trait Loci (QTL) for complex traits such as NUE (Hirel *et al*., 2007) and underlying traits. The QTL can then be used to develop molecular markers with which to screen breeding lines for traits underlying NUE.

However, breeding strategies in the UK in recent years have been to select varieties at high levels of fertiliser N supply (Foulkes *et al*., 1998; Sylvester-Bradley and Kindred, 2009). This has potentially resulted in an increase in the uptake and accumulation of canopy N without immediate function (i.e. photosynthetic or structural N), and the accumulation of reserve N in the green canopy, most probably in stems and leaf sheaths (Foulkes *et al*., 1998). Such cultivars may therefore have a high efficiency of N recovery, but a low conversion efficiency of crop N into grain dry matter under high N supply, and may perform poorly under limited N supplies. Although crop reserve N accumulation does have an important role in the N economy of the plant, breeding for an optimisation of reserve N accumulation in wheat canopies would offer the prospect of the reduction in crop N fertiliser requirement through higher utilisation efficiencies, that is, producing higher yields from the same nutrient levels, or the same yield with lower nutrient levels.

1.6 AIMS OF THE THESIS

The challenge to world agriculture is to optimise the trade-off between grain yield and economic profit and the negative environmental impacts associated with fertiliser N use in intensive agricultural systems. High crop NUE is required, and the breeding of new N-

efficient varieties of wheat will be critical in meeting these challenges and concerns, whilst maintaining increases in grain production to ensure food security.

To meet these challenges, new wheat varieties must be bred requiring lower N inputs whilst maintaining, or ideally increasing, grain yields (possibly of low protein concentration). This increase in the crop NUE would be achieved, at least partly, through an increase in crop N-utilisation efficiency (UTE; grain DM yield/ crop N uptake).

The overall aims of this thesis are therefore to:

- Increase the understanding of the physiological processes determining the requirements of the crop for N during vegetative and reproductive growth which affect crop NUE, including physiological mechanisms for improving N uptake, use and partitioning within the crop canopy.

- Identify and quantify physiological traits of existing UK wheat varieties at the canopy level which are associated with improved crop N utilisation efficiency, and tolerance of low to moderate N availability which could provide breeding opportunities for new, more N-efficient cultivars.

- Improve the understanding of environmental variation on NUE and underlying traits, particularly those affecting crop N-utilisation efficiency, with reference to the UK and New Zealand environments.

- Produce a framework to quantify the crop N status according to structural, photosynthetic and reserve N content of the canopy, which can be applied to the investigation of target traits.

2 LITERATURE REVIEW

2.1 INTRODUCTION

The physiological N requirement of wheat is the amount or concentration needed to satisfy each of the plant processes affecting growth up to grain maturity. The crop requires sufficient N to produce and maintain a green canopy area capable of intercepting and utilising solar radiation to optimise biomass production, grain growth and quality. Wheat crops in the UK require large quantities of N to produce high grain yields (of around 10 t ha^{-1}) and of high quality (of around 12% protein) (Greenwood, 1982). The rationale for optimum N fertilisation requires a fundamental understanding of the complex inter-relationships between N availability, uptake and utilisation during crop growth.

This study examines the requirement of wheat for N, addressing how N is used within the canopy (functional and non-functional roles), how it is distributed between the components of the canopy, and how this changes during the growth of the crop. The effects of the availability of N and variety are quantified in field experiments, which analyse the distribution, function and determination of canopy N, and the allometric relationships of allocation and partitioning of N between the canopy components. Particular focus will be placed on the N content in the non-leaf components of the canopy in relation to their possible non-functional roles and whether physiological traits can be identified which could be manipulated to increase the efficiency of N use.

This chapter reviews the literature regarding the physiological bases of the efficiency of the use of N by wheat crops. It starts by examining the evidence for variation in N-use efficiency and its two sub-components, UPE and UTE, with N availability and variety. The current approaches to defining limitations to whole-crop growth according to crop N status, and the distinction between N-deficient and N-sated crops are discussed. Then the current knowledge and understanding of the physiological role of N at the leaf and canopy level is reviewed for productive and efficient crops, separately for the vegetative

(up to anthesis) and the reproductive (anthesis to harvest) phases. Finally, how wheat simulation models have interpreted these canopy N traits in sensitivity analysis to predict growth and yield, and the potential for model improvement is considered. The chapter concludes with the study objectives and hypotheses to be tested in this thesis.

2.2 N-USE EFFICIENCY AND COMPONENTS

2.2.1 Definition of N-use efficiency

In order develop strategies to improve the efficiency of N fertiliser use, it is first important to clearly define N-use efficiency (NUE). Moll *et al*. (1982) defined NUE as 'grain production per unit of N available in the soil'. This is represented by the grain dry matter yield (Y) divided by the amount of N supplied (N) to the plant by the soil (including the residual N present in the soil and the fertiliser N);

$$NUE = Y\ (kg\ ha^{-1}) / N\ (kg\ ha^{-1}) \qquad \textbf{Equation 2-1}$$

NUE encapsulates the whole plant N uptake and the utilisation of N to produce grain from the sum of the processes associated with the absorption, translocation, assimilation, and redistribution of N (Moll *et al*., 1982). NUE can therefore be further divided into two primary components: (i) the N-uptake efficiency (UPE; the efficiency with which the plant takes up N from the soil), and (ii) the N-utilisation efficiency (UTE; the efficiency with which the absorbed N is used to produce grain dry matter) (Le Gouis *et al*., 2000);

$$UPE = AGN\ (kg\ ha^{-1}) / N\ (kg\ ha^{-1}) \qquad \textbf{Equation 2-2}$$

$$UTE = Y\ (kg\ ha^{-1}) / AGN\ (kg\ ha^{-1}) \qquad \textbf{Equation 2-3}$$

Where, AGN is the total N in the above-ground plant at maturity. Hence;

$$NUE = (AGN/N) \times (Y/AGN) \qquad \textbf{Equation 2-4}$$

These definitions of NUE and its two main sub-components will be used hereafter in this thesis.

2.2.2 Evidence for genetic variation in N-use efficiency

Differences in NUE due to differential responses to N fertiliser are well documented, and experiments examining NUE at different levels of N supply nearly always show that the highest efficiency of fertiliser N is achieved with the first increments of added N, while NUE declines with higher levels of application (Chamorro *et al*., 2002). Low NUE is typically the product of over fertilisation, sub-optimal yields, N losses, and disproportionate increases in maintenance costs of large canopy N contents. However, as the level of N supply changes, the relative contributions of the component NUE traits (UPE and UTE) to genetic variation in NUE have been found to be considerably different. Genetic variation in NUE, and the interaction between N response and variety, has been demonstrated for wheat (Ortiz-Monasterio *et al*., 1997; Le Gouis *et al*., 2000) and for other important crops such as maize (Moll and Kamprath, 1977; Moll *et al*., 1982) and rice (Borrell *et al*., 1998). Even where similar levels of NUE are found between varieties, differences in the component traits may exist, and such genetically controlled factors provide potentially selectable traits for breeding for improved uptake and/or utilisation efficiency.

Following an experiment by Moll *et al*. (1982) on maize, Dhugga and Waines (1989) studied the accumulation and use of N in 12 bread and 2 durum wheat varieties. It was found that the uptake efficiency became more important than the utilisation efficiency in determining NUE at increasing soil supply. Ortiz-Monasterio *et al*. (1997) also found significant variation in NUE with N supply between varieties of bread wheat cultivars grown in northern Mexico, however in contrast to the findings of Dhugga and Waines (1989). Ortiz-Monasterio *et al*. (1997) observed an inverse relationship; at low N supply differences in NUE between varieties was due largely to UPE, whilst at high N supply the variation was largely due to UTE. Le Gouis *et al*. (2000) working on a set of 20 winter wheat cultivars in northern France, also found that UPE accounted for more of the

variation in NUE than UTE at low N supply. Overall these investigations showed that when N is limiting the ability to explore the soil and absorb N is of greater importance to the crop, whereas when N is not limiting sufficient N will be available within the crop independently of the efficiency of the root system, and UTE would be of greater importance in determining NUE.

2.2.3 N-uptake efficiency

N-uptake efficiency is the ability of the plant to remove N (as ammonium or nitrate ions) from the soil in relation to that available (soil mineral N and applied N). It is normally assumed that soil mineral N is recovered by the crop in preference to applied N, although full recovery of available N by fertilised cereal crops is never achieved (Vaidyanathan, 1984). From a summary of published data on N efficiencies in cereal crops, Ladha *et al.* (2005) found that the average recovery efficiency of fertiliser N was 54% in wheat. Field trials in Europe have recorded an average range of 50-60% recovery of fertiliser N applied to winter wheat (grain and straw) (Bloom *et al*., 1988; Powlson *et al*., 1992; Blankenau *et al*., 2002; MacDonald *et al*., 2002).

In high-input systems the ability to absorb and accumulate high concentrations of N is a desirable trait and the selection of such genotypes would reduce leaching losses from applied N during periods of abundant supply. Field experiments have shown significant differences between varieties for N-uptake in wheat (Halloran and Lee, 1979; Ortiz-Monasterio *et al*., 1997; Le Gouis *et al*., 2000). Some cultivars are genetically better able take up N more efficiently, indicating the influence of genetic control for N uptake (Le Gouis *et al*., 2000). Differences in UPE may result from variation in the ability to absorb N from various soil depths which affect the quantity, rate and duration of N uptake. These differences may be related to: (i) rooting characteristics such as length, density and distribution with depth, and longevity, (ii) the efficiency of absorption and assimilation of ammonium and nitrate at the root surface, (iii) root-induced changes in the rhizosphere affecting N mineralization, transformation and transport (Kundu and Ladha, 1997), (iv) the soil texture, climate conditions, interactions between soil and bacterial processes

(Burger and Jackson, 2004), and (v) the nature of organic or inorganic N sources (Schulten and Schnitzer, 1998). The applied N not recovered by the plant during the growing season is either incorporated in soil organic matter (immobilised) (typically 8 to 21%), lost by denitrification (typically 2 to 18%) (Owen and Jurgens-Gshwind, 1986) or comprises a variable amount lost to volatilisation depending on environmental conditions (King *et al.*, 2001).

2.2.4 N-utilisation efficiency

N-utilisation efficiency is the ability to use the N taken up by the plant to produce grain yield, and is the product of: (i) the amount of above-ground biomass produced per unit of crop N uptake (BPE, above-ground biomass production efficiency; Ortiz-Monasterio *et al.*, 1997) and (ii) the partitioning of this biomass to the grain (harvest index) at harvest. UTE therefore concerns yield determining processes, including the N required for canopy formation and survival, the construction and maintenance of photosynthetic processes, grain filling through carbohydrate and N remobilisation, and the grain storage capacity. Crops with higher UTE will produce higher yields from the same N uptake, or the same yield with lower N uptake.

Many of these yield-determining steps are genetically controlled, and genotypic variation in UTE in wheat has also been reported in several studies (Cox *et al.*, 1985b; Van Sanford and MacKown, 1987; Dhugga and Waines, 1989; May *et al.*, 1991). HI has generally been the component most associated with genetic gains in UTE in the past (Riggs *et al.*, 1981; Austin *et al.*, 1989; Ortiz-Monasterio *et al.*, 1997, 2001). For example, Austin *et al.* (1989) reported that the progressive increase in grain yield (from 5.1 to 8.1 t ha^{-1}) for cultivars introduced from 1900 to 1986 was almost entirely accounted for by the increase in the HI from 0.34 to 0.51, whilst total biomass production was broadly maintained.

Improvements to BPE also provide potential utilisation efficiency increases, especially as physiological avenues to further increase HI above the theoretical maximum of about

0.62 in winter wheat (Austin *et al*., 1980) may be limited in countries where HI is already approaching this level (Fischer, 1981; Calderini *et al*., 1995). Genetic differences in uptake, reduction, assimilation and storage of N over the vegetative and reproductive periods can affect the amount of N in the shoot, and the partitioning between the shoot component organs, at both anthesis and harvest respectively (Cox *et al*., 1985a; Loffer *et al*., 1985).

Increased rates of nitrate assimilation to ammonium, amino compounds and proteins could be achieved through improvements to enzymes such as nitrate reductase (converts nitrate to nitrite), nitrite reductase (converts nitrite to ammonium), and the glutamine synthetase/glutamate synthase enzyme system (GS/GOGAT; converts ammonium to glutamine or glutamate) (Lea and Ireland, 1999). However, recent studies on altering the nitrate reductase encoding genes have resulted in no change in plant growth (Crawford, 1995), and studies have failed to find a correlation between leaf nitrate reductase activity and yield in wheat (Kelly *et al*., 1995) and maize (Masclaux *et al*., 2001). Manipulation of glutamine synthetase, which is also important in the internal recycling and remobilisation of N, has been shown to improve grain yield and protein concentration in wheat (Habash *et al*., 2001). At the crop/plant level of organisation, increased biomass production from accumulated N could be achieved through exploiting genetic differences, to: (a) increase the photosynthetic rate per unit leaf N (Hirose and Werger 1987b), (b) optimise the distribution of N within the canopy to maximise net assimilation, (c) increase the 'sink' capacity of the crop to assimilate and incorporate N and DM during vegetative growth, (d) reduce the non-functional labile N accumulated in the canopy, (e) optimise N remobilisation and re-distribution during grain filling, and (f) select for cultivars with low grain N concentrations.

2.3 QUANTIFYING THE N STATUS OF WHEAT CROPS

Crop N uptake in wheat is co-regulated by the soil N concentration and the potential rate and duration of growth of the crop (Lemaire *et al*., 2004). The crop biomass provides a strong sink for N uptake and the crop N content is therefore related to crop biomass. The

relationship is not linear and the proportion of additional N uptake per unit of biomass declines as the crop becomes larger (Gastal and Lemaire, 2002).

The main effect of an increased N supply on growth is through increased canopy green area (rather than through increased net assimilation rate or leaf net photosynthesis) from both higher tiller survival (Hirel *et al*., 2007) and a higher leaf area per shoot, leading to an increased light interception and production of dry matter (Grindlay *et al*., 1997; Olesen *et al*., 2002). The maximum canopy green area normally occurs at around ear emergence (Sylvester-Bradley *et al*., 1997). However, N deficiency reduces leaf expansion and canopy green area (Fischer, 1993), producing pale foliage (Milford *et al*., 1985), diverting resources to promote root extension (Brouwer, 1966), and slowing the production of dry matter (Biscoe and Willington, 1985). In N deficient crops, the N supply (a function of soil mineral N content and root growth) determines the amount of N taken up (Justes *et al*., 1994). A close relationship exists between the N status of a crop and the size and productivity of its canopy in the vegetative phase of crop growth. Two alternative models have been proposed to quantify this nutritional relationship: (i) the 'crop N requirement' (based on canopy green area), and (ii) the 'critical N concentration' (based on crop biomass):

2.3.1 The crop N requirement

Sylvester-Bradley *et al*. (1990a) observed that in a well managed crop, a relatively constant relationship exists between the canopy green area and the N content per unit ground area of UK grown winter wheat (excluding the ears). In experiments at Broom's Barn Experimental Station, Suffolk, cv. Avalon was grown at a range of N rates for two seasons: 1981/82 and 1984/85. The slope of the linear relationship was termed the 'canopy nitrogen requirement' (CNR; g N per m^2 green area). It was found that approximately 3g of N in the shoot was required to build 1 m^2 of canopy green area. Studies on other crops have found a similar relationship in lucerne (Lemaire *et al*., 1997) and in maize (2.9 g N m^{-2}; Plénet and Lemaire, 1999).

Grindlay *et al.* (1993) in an experiment at ADAS Cambridge, found that the majority (range 87-97%) of the variation in shoot N of winter rye (*Secale cereale*) and winter barley (*Hordeum vulgare* L.) was accounted for by the green area across a range of plant sizes and N supplies, but excluding supra-optimal N rates where luxury consumption of N can occur. Sylvester-Bradley *et al.* (1990b; 1997) found that this proportionality between crop N content and canopy green area for winter wheat (cv. Norman) was consistent throughout development, and across different sowing dates, sites, growing conditions and levels of N (excluding excess N supplies). The stability of the relationship can be understood through the physiological roles of N in the formation, support and maintenance of the crop canopy (Critchley, 2001). During the vegetative phase, the canopy can be viewed conceptually as a combination of 'metabolic' and 'structural' components. The metabolic component corresponds to the leaf lamina tissues which contain large quantities of reduced N in the form of photosynthetic proteins (enzymes and other compounds such as chlorophyll). The structural component corresponds to the supporting tissues and vascular connections of the stem and leaf mid-rib.

As the crop canopy grows the balance between the metabolic and the structural components changes:

1. As the lamina area becomes larger an increasing proportion of the leaves are self-shaded, inducing a vertical leaf N content distribution from the top of the canopy (high N concentration) to the shaded lower layers (low N concentration). Consequently, average N content per unit leaf area decreases in tandem with increasing canopy area (Field, 1983; Hirose and Werger 1987b; Sinclair and Horie, 1989; Pons and Percy, 1994).

2. The increasing lamina area increases the need for structural support and with vascular connections. This increases the proportion of the structural component with a low N content (Grindlay, 1997).

Thus, the decrease in the metabolic N is balanced by the increase in the structural N, maintaining the stability of the crop N to green area ratio.

However, the consistency in canopy N requirement may only be applicable within specific bounds (Sylvester-Bradley *et al.*, 1997). Since several studies have shown significant effects of N treatment, cultivar, and environmental conditions (such as light intensity) on the CNR (Foulkes *et al.*, 1994; Stokes *et al.*, 1997; Foulkes *et al.*, 1998; Scott *et al.*, 1998). In particular, the CNR has been found to vary in relation to the N availability. Grindlay *et al.* (1997) found that the CNR of unfertilised, N deficient wheat was reduced to 2.2 to 2.5 g N m^{-2}, whilst that of wheat under high N could be far higher at 5.1g N m^{-2} (Grindlay *et al.*, 1993). The N present in the crop may therefore represent a 'content' rather than a 'requirement', with an increased N content representative of additional uptake rather than functional requirement (Critchley, 2001).

2.3.2 The critical N concentration

Alternatively, the N nutritional status of a crop can be assessed through the N concentration of its biomass. For a crop of given biomass, there is minimum concentration of N needed to achieve the maximum growth rate (at a given time and field situation) (Ulrich, 1952; Greenwood *et al.*, 1991) and this is termed the 'critical N concentration' (Lemaire *et al.*, 1991). Lemaire and Salette (1984) demonstrated that for two grass species (tall fescue and cocksfoot) growing with a non-limiting N supply, the plant N% was related to the dry matter accumulation through the negative power equation:

$$N\% = a\,(W)^{-b} \qquad \textbf{Equation 2-5}$$

where W is the weight of above-ground dry matter (t ha^{-1}), N% is the N concentration as a percentage of dry matter, and coefficients *a* and *b* represent the plant N% for an above-ground biomass of between 1 (below which plants behave as individuals and little self-

shading occurs, this is 1.55 for wheat; Lemaire *et al*., 1991) and initially to 12 t ha^{-1}, and characterise the pattern of decrease in N% during growth, respectively.

However, as the crop grows, the N concentration of the crop biomass was found to decline, even under a non-limiting supply of N (Lemaire *et al*., 1985; Lemaire and Gastal, 1997; Plénet and Lemaire, 2000). As with the CNR, this is accounted for by a reduction in the proportion of metabolic tissues (with high N) and increase in the proportion of structural tissues (with low N) with crop canopy growth (Grindlay, 1997). The decline in the concentration of N in the crop was termed the 'dilution law of critical N%' by Justes *et al.* (1994). Greenwood *et al*. (1991) plotted the critical N% of many different C_3 species on the same graph and found that they fell on approximately the same curve. Other studies on individual crops also demonstrated the same pattern and confirmed the 'law' for C_3 species: grassland (Lemaire and Salette, 1984), potatoes (*Solanum tuberosum*; Greenwood *et al*., 1990), wheat (Justes *et al*., 1994) and oilseed rape (*Brassica napus* L.; Colnenne *et al*., 1998), but not for C_4 species: maize (Plénet and Lemaire, 1999), sorghum (*Sorghum bicolour*) and *Setaria anceps* (Lemaire and Gastal, 1997). Thus from Equation 2-5, the coefficient *b* is roughly similar between species and the coefficient *a* is different between the C_3 and C_4 metabolic groups.

The critical N dilution curve for wheat was determined statistically by Justes *et al.* (1994) from several experiments with increasing levels of N supply (from deficient to supra-optimal) and different varieties of winter wheat (Figure 2.1). That study showed that crops with optimal N supply conform to the line, whilst crops with supra-optimal N nutrition have N contents in excess of that required to maximise productivity, which is independent of experimental conditions (Lemaire and Denoix, 1987). Justes *et al.* (1994) showed that at the supra-optimal N fertilisation rates, the plant N% could be up to 60% higher than the critical concentration as a result of 'luxury uptake' without providing additional growth. It was also found that this extra N was not evenly distributed across the whole canopy, but accumulated in specific canopy components, such as the upper leaves.

This ‘critical dilution curve’ was described by the equation (Justes *et al*. 1994):

$$N\%_{ct} = 5.35\ DM^{-0.442} \qquad \textbf{Equation 2-6}$$

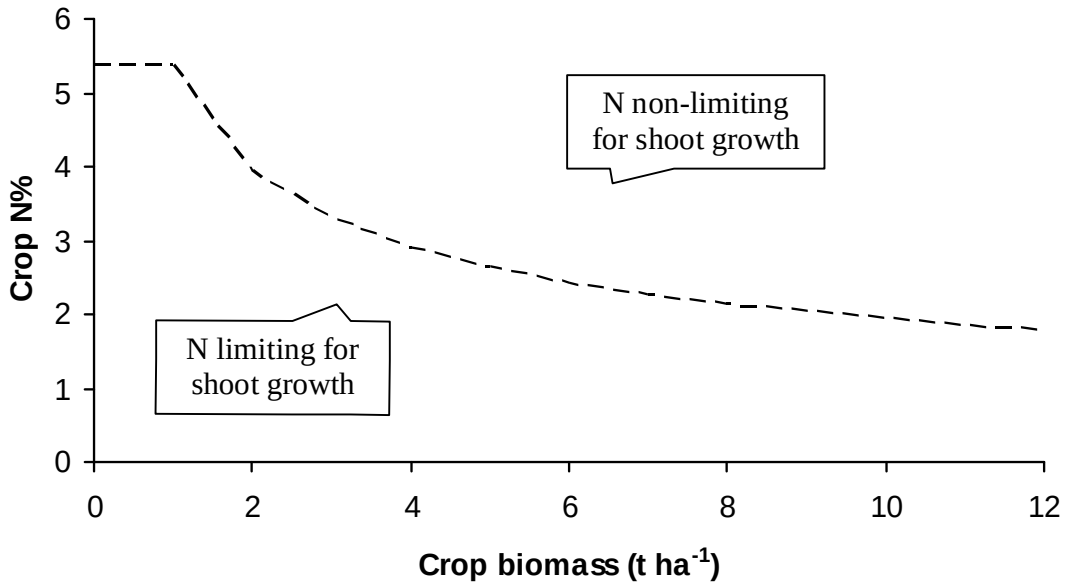


Figure 2.1 Critical dilution curve for winter wheat (from Justes *et al*., 1994)

Wheat crops of given biomass can have a range of N concentrations depending on the level of N fertilisation. Crops with an N% below the critical line can be considered to be deficient in N with this limiting growth, whilst crops with N% at or above this line can be considered to have a canopy N sufficient to or in excess of that required for non-limited growth. Lemaire *et al*. (1989) proposed the ‘N nutrition’ index’ (NNI) to quantify the ratio between the actual N% and the critical N% for diagnosing the N status and N stress intensity of the crop:

$$NNI = N_{actual} / N_{critical} \qquad \textbf{Equation 2-7}$$

where N_{actual} is the total N% measured and $N_{critical}$ is the critical N% at that dry mass (DM). Such an evaluation is only valid during vegetative growth, as the accumulation of

storage materials (mainly starch) in the reproductive tissues during grain filling makes the N% decline more rapidly.

The instantaneous crop N nutrition is considered to be 'optimal' when the NNI = 1. A crop whose NNI is maintained at or above 1 should accumulate the maximum dry matter allowed by the environmental conditions, whilst a crop with periods of deficiency (as; 'time x (1-NNI)') will show a reduced productivity by anthesis. Periods of excess canopy N can occur transiently (such as immediately after fertilisation application) or for longer periods (such as at supra-optimal fertilisation N rates) when continued excess N uptake will occur.

2.4 THE ROLE OF N IN THE VEGETATIVE GROWTH PHASE

During the vegetative growth phase (emergence to anthesis), three conceptual pools of N within the canopy have been suggested (Lemaire and Gastal, 1997) to enable crop growth: (i) the structural N - composed of N containing structures required to physically support the photosynthetic surfaces and supply them with vascular connections, (ii) the photosynthetic N - composed mainly of the proteins of the photosynthetic machinery, and (iii) the reserve N - composed of a 'reservoir' of N accumulated by the crop in excess of that required to satisfy the demands of (i) and (ii). As the majority of the N which enters a plant is retained and re-used, the N in the pools is dynamic, changing in relation to the patterns and conditions of growth.

The two models described in section 2.3 described the N status at the whole crop level by averaging the crop N content over the whole shoot. However, a more functional analysis should aim to take account of the component organs (leaf laminae, leaf sheaths, true stem and ears) – and to consider the N content of each organ in relation to that required to fulfil its function. The following sections will consider the role of N in relation to photosynthetic, structural, transport and reserve functions in the major physiological processes of growth.

2.4.1 Photosynthetic requirement for N

The capacity of a leaf for photosynthesis is related to the amount of light incident upon the leaf and the N content of the leaf. Net carbon gain (productivity) at the whole canopy level therefore depends on the quantity and distribution of light in relation to the photosynthetic tissues and their capacity for photosynthesis. Over the whole growing season, the productivity of the canopy and yield is therefore a function of the total amount of light which is intercepted and the efficiency with which it is converted to DM (Monteith, 1977; Hay and Porter, 2006).

2.4.1.1 Light interception

Solar radiation (light) provides the energy to drive photosynthesis. Of the spectrum of solar radiation, the proportion that can be used by plants has wavelengths between 400 (blue) and 700 nm (red) and is termed the 'photosynthetically active radiation' (PAR). When absorbed by a chloroplast, the photon provides the energy to excite the electrons that drive the reactions of the Calvin cycle, the first few steps of photosynthesis. The PAR is normally taken to be 50% of the total solar radiation (Monteith, 1972), as an approximate average of a direct beam of light (45% PAR) and diffuse light within a canopy (60% PAR). The rate of light energy delivery to the canopy per m^2 of ground area is composed of the number of mol of quanta per second per unit of leaf area, commonly referred to as the 'photosynthetic photon flux density' (PPFD), and the irradiance can be expressed as the amount of light energy (E) in joules per unit area per unit time ($J\ m^{-2}\ s^{-2}$) and is proportional to the frequency of light (Montieth, 1984).

At a particular moment, the amount of incident light radiation intercepted depends on the green area index (GAI; i.e. the area of the crop green surfaces - leaves, leaf sheath and other green tissues per area of ground) and how the leaves are geometrically arranged in the canopy. For cereal crops, there is a diminishing increase in the proportion of radiation intercepted as the green area increases (Hay and Walker, 1989), and for winter wheat crops with a GAI of 5, more than 95% of the incident PAR will usually be intercepted (Sylvester-Bradley *et al.*, 1997); typical of a UK winter wheat crop in May. Large

canopies have the potential to intercept more radiation, but do not do so efficiently in relation to the green area and N required to produce them. However, more rapid canopy closure during the spring can significantly increase the total amount of light interception during the stem-elongation phase (and is promoted by N fertilisation and strongly linked to biomass at anthesis and final grain yields; Scott *et al.*, 1994). Over the season, the total amount of light intercepted by a crop canopy is therefore a function of its size, longevity, optical properties and structure.

As light passes through the canopy it is absorbed, and the remaining light is transmitted to the lower leaves. It is assumed that the attenuation of light through the canopy is similar to the passage of monochromatic light through a homogenous light absorbing solution approximated by Beer's law. The amount of light available at depths within the canopy can be described by a form of Beer's Law (Monsi and Saeki, 1953):

$$I = I_o \, e^{-k L} \qquad \textbf{Equation 2-8}$$

Where I_o is the radiation above the crop canopy and I is the radiation at a point within the canopy above which there is a GAI of L. If the light extinction coefficient (K) is known, the fraction of the light intercepted by the canopy (F) can be calculated from L assuming that the leaves are randomly arranged:

$$F = 1 - e^{-K L} \qquad \textbf{Equation 2-9}$$

The geometry of the canopy is described by K, which is analogous to the adsorption coefficient in Beers Law (Baret *et al.*, 1993). The most important attribute affecting K is leaf angle, but K is also affected by leaf surface properties, thickness, size, shape, degree of dissection, phyllotaxis, and the vertical stratification of the leaf area (Hay and Porter, 2006). There are substantial differences in the extent of light radiation penetration into the canopy with leaf angle. Canopies with more erect leaves will intercept less light per GAI and therefore have a lower K, resulting in less saturation of the upper leaves and more light available to the lower leaves and a more efficient canopy. Reported K values

for PAR (K_{PAR}) in winter wheat demonstrate genetic variation for this trait; between 0.38 to 0.76 (Hay and Porter, 2006), 0.40 to 0.70 (Azam-Ali *et al*., 1994), 0.44 to 0.57 (for 8 UK winter wheat cultivars at GS 39; Shearman *et al*., 2005), 0.41 (for cv. Avalon; Sylvester-Bradley *et al*., 1990a), and 0.46 (which was unaffected by growth stage and N treatment; Thorne *et al*., 1988).

2.4.1.2 Leaf photosynthetic rate and radiation-use efficiency

The net rate of photosynthesis increases with increasing irradiance until the light saturation point which gives the maximum rate of photosynthesis (P_{max}), which is affected by temperature and cultivar (Fischer *et al*., 1998). A typical C_3 crop leaf, arranged horizontally, would be saturated at around 100 W m^{-2} PAR, less than half of the maximum irradiance in midsummer in north temperate zones, and produce a maximum rate of net photosynthesis (P_{max}) of the order of 20 μmol CO_2 m^{-2} s^{-1} at leaf N concentrations of 2 g m^{-2} under favourable conditions (Foulkes *et al*., 2006). While the photosynthetic efficiency of the leaf decreases with increased irradiance, this is most obvious after the point of saturation when increasing irradiance gives little or no further increase in the rate of net photosynthesis.

However, the photosynthetic efficiency of individual leaves within a canopy can vary significantly. The upper leaves of the canopy of most cereals are not capable of utilising all the incident radiation they receive due to light saturation, whilst the photosynthesis of lower leaves may be light limited. The amount and distribution of light penetrating a crop canopy depends on the incident radiation, canopy size and geometrical arrangement of the leaves. Leaf photosynthetic efficiency is also related to leaf N content (Dreccer *et al*., 2000), given that a substantial fraction of the leaf N is associated with the photosynthetic apparatus, although the relationship is hyperbolic (Sinclair and Muchow, 1999). The radiation-use efficiency (RUE; i.e. efficiency of conversion of the intercepted light radiation into above-ground crop dry matter) is predicted to increase with the optimal distribution of both light and N in the canopy, such that more N is allocated to the more illuminated leaves, reducing light saturation (Dreccer *et al*., 1998). For large wheat

canopies, RUE is higher with more erect leaves, associated with reduced light saturation of the upper leaves (Evans, 1983; Araus *et al*., 1993), and most modern wheat cultivars worldwide have semi-erect or erect flag leaves.

Radiation-use efficiency is expressed at the crop scale as the above-ground biomass per unit of solar radiation or PAR intercepted (g MJ^{-1}), and can be calculated from the difference in biomass between two consecutive harvests divided by the corresponding amount of radiation interception. The slope of this linear relationship is often assumed to be constant for a non-stressed crop species; stresses include drought and mineral nutrient deficiency (Gallagher and Biscoe, 1978). The net or 'apparent' rate of photosynthesis for the dry matter production of a crop, gives a balance between photosynthetic production and respiratory losses (photorespiration and 'dark' respiration). Reynolds *et al*. (2000; 2005) propose RUE as an avenue for genetically improving yield, given that RUE can theoretically be enhanced at the canopy, leaf and biochemical levels.

Sinclair and Muchow (1999) reported the range of maximum values for RUE in wheat to be 1.46 to 2.93 g MJ^{-1} PAR, affected by genotype and environment, and Kiniry *et al*. (1989) reported the average RUE for wheat to be 2.8 g MJ^{-1} PAR (± 0.2) from a review of five papers. These results are similar to the average RUE of 2.8 g MJ^{-1} PAR measured for temperate C_3 crops by Monteith (1977), and those published for other cereals, such as rice of 2.2 (Kiniry *et al*., 1989) to 2.8 g m^{-2} (Sinclair and Horie, 1989); although rice is generally lower than wheat due to higher losses from photo-respiration (Mitchell *et al*., 1998; Shearman *et al*., 2005). Assuming that the energetic content of carbohydrate-based dry matter is 17.5 kJ g^{-1}, a wheat crop with an RUE of 2.8 g MJ^{-1} PAR would have an average efficiency of just under 5% of the incident PAR that fell on the crop (Hay and Porter, 2006) typical of that found in experiments conducted in optimal conditions. In the post-anthesis period RUE declines (Fischer, 1993) possibly due to a decline in canopy photosynthetic efficiency, the end of leaf production, reduction in photosynthetic capacity of the existing leaves as a consequence of progressive senescence (Gallagher and Biscoe, 1978), or through losses in leaf N content due to remobilisation (Sinclair and Horie,

1989). However, studies suggest that this decline in RUE does not occur until late the grain-filling stage in barley (Bingham *et al*., 2007).

2.4.1.3 Relationship between leaf N and photosynthetic rate

The net photosynthetic rate of a leaf is strongly related to its N content and the amount of incident radiation upon the leaf under given environmental conditions (Werger and Hirose, 1991). Leaves of higher N content show a greater photosynthetic response to increased PPFD and therefore greater assimilate production. However, there are potential costs to the plant in having leaf N contents less than or greater than the optimum.

The leaf N in C_3 plants is used for proteins (70-80%; approximately divided into: 'Rubisco' (Ribulose 1-5-Bisphosphate carboxylase/oxygenase) (25%), light-harvesting complexes (25%), structural N (20%), biosynthesis N (20%), and Calvin-cycle N (10%) (Lawlor *et al*., 2001)), nucleic acids (10%) and lipoproteins (10%), with the remainder mostly in free amino acids (Field and Mooney, 1986; Evans, 1989). More than three-quarters of the total leaf N may be connected with photosynthesis (Field and Mooney, 1986). The photosynthetic N components can be divided into two parts; the soluble proteins involved in photosynthetic capacity (dominated by the enzymes involved in carbon dioxide fixation; i.e. Rubisco), and the protein in the thylakoid membranes of the chloroplast that are associated with the light capture, the pigment-protein complexes (including chlorophyll and chlorophyll proteins) and the various components of the electron transport chain (Evans, 1989). This division into thylakoid and soluble proteins is convenient because it functionally represents the light and dark reactions of photosynthesis (Evans, 1989).

In C_3 plants the most abundant soluble protein is the enzyme Rubisco which is required in large quantities to maintain adequate carboxylation rates due to its low catalytic rate and poor affinity for CO_2 (Evans, 1989; Irving and Robinson, 2006). Rubisco accounts for up to 30% of total leaf N (Hay and Porter, 2006) and 40 to 70% of total soluble protein (Evans, 1989) in wheat, and 25 to 32% of total leaf N and about 56% of total

soluble protein in rice (Mae *et al.*, 1983). A wheat leaf can accumulate 14 g m^2 of soluble protein (Lawlor *et al.*, 2001), of which Rubisco constitutes between 2.8 and 7.0 g m^2 (Lawlor *et al.*, 1987a; 2001). Across a large number of species, Björkman (1981) found a correlation coefficient of 0.96 between the light saturated rate of CO_2 assimilation in air and the fully activated Rubisco activity. A similar relationship was found in wheat by Evans (1989).

Chlorophyll is the light-harvesting pigment and can account for 60 to 85% of the thylakoid protein (Heldt, 1997) and 20 to 60% of photosynthetic N, depending on irradiance (Hikosaka and Terashima, 1995). The ratio of Rubisco to leaf N, and Rubisco to chlorophyll, is relatively constant at increasing and decreasing (senescence) leaf N contents (Evans, 1983; Lawlor *et al.*, 1987a). Within species there are strong linear relationships between leaf N and both Rubisco and chlorophyll (Evans, 1989), and in rice (Mae, 1997). However, plants are able to change the relative investment in N between photosynthetic components (Evans and Poorter, 2001). Thus, plants grown in lower light irradiance have been observed to increase the proportion of N allocated to the thylakoids in relation to Rubisco, whilst at high light irradiance the opposite has been observed to occur (Medina, 1971; Björkman, 1981; Evans, 1989) due to the carboxylation capacity of Rubisco then being the limiting factor (Farquhar *et al.*, 1980).

Given that the majority of leaf N is in the form of photosynthetic proteins, it might be expected that there would be a relationship between leaf photosynthesis and leaf N content. All things being equal, the light-saturated net photosynthetic rate (P_{max}) tends to increase in a roughly linear fashion as the specific leaf N increases, up to a certain value characteristic of the species (Field and Mooney, 1986; Grindlay, 1997; Shangguan *et al.*, 2000) irrespective of nutrient supply, leaf age, or season (Evans, 1983) indicating enzyme-limited fixation in irradiance-saturated conditions. The linear part of the relationship shows that the photosynthetic capacity of leaves and the irradiance at which they saturate increases linearly with increased leaf N per unit leaf area (specific leaf N; SLN) (Mooney and Gulman, 1979; Field and Mooney, 1986), so at a given irradiance there is a certain minimum leaf N content required to maximise carbon gain (Hirose and

Werger, 1987a; Werger and Hirose, 1991). Rubisco activity also increases linearly with the leaf N content of wheat (Evans, 1983), except at very high N content (Lawlor *et al*., 1987a).

When individual species are plotted (rather than diverse species plotted together which can give a continued linear relationship; Makino *et al*., 1988), this shows that as the leaf N content increases there is a non-linear, asymptotic, increase in the rate of photosynthesis, which was observed in wheat above 1.5 g m^{-2} (Evans, 1983). Therefore, rather than a precise optimum leaf N for a given PPFD, there is a range over which relatively large increases in leaf N per unit leaf area will give only marginal increases in photosynthesis rates (Hirose and Werger, 1987a). This asymptotic response indicates that crop species have the capacity to accumulate N to luxury levels, in which case an asymptotic relationship seems likely over an extended range of N supply (Evans, 1983; Sinclair and Horie, 1989).

For winter wheat in light-saturated conditions, the optimum leaf N is in the range 1.5 to 2.0 g m^{-2} (Evans, 1983; Jamieson and Semenov, 2000), similar to that reported for soybean (2.4 g m^{-2}), rice (1.6 g m^{-2}) and maize (2.1 g m^{-2}) by Sinclair and Horie (1989). Of the C_3 species, wheat and rice have the greatest rate of CO_2 assimilation for leaf N contents up to 1.68 g m^{-2}; evergreens and sclerophylls have the lowest (Evans, 1989). Genetic variation in SLN amongst 17 durum wheat cultivars was observed from 2.1 to 2.4 g m^{-2} (Giunta *et al*., 2002) and in leaf N concentration amongst eight wheat cultivars from 43.7 to 47.6 mg g^{-1} (Fischer *et al*., 1998). However, with an ample N supply, leaf N may continue to increase above the optimum value without significant increases in photosynthetic rate (Sinclair and Horie, 1989; Muchow and Sinclair, 1994). Wheat leaves commonly accumulate contents in excess of 2.5 g m^{-2} (about 4% N in dry matter) especially in the upper leaves of the canopy (Grindlay 1997; Critchley, 2001).

At the other extreme of the relationship, the leaf N content where the rate of photosynthesis equals zero corresponds with a zero Rubisco content of the leaf. The residual leaf N content at this point can therefore be taken to indicate the non-

photosynthetic N required for functions associated with the epidermal and vascular tissue, cell walls, and the nucleic acids and amino acids involved in primary metabolism (Evans and Porter, 2001). Sinclair and Horie (1989) reported the non-photosynthetic leaf N contents for soybean (1.0 g m^{-2} leaf area), maize (0.2 g m^{-2} leaf area) and rice (0.3 g m^{-2} leaf area), and suggested that response of wheat would be very similar to rice. Evans (1983) observed wheat to have an intercept of 0.2 g m^{-2}, as shown in Figure 2.2.

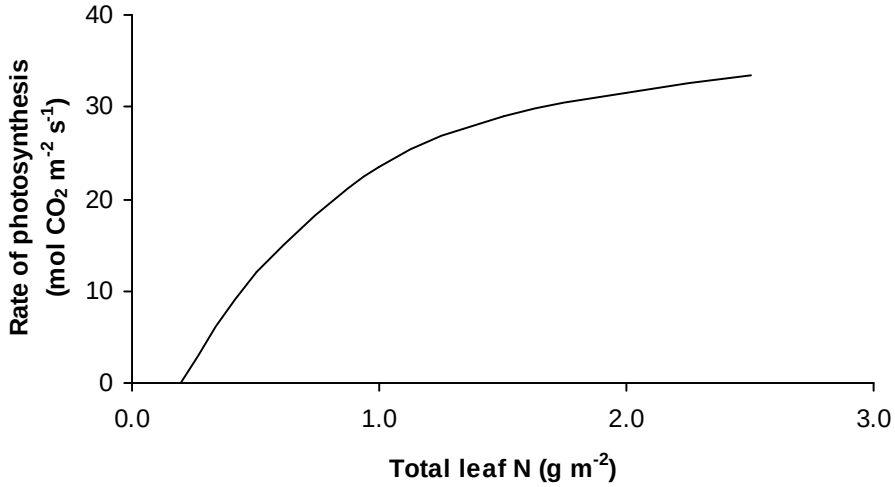


Figure 2.2 Light saturated rate of photosynthesis against total leaf N (g N m^{-2} leaf area) in the flag leaf of wheat. All measurements made with PAR flux at leaf surface of 1800 µmol m^{-2} s^{-1} and leaf temperature of 23ºC (Redrawn from Evans, 1983).

2.4.1.4 Relationship between canopy photosynthesis and canopy N

The relationship between the amount and rate of photosynthesis and the N content of the photosynthetic tissues is intrinsically complex as photosynthesis represents an integrated series of processes sensitive to environmental factors as well as to leaf physiology and structure (Field and Mooney, 1986).

As crops construct a stand, a gradient of irradiance (PPFD) develops down the canopy (Monsi and Saeki, 1953) such that the most illuminated leaves are at the top of the canopy and have the highest photosynthetic potential, whilst the leaves below are

progressively shaded by the leaves above and become limited by light. In parallel to this irradiation gradient is a gradient of specific leaf N, formed from the acclimation of the leaf N content and relative partitioning of leaf N according to that needed at each point to maximise *in situ* net photosynthesis (Evans and Poorter, 2001). The non-uniform distribution of N within the canopy is found more strongly in dense stands than in open stands (Werger and Hirose, 1991), and it is probable that in closed canopies at high leaf N contents, photosynthesis is not maximal. The N within the photosynthetic tissues is strongly correlated with both light exposure and photosynthetic capacity.

The non-uniform distribution of leaf N within the canopy reduces incidences where upper leaves are light-saturated through N limitation, whilst lower leaves have excess leaf N photosynthetic capacity (light limited). Grindlay *et al*. (1997) observed non-uniform leaf N partitioning in wheat crops, with the leaves at the top of the canopy having N contents of 1.9 g m^{-2} declining to 0.7 g m^{-2} at the bottom. In between, a more or less exponential decline in leaf N per unit leaf area with accumulated leaf area index develops (Hirose and Werger, 1987b; Lemaire *et al.*, 1991).

At non-limiting N availabilities, plants distribute leaf N within the canopy to maximise the photosynthetic efficiency and therefore DM production. Several studies have found that the whole canopy photosynthesis is maximised when leaf N is optimally rather than uniformly distributed (Field, 1983; Werger and Hirose, 1991), when the leaf N distribution pattern within the canopy is similar to that of light (Field, 1983; Goudriaan, 1995), increasing the efficiency of N canopy use by making fuller use of intercepted radiation. This vertical relationship between light (K_L) and N (K_N; i.e. the cumulative increase in canopy N with GAI) distribution could be described by K_N/K_L and assumed to be optimum when $K_N/K_L=1$ (Anten and Werger, 1996). The 'optimal' distribution of leaf N within the canopy can be defined as 'the point when the increase in photosynthesis by one leaf due to withdrawing N from another is cancelled out by the reduction in photosynthesis of the leaf which lost the N' (Evans, 1989).

The evidence from the literature indicates that plants have a canopy N distribution closer to the optimum (based on maximising NUE) than a uniform distribution (Field, 1983; Pons *et al*., 1989; Grindlay, 1997; Critchley, 2001) and that this results in a net carbon gain over non-optimal distributions (Hirose and Werger, 1987b). However, often the plant does not show a completely optimal distribution of leaf N within the canopy, with the actual N content being lower at the top and higher at the bottom of the canopy than the optimum predicted from light-photosynthesis models. This can arise from other factors influencing N distribution, such as response lag-time to changes in the canopy light environment (Pons *et al*., 1989), the linear relationship between leaf N and dark respiration rates (Hirose and Werger, 1987a), the metabolic cost of redistributing and re-assimilating N compounds, risk of herbivory N loss of high N leaves, the minimum N concentration required for maintenance purposes (Hirose and Werger, 1987a), maintaining the ability to use sun-flecks, the effects of leaf senescence and nutrient storage, and excess/deficient N supply (when N is limiting the N is preferentially partitioned to the upper leaves; Grindlay *et al*., 1997).

2.4.2 NON-PHOTOSYNTHETIC FUNCTIONS OF CANOPY N

Although the majority of the canopy N during the vegetative phase is associated with the photosynthetic machinery, a significant amount of N is used for structural, transport, metabolic, and/or storage functions. This non-photosynthetic N is located in the cell contents, as a physical part of the cell itself, or in the extra-cellular fluids (such as the xylem and phloem saps) and can be found in all component parts of the shoot.

2.4.2.1 The role of structural N

The height of wheat plants can range from 30 cm in the extreme dwarf varieties to 150 cm in some traditional long-strawed European cultivars, with modern semi-dwarf cultivars averaging about 75 cm. The stem is the main structural element of the wheat plant, providing structural support for the leaves and ear. The stem grows concurrently with the leaves, roots, sheath and ear, and like a leaf grows from an intercalary meristem.

A mature wheat stem typically consists of six internodes extending from nodes (Knapp *et al*., 1987) and the uppermost internode (peduncle) may account for as much as half of the total shoot height (Rawson and Evans, 1971) and may continue to extend until after anthesis, so perhaps competing with the ear under limiting assimilate supply conditions. Although the majority of the stem is enclosed by the leaf sheath, the exposed parts (generally the top two internodes) contain chloroplasts located in the epidermis and are capable of photosynthesis (Murthy and Singh, 1979) which may be of some value for photosynthesis under stressed conditions, or during grain filling.

The stem is a compact structure of cellulose (35-40%), hemicellulose (20-30%) and lignin (8-15%) (Klinke *et al*., 2002), and is composed mainly of structural polysaccharide compounds, with only the lignin containing a small quantity of N. The basic structure of the stem is a 'composite tube'. A cross section of the internode reveals four distinct tissue types; epidermal (skin tissue), hypodermal (beneath the epidermal), vascular (plumbing system), and parenchyma (packaging which breaks down during growth to leave a hollow stem). The strength of the stem comes from the sclerenchyma fibres which surround each of the vascular bundles running through the parenchyma. This sheath of elongated cells forms thickened and toughened structural fibres composed of ligno-cellulose (cellulose and lignin) which form the main component of wheat straw at harvest.

Plants allocate relatively little N to the cell walls for structure (Bacon, 1988) as the cell wall of higher plants consists of more than 90% carbohydrates and less than 10% proteins (Heldt, 1997). Lawlor (2002) identified a critical amount of 3 g m^{-2} of protein (20% of leaf protein content) required for basic cell structures in the leaf without which other structures cannot be formed. These cell wall proteins may be divided into two classes: enzymes and structural proteins. The enzymes, generally oxides, are involved with the making and breaking of glycosidic bonds allowing cell growth and expansion. The structural proteins are glycoproteins (composed of carbohydrates and proteins) and (along with the pectins) hold together the polysaccharide fibres of the cell wall to form a scaffold structure (Lamport, 1965). The structural component of the plant is therefore

essentially low in N, one the main assumptions of the nutritional models discussed in section 2.3.

2.4.2.2 The role of transport N

N is actively taken up by the roots of wheat (Gregory *et al*., 1979), and is transported via the transpiration stream of the xylem to the growing parts of the shoot (Simpson and Dalling, 1981). N in the xylem is in the form of nitrate and amino acids, although some of this amino acid content may be the result of the recycling of amino acids produced in the leaves (Simpson *et al*., 1983). The principal amides found in the xylem sap of wheat plants are asparagines, aspartate, glutamate, glutamine and arginine (Simpson and Dalling, 1981; Goodwin and Mercer, 1983), which are transported to and unloaded in the sinks (such as active leaves, apices or developing reproductive structures).

N redistributed within the wheat canopy is loaded into the phloem. During the pre-anthesis phase, redistribution may be both within the canopy (e.g. between leaves) or from the canopy to the roots. The constant redistribution of N between plant parts means that a certain amount of N will also be in transport, although the amount is likely to be relatively insignificant. In a study of young wheat plants, Hayashi and Chino (1986) found that significant amounts of twenty different amino acids (261.7 mM) were present in phloem sap, of which the dominant were glutamic acid (30%), aspartic acid (19%) and serine (7%). These amino acids are also important for the transport of carbon (carrying about half that of sucrose) to the meristematic tissues (Schenk, 1996). Hayashi and Chino (1986) also found small amounts of nitrate in the phloem sap of wheat plants, but at low concentrations (8.1 mM). Other studies have reported trace amounts of organic acids, protein-amino acids, non-protein-amino acids, nucleotides, peptides, proteins, plant growth regulators and phyto-hormones (Atkins and Beevers, 1990; Heldt, 1997).

During the reproductive phase, the supply of N (and C) to the developing grains is almost entirely (>99%) in the phloem (Simpson *et al*., 1983; Wardlaw, 1990). N is supplied from both the roots and the vegetative organs. N in the xylem from the roots is first imported

by the leaves and glumes where it is transferred into the phloem and re-exported. However, the largest contribution of N for grain protein synthesis comes from the degradation of the soluble leaf proteins, notably Rubisco (Michael *et al*., 1960).

2.4.2.3 The role of metabolic N

A metabolic pool of N exists to allow plants to grow, reproduce, maintain their structures, and respond to their environment. Although the vast majority of this metabolic N in wheat is concerned with photosynthesis, other functions of wheat proteins are to catalyse metabolic reactions in the cell, enable cell signalling, cell immune response (protection against pathogens, micro-organisms and animals), cell adhesion and the cell cycle. In some situations, proteins can be used as a respiratory substrate, although very little of the protein in wheat leaves is respired (Helleburst and Bidwell, 1963).

In the chloroplasts, the pigment-protein complexes (chlorophylls) absorb energy and pass it to membrane-bound electron carrier proteins of the electron transport chain, predominantly the cytochromes, ferredoxin, and plastocyanin (Goodwin and Mercer, 1983). The 'light-independent' phase of photosynthesis, located in the chloroplast stroma, involves the carboxylase enzyme Rubisco, and several other enzymes such as phosphorylase and ATP synthase. Other proteins in the plant function as enzymes (such as proteases and peroxidases), enzyme inhibitors (such as trypsin and chymotrypsin), in the transcription and replication of DNA (nucleic acids and proteins), and as recognition and regulatory proteins. These proteins are present in other organelles and the cytoplasm of the cell.

2.4.2.4 The role of reserve N

The formation of N reserves within plants can occur during the pre-anthesis phase as a saving for the future nutritional needs of the plant (i.e. root or canopy reserves) or during the post-anthesis phase as a saving for the next generation (e.g. grains, tubers and stolons). However, N reserve can also increase the 'sink' capacity of the plant allowing uptake of N which may otherwise be lost through leaching, and can buffer high grain N

demand during grain filling. In understanding the N economy of the crop during growth and reproduction, it is necessary to account for the requirement for formation and use of reserve N.

N reserve formation during in the pre-anthesis phase is found in several important grain crops; including oil seed rape (OSR, *Brassica napus* L.; Rosatto *et al*., 2001), maize (Plénet and Lemaire, 2000), and wheat (Grindlay, 1997). Millard (1988) proposed two distinct mechanisms to account for the formation of reserve N: (i) N accumulation through 'luxury uptake' when the supply of N exceeds the crop N demand for growth and maintenance, or (ii) N *storage* when N is deposited specifically for later redistribution and use (e.g. for grain development). The accumulation of N occurs in relation to the canopy demand and the uptake of soil available N. Plants able to take up N faster than demanded by current growth and maintenance accumulate N in the tissues. This can occur at any point of crop growth - at both low (such as when growth is constrained by low light, low temperature or another macro-nutrient) and high growth rates (such as when supra-optimal N fertilisation is applied). Accumulation can be quantified from the amount by which the plant N% is above the critical N% required for maximal growth (as seen in the critical N% model of Justes *et al*. (1994)).

N can accumulate in several forms within the plant tissues. Nitrate accumulation has been observed in sugar beet (Armstrong *et al*., 1986), potatoes (Millard and Marshall, 1986), maize (Plénet and Lemaire, 1999), rice (Fan *et al*., 2007), barley (Granstedt and Huffaker, 1982) and wheat (Zhen and Leigh, 1990; Abrol *et al*., 1999; Gastal and Lemaire, 2002) particularly in the stem phloem cell sap and cell vacuoles of the leaf lamina (Granstedt and Huffaker, 1982). For hydroponically grown wheat, the growth requirements for N were satisfied when nitrate started to accumulate in the shoot (Zhen and Leigh, 1990), and for field-grown wheat, plants abundantly supplied with N contained accumulated nitrate in their shoots whereas unfertilised plants contained no nitrate (Barraclough, 1993). Chatterjee *et al*. (1981) found that for fertilised barley plants, nitrate accumulated in the flag and second leaf during the pre-anthesis period where it was actively reduced. While Abrol *et al*. (1990) observed that wheat and barley in well

fertilised soils accumulated nitrate in the stem internodes and/or stem base in wheat (Satorre and Slafer, 1999).

Nitrate accumulation occurs especially in the leaves of young crops with low crop biomass at the beginning of the season (Gastal and Lemaire, 2002). During the early stages of growth, nitrate is generally more readily available to crop plants and the young plant is limited in C supply to assimilate these nitrates into other forms. Therefore, in young plants the accumulation of nitrate may function as a short-term reserve before assimilates are available for nitrate reduction (Leigh and Johnson, 1987). Whereas in more mature crops, nitrates are a transient form of N as they are readily reduced in the photosynthetically active tissues of the upper canopy where nitrate reductase is stimulated by light.

Amides and amino acids (such as glutamine and asparagine) have also been found to accumulate to high concentrations in vegetative tissues (Pate, 1983), predominately in the cell vacuoles (Boudet *et al*., 1981). Amino acid-N accumulation may occur in the glumes of the ear in the period just prior to rapid grain filling, providing a temporary sink for N that would soon be mobilised to the grain (Waters *et al*., 1980). A major problem with accumulating both nitrates and amides at high cellular concentrations is that both forms contribute to cell solute potential, and this can both make them difficult to withdraw from the cell and lead to significant osmotic imbalances and eventually cell death (Staswick, 1994).

However, soluble proteins are the most common form of accumulated N in the vegetative tissues, particularly in more mature plants which have available assimilates to synthesise proteins without an immediate metabolic or structural role. All proteins contain amino acids which can in turn be used in re-synthesis as proteins turn over, but certain proteins are particularly suited to the role which have a high proportion of N-rich amino acids (i.e. arginine and amide) such as Rubisco. Storage proteins should be quickly and effectively formed and controlled to react to excess or lack of available N. Soluble proteins have a number of advantages over both nitrates and amides as reserve N, primarily that soluble

proteins can accumulate to high cellular concentrations with a much smaller effect on osmolarity.

Synthesising proteins may be an important strategy for accumulating reserve N. Many of these soluble proteins may be important metabolically, of which the most important is the photosynthetic enzyme Rubisco. Experiments by Evans (1989) and Lawlor *et al*. (1987a) have found that plants with non-limiting N supplies have high leaf N contents, with an increasing proportion of the total leaf N in the soluble proteins (i.e. Rubisco) compared with the membrane-bound thylakoid proteins (such as chlorophyll), particularly in the upper laminae (Sivasankar *et al*., 1998). Wheat leaves with high soluble protein contents have also been shown to have concentrations of Rubisco far beyond that required to maximise photosynthesis, and of which up to 50% of the Rubisco of is not active (Lawlor *et al*., 1987a) or that only half of the active sites were functional, suggesting the accumulation of the protein. Studies using transformed tobacco (*Nicotinia tabacum*) plants showed that as much as 43% of the Rubsico content of the leaves of wild-type plants could be lost with out reducing photosynthesis (Clarkson and Hawkesfield, 1993). Rubisco therefore fulfils a dual role, primarily as a CO_2 fixing enzyme but also as a major source of reserve of N in wheat (Millard, 1988).

Using metabolically active proteins, such as Rubisco, for a N reserve function has several advantages to the plant. The existence of high levels of metabolically important proteins within the photosynthetic tissues increases the instantaneous capacity for photosynthesis should light levels increase. Also, these proteins are located in or near tissues where N demand will be the highest during growth, and are able to be rapidly degraded and the reserve N used for protein synthesis. However, there are also disadvantages of using soluble proteins for N storage. Firstly, it is energetically costly to synthesise proteins and then break them down into amides for transport (Millard, 1988), although there is a potential metabolic advantage from increased C assimilation, and Ferreira and Davis (1987) found there is relatively little turnover of Rubisco in C3 plants before leaf senescence. Also, since leaf N is linearly correlated with photo-respiration (Lawlor *et al*.,

1987b) and dark respiration (Hirose and Werger, 1987a), high leaf N contents may increase leaf gross C losses.

Vegetative storage proteins (VSP) have been identified in some plant species as a means to stably store N to high concentrations, often deposited within the cell (or vacuole) as membrane-bound 'protein bodies'. Although VSPs are frequently found in the reproductive structures; tubers (e.g. potatoes contain Patatin), stolons (e.g. spurge, *Euphorbia esula* contains a 26-KDa protein), seeds and grains (e.g. wheat contains gliadins and glutenins), they also occur in the vegetative tissues (leaves, stems and roots). Staswick (1994) studied soybean (*Glycine max* L.) and found that VSPs are synthesised in expanding young leaves and pods, where they accounted for up to 50% of the soluble protein (Wittenbach, 1983). The deposition of storage proteins near xylem and phloem appears to facilitate synthesis and mobilisation (Staswick, 1994; Shewry *et al*., 1995). However, although other legumes (peas, beans and alfalfa), maize and tobacco have shown the presence of some VSP-like proteins, it is likely that the significance of VSP in soybean is related to the unusually high requirement for N during seed development (the mature soybean containing about 40% proteins) (Staswick, 1994). VSPs have been identified in the sheaths, stems and roots of perennial grasses such *Lolium perenne* and *Festuca rubra* (Louahlia *et al*., 1999), poplar (*Populus deltoids*; Wetzel *et al*., 1989). Rossato *et al*. (2002) have shown that a VSP exists in the taproot of oilseed rape which could be used as a storage buffer between N losses from senescing leaves and later grain filling. However, there appears to be no literature to identify VSPs in the vegetative tissues of cereals.

In wheat, there is the potential for reserve N which is not required for immediate growth, maintenance or structure. The difference between '*accumulated* N' and '*storage* N' is in the conditions under which they are formed (Critchley, 2001), particularly in relation to the availability of N. Synthesis of soluble proteins to provide N for another tissue at a later developmental stage, can be regarded as *storage*, and a particularly important role of stored N in cereals is to buffer N demand during grain filling when large quantities of N

are required by the grain, and liberated stored N can be used in preference to the proteolysis of metabolically active proteins, thus maintaining canopy function.

Several studies have referred to the soluble protein of wheat leaves (i.e. Rubisco) as a potential storage pool of N. However, there appears to be little literature on N storage in other components of the canopy, despite their high N contents. Vouillot and Devienne-Barret (1999) discuss the significance of the root system as a temporary N storage pool. Triboi and Ollier (1991) identify the stem to have a potential storage function, with variation in storage capacity between varieties. The stem is recognised to have significant C stores for grain filling (Blum, 1998), and would also appear to provide an opportunistic location for N storage as it is located near to transport systems for rapid mobilisation to the growing tissues (e.g. ear) and connected to lower leaves transiently to store N from senescing lamina tissues. The physical size of the stem would also indicate the capacity to store considerable amounts of N as soluble proteins in the stem cell contents. Increasing the size of the stem would allow N to be taken from the soil more quickly, because of higher demand for N, therefore reducing potential losses to the environment by leaching and denitrification.

N accumulation and storage would appear to have an important role in crop ontogeny; allowing young plants to take up N when the soil N is high ('sink' function) such as immediately after fertiliser application, and later providing N for periods when grain demand exceeds supply (such as during grain filling) ('source' function). Reserve pools of N provide a means of allowing grain filling and senescence to proceed (at least in part) more independently of each other. On the other hand, the excessive accumulation of non-functional N within the crop would reduce NUE by increasing crop N demand with little or no increase in yield or quality, pointing to a requirement for the optimisation of storage through breeding.

2.5 THE ROLE OF N IN THE REPRODUCTIVE GROWTH PHASE

Anthesis represents the transition from vegetative to reproductive growth (grain filling). In the period leading up to anthesis, the plant N status has a significant influence on the number of grains set per m^{-2} and hence the post-anthesis sink capacity of the crop, through the number of grains set per ear (Fischer, 2008) and the number of fertile shoots per unit area (normally fixed following the tiller survival phase). After anthesis, N uptake may continue as the root system remains active (Andersson and Johansson, 2006). However, the majority of the N required for grain filling is translocated from the vegetative organs. The assimilates required by the grain are derived both from pre-anthesis storage and from current photosynthesis. Post-anthesis canopy longevity and the remobilisation and translocation of N are therefore important factors in the production and determination of grain yield and grain quality.

2.5.1 N re-mobilisation to the grain

Wheat remobilises a significant proportion of the N accumulated by the vegetative tissues of the plant to the grain, and the majority of N transport during the grain filling period is the result of this redistribution of N. The leaves and stems have been found to be the most important sources of N for the grain (Critchley, 2001), with smaller amounts contributed by the glumes (about 15%) and the roots (about 10%) (Dalling, 1985), and continued N uptake by the roots can contribute between 5 to 50% of grain N (De Ruiter and Brooking, 1994; Andersson, 2005) depending on the environmental conditions and soil N availability. High N supply and late N applications (such as dry top dressing near anthesis or foliar sprays at the milky-ripe stage, GS75) can significantly increase grain protein content (Gooding and Davies, 1992; Palta and Fillery, 1993; Bly and Woodward, 2003).

In the pre-anthesis phase, there is a net increase in the N content of the leaves, stems and ears through nutrient assimilation; most of the N which enters the leaves and stems is retained and most of this can be remobilised. At anthesis, these organs change from ‘sinks’ to ‘sources’ as remobilisation ‘unloads’ N (and C) into the developing grain

during grain filling. The developing grains are a strong sink for N, and monopolise all the nutrients that are remobilised from senescing parts of the plant, from storage sources, and from root uptake. Accumulation and redistribution of N are important processes in determining yield quantity and quality in wheat (Simpson *et al*., 1983; Le Gouis *et al*., 2000). Over 80% of the above-ground N at harvest is present in the above-ground crop at anthesis (Cregan and Van Berkum, 1984), and can account for as much as 50-100% of the grain N content at harvest (Austin *et al*., 1977; Cox *et al*. 1985b; Sarandon and Caldiz, 1990; Gebbing and Schnyder, 1999). At harvest the grain can contain 57 to 76% of the above-ground plant N content (N harvest index, NHI; the proportion of N in above-ground plant which is in the grain at harvest) (Xu *et al*., 2005) depending on the variety and environmental conditions. Similar results were found in rice, where 70 to 90% of the total panicle N was remobilised from the vegetative organs, especially the leaf lamina (Mae, 1997) and canola where 60-65% of the N was remobilised from the leaves and stem after anthesis (Hocking, 1997).

N remobilisation is an important trait affecting the utilisation of canopy N, and the efficiency of the N translocation in the above-ground parts to the grain can be measured by the NHI. NHI is a very conservative trait (White *et al*,. 1998) and a heritable characteristic (Cox *et al*., 1985b). NHI for field-grown wheat are typically high, in excess of 0.70 (Austin *et al*., 1977) to around 0.80 (White *et al*,. 1998; Andersson, 2005). Loffler *et al*. (1985) and Paccaud *et al*. (1985) reported that NHI was positively correlated with grain N concentration amongst a range of wheat genotypes (when yields are maintained). The uptake and accumulation of canopy N during the vegetative phase therefore has a significant effect on grain N supply. Hence, N partitioning to the stem reserve during vegetative growth and the subsequent ability to redistribute this N to the grain may be an important factor in crop NHI (and NUE).

During grain filling, the non-photosynthetic N is the first to be redistributed followed by that of the photosynthetic proteins, especially Rubisco (Peoples and Dalling, 1988; Sarandon and Caldiz, 1990). Xu *et al*. (2005) found that the stem is also a significant source of N for the grain, and Tahir and Nakata (2005), in a study of 18 bread wheat

varieties, found genotypic variation in the quantity of N accumulated in the stem at anthesis and the efficiency of stem N remobilisation during grain filling. The remobilisation of N from the leaf lamina reduces SLN and is associated with accelerated leaf senescence, resulting in a rapid decrease in photosynthetic activity and RUE in the latter stages of grain filling in barley (Bingham *et al.*, 2007) (see also the 'self-destruction hypothesis; Sinclair and De Wit, 1975). So the use of stem N as a buffer may reduce the rate of leaf senescence and loss of chlorophyll. Thus stem N may have an important role in maintaining green canopy area and/or photosynthetic efficiency during grain filling. N from the vegetative organs first accumulates temporarily in the glumes before it is transported via the phloem to the grains.

In wheat N remobilisation appears not to be driven by 'demand' for N by the grain, but by the source supply from the vegetative tissues (Martre *et al.*, 2003). The amount of N remobilised (NR) depends on the N remobilisation efficiency (NRE) and the amount of N available. The NRE is calculated as the fraction of N in the whole plant or organ at anthesis which is not recovered in the straw at harvest (Cox *et al.*, 1986). Although affected by variety (Cox *et al.*, 1986; Barbottin *et al.*, 2005), remobilisation is also influenced the availability of soil N (Palta and Fillery, 1993; Sinclair *et al.*, 2000) and by the growing conditions during grain filling. Cox *et al.* (1986), working on wheat in the Central Valley of California, showed that high levels of N fertilisation before flowering led to a decrease in remobilisation as increased post-anthesis N uptake renders N remobilisation less necessary, whereas low N conditions have been shown to increase remobilisation (Barbottin *et al.*, 2005). Palta *et al.* (1994) observed that conditions of heat and water stress during the grain filling period (such as in the Mediterranean or Western Australia) increased N remobilisation efficiency from the vegetative organs to the grain, as the plant is forced to make greater use of its accumulated N at anthesis. However, if environmental conditions cause accelerated senescence this can reduce NRE (Tahir and Nakata, 2005; Xu *et al.*, 2005), e.g. effects of high temperatures (Heitholt *et al.*, 1990). Foliar diseases, such as brown and yellow rusts (caused by *Puccinia spp.*) and septoria blotch (caused by *Septoria tritici*), have been shown to reduce translocation of N from the vegetative tissues during grain filling (Dimmock and Gooding, 2002b).

The NRE and rate with which the N is transferred to the grain also depends on the organ, and the function of the N in that organ. Studies by Zhen-Yuan *et al.* (1996) found that between anthesis and maturity, the roots, stem and leaves of wheat exported 47%, 43% and 75% of their total N, respectively. This contributed 3%, 11% and 18% to ear N accumulation, respectively, and the remaining 67% came from soil N uptake. De Ruiter and Brooking (1994) also observed significant quantities of post-anthesis root N uptake in barley. Changes in N metabolism, the catabolism of cell constitutes, and the formation of transport compounds are important steps in the re-distribution of N during senescence. NRE is therefore an important aspect of the crop N utilisation efficiency.

2.5.2 Factors influencing senescence

Although ultimately leading to canopy death, the senescent phase of plant development is a highly organised and well regulated process (Hörtensteiner and Feller, 2002) during which the proteins in the vegetative organs are degraded to provide N for grain filling. In particular the stromal enzymes (such as Rubisco, glutamine synthetase and glutamate synthase) are degraded early in senescence leading to a decline of photosynthetic capacity (Hörtensteiner and Feller, 2002). In wheat, the oldest leaves senesce first and the uppermost leaf remains active for the longest period. This process can start before anthesis with the N in the lower leaves being remobilised to the upper expanding leaves. The three uppermost leaves, in particular the flag leaf, contribute the most assimilates to grain filling and so are maintained the longest (Rawson *et al*., 1983). Leaf senescence (yellowing) starts at the tip and progresses towards the base, finally reaching the leaf sheath. A certain amount of non-remobilisable structural N will be lost when these leaves abscise, suggested to be about 1% DM (Sinclair and Amir, 1992), whilst the fraction remobilised corresponds to the metabolic and storage N pools. Culms and spikes (glumes and awns) remain green for longer, and besides producing the energy for N remobilisation, are the last source of protein accumulation to be incorporated in the grain (Simpson *et al*., 1982; 1983). The roots are the last vegetative part to senesce (Peoples and Dalling, 1988) and remain active during grain filling (Andersson *et al*., 2004).

With the onset of senescence, sequential patterns can be observed at the cellular and biochemical level. Component tissues of the same leaf senesce at different times, for instance the guard cells remain active for longer than the mesophyll cells. Photosynthetic processes decline early in senescence as the chloroplasts are the first organelles to be dismantled (Hörtensteiner and Feller, 2002). The photosynthetic pigments and enzymes are degraded first, and then the chloroplast envelope is hydrolysed. Up to 75% of the N in the mesophyll cells is contained in the chloroplasts (Peoples and Dalling, 1988) so considerable quantities of amino acids are derived, particularly from the degradation of the stomal enzyme Rubisco which constitutes a large proportion of the soluble leaf protein in wheat plants. The mitochondria and metabolic processes are maintained for longer during senescence, and cellular metabolism may even rise slightly during the initial stages, to provide respiration and energy for the remobilisation when photosynthesis is no longer functional. Protein hydrolysing enzymes (i.e. endo- and exo-peptidases) frequently reach their highest activities during senescence. Amino acids derived from proteolysis may be further metabolised (e.g. production of amides from amino acids) or loaded directly into the long distance transport (phloem) system. Proteolysis may be dependent on the growth conditions (e.g. light availability, temperature, water supply) and the source-sink relations of the plant.

The initiation and rate of senescence are controlled by a variety of factors. Initiation may be controlled by phytohormones; exogenous hormones applied to detached leaves in experimental conditions are very effective in promoting (e.g. abscisic acid) or delaying (e.g. cytokinins) senescence. Plant N status, grain demand and depletion of soil N sources may regulate senescence through interactions between N source, sink and transport systems. However, leaves of de-eared and de-grained plants also senesce, with the N accumulating in other tissues of the same leaf or in other vegetative organs, so leaf senescence is not necessarily entirely controlled by the demand of the grain.

Prolonged green leaf area duration through delayed leaf senescence (‘stay-green’) allows photosynthetic activity to continue, enables the plant to assimilate more carbon and use

more N for biomass production (Borrell and Hammer, 2000) increasing yield and grain weight (Dimmock and Gooding, 2002a). Stay-green properties would be beneficial in feed wheat cultivars (Christopher *et al.*, 2008), but may not be desirable in bread-making cultivars as they could be associated with reduced N relocation to the grain. Stay-green traits have been identified in durum wheat (Spano *et al.*, 2003), bread wheat (Foulkes *et al.*, 2007), and other major crops such as maize (Rajcan and Tollenaar, 1999) and sorghum (Borrell and Hammer, 2000), with mapping populations and associated QTLs identified in wheat (Verma *et al.*, 2004) and sorghum (Borrell and Hammer, 2000). Investigation of stay-green mutant genotypes in grasses has shown that soluble protein degradation during senescence may be close to normal, but the light harvesting and reaction centre thylakoid membrane proteins are much more stable (Thomas *et al.*, 2002). This may indicate that, with the dismantling of the biochemical apparatus, the leaves cease to function (Hay and Porter, 2006), but remain green owing to the protection of the chlorophyll pigments (Thomas and Howarth, 2000).

However, a report by Subedi and Ma (2005) showed that the stay-green phenotype in maize was exhibited only when there was an adequate supply of N. In sorghum, stay-green was associated with greater leaf N concentration at anthesis and crop N uptake during grain filling (Borrell and Hammer, 2000). Further work is required on the physiological and molecular basis of the stay-green to determine whether the phenotype is beneficial when N fertilisation in reduced (Hirel *et al.*, 2007) and the longer term scope to improve N economy through understanding patterns of N uptake, leaf to grain remobilisation and the manipulation of senescence profiles.

2.5.3 The determinants of grain N concentration

Carbohydrates and proteins are accumulated in the grain during the grain filling period, but C and N accumulation are regarded as independent events controlled by separate mechanisms (Jenner *et al.*, 1991). The carbohydrate (starch) is synthesised in the grain from the sucrose derived mostly from assimilation during grain filling (Rawson and Evans, 1971), with a smaller contribution (typically 10 to 30%) (Gebbing *et al.*, 1999)

from the remobilisation of stored soluble carbohydrates in the stems and leaves (Blum, 1998). Protein is synthesised in the grain after anthesis from the amino acids derived from senescence of vegetative proteins and reserve N accumulated before anthesis, and from continued root uptake. The qualitative composition of the grain protein is a genetic characteristic, caused at least in part by differences in protein synthetic capacity (Shewry and Halford, 2002), whilst the rate, duration and grain protein quantitative composition (i.e. the ratio between the different protein fractions; Martre *et al*. 2003) can be modified by environmental conditions. The duration of grain filling is relatively constant in thermal time, and allows good translocation of N stored in vegetative tissues to the grain during hot summers (Triboi *et al*., 2006).

Grain protein concentrations range between 7% and 15% of the dry mass (1.5 and 2.7 %N), and experiments have shown that this concentration does not increase further even when excess soil N is available, or a substantial fraction of the straw N was not mobilised (Barneix *et al*., 1992). However, an inverse relationship exists between the grain protein concentration and yield (Cox *et al*., 1985a; Johansson and Svensson 1997; Triboi *et al*., 2006), making the simultaneous genetic improvement of yield quantity and quality a difficult task (Cox *et al*, 1985a), a particular issue when breeding bread-making wheats. This is possibly due to the bioenergetics of carbohydrate and protein synthesis, as almost twice as much energy is required for protein synthesis as for starch synthesis (Penning de Vries *et al*., 1974), and the competition for assimilates and energy results in increasing grain protein concentrations decreasing grain dry matter yields. However, it is possible to identify wheat lines that have a higher grain protein content than predicted from the overall negative linear regression to grain yield amongst groups of lines (Oury *et al*., 2003; Kade *et al*, 2005).

The environmental conditions during grain filling can also have major impacts on grain protein concentration, particularly temperature, light and water availability, and foliar diseases. Temperature has a strong influence on developmental rate, for instance, an increase of 5^{o}C during the grain filling period can reduce the duration from 56 days to 36 days. Increases in C supply to the grain are mostly due to increases in growth duration,

especially after anthesis (Triboi *et al*., 2006), and reduce the grain protein concentration. This is particularly relevant as, in most growing regions, grain filling occurs when physical and biotic stresses are increasing. Thus, high temperature (above 20^{o}C) during grain filling has a greater effect on C accumulation than on N accumulation, and generally gives smaller grains with high N concentrations (Gooding *et al*., 2003). This explains most of the environmental variations in wheat yield protein concentration in Europe: in the northern and western regions there is generally high yield potential (>8 t ha^{-1}) with low grain protein (10-12%), whilst in the southern and eastern regions there is lower yield potential (<5 t ha^{-1}) with high grain protein (about 15%).

Grain N accumulation is driven by the availability of N from the sources (Martre *et al*., 2003), defined as the total non-structural crop N at anthesis. Therefore, increasing N supply to the grain will result in an increased grain protein content (Triboi and Triboi-Blondel, 2002). This was demonstrated in experiments by Martre *et al*. (2003) on four wheat varieties in which the N source-sink balance was manipulated by removing the top half of the ear at anthesis. This manipulation resulted in a significant increase in the N concentration of the grain, particularly the storage proteins, showing that the grain N accumulation is regulated by the source and not by the activity of the grain (sink regulated). Borghi *et al.* (1986) also showed that grain N could be increased by source-sink manipulation, through the removal of 50% of the spikelets at heading which resulted in a 65% reduction in grain yield but a 12 to 17% increase in grain protein. Other studies have also shown control of grain N accumulation by the level of N supply for wheat (Ma *et al*., 1996), barley (Dreccer *et al*., 1997), maize (Wyss *et al*., 1991) and soybean (Nakasathien *et al*., 2000).

For each cell in the grain there appears to be a minimum obligatory, quantitative requirement for N for the synthesis of essential amino acids and structural and metabolic proteins. This gives grain a minimum N concentration of approximately 1.5% (Sinclair and Amir, 1992), after which, the synthesis of grain storage proteins typically increases the grain N concentration to 2.1% (about 12% protein, typical of milling wheat). The accumulation of the different protein fractions is highly asynchronous. During the early

stages of grain growth, the structural and metabolic fractions accumulate and consist of albumin, globulin and amphiphilic proteins which are divided into two broad categories: the gliadins (monomers) and the glutenins (polymers). Then, once the cell division has stopped and grain growth is only due to cell expansion, the storage fractions accumulate (Triboi *et al*., 2003).

The concentration and composition of these proteins are major determinants in the nutritional value of the grain (Feil, 1997) and flour functional properties (Shrewry and Halford, 2002). The bread making quality of the grain is determined by its protein concentration and composition (gliadin : glutenin ratio). Gliadins and glutenins are the main components of gluten, which is the main contributor to the rheological and bread making properties of wheat flour (Branlard *et al*., 2001). Grain quality reaches a peak at a N supply above that needed to achieve maximum yield, after which further increases in N supply result in increased grain protein content through 'luxury consumption', but a reduction in protein (and bread making) quality as the additional N accumulated is represented by glaidins or non-protein N (Borghi *et al*., 1986).

2.6 USE OF CROP SIMULATION MODELS

Wheat simulation models attempt to predict the biomass accumulation, grain growth and grain protein content of a crop from the effects of environmental variation (weather, water supply and nutrient availability) on the dynamic plant processes which lead to yield formation. Several models have been produced: AFRCWHEAT2 (Porter, 1993), CERES-Wheat (Richie and Otter, 1985), SWHEAT (Van Keulen and Seligman, 1987), and SIRIUS (Jamieson *et al*., 1998). They treat crop N demand either as a function of the dry matter (i.e. the plant N concentration) or as a driver of green area production. The plant N concentration is compared to an ontogenetically changing optimum, minimum and maximum averaged over the whole plant, similar to the 'critical N concentration' and 'N nutrition index' approaches discussed in section 2.3.

However, according to the experimental data (from 9 years of observations) and the model of Sinclair and Amir (1992), Jamieson and Semenov (2000) recognised the limitations of the previous models in averaging crop N content over all shoot biomass, and proposed a new version of SIRIUS which disaggregated shoot tissue into the component organs (leaf, stem and grain) with different N requirements. The model was based on the assumption that the N in the green tissue can be assumed as constant per unit leaf area, and the crop N demand is set according to the need to maintain the N content of the leaf lamina at the optimum for maximum net assimilation. New leaf laminae can only be produced if sufficient N is available from the soil or within plant reserves, and an inadequate N supply results in the development of less leaf area or premature senescence, rather than affecting the rate of photosynthesis per unit leaf area (Grindlay, 1997).

The N requirements of the component organs during growth and remaining in the dead tissue at senescence were set as;

i. leaf lamina has a N concentration of 2.0%, with a minimum SLN of 0.8 g m^{-2} (based on a specific leaf weight of 40 g m^{-2}; Sinclair and Amir, 1992) and an optimum SLN of 1.5 g m^{-2} (or 15 kg ha^{-1} of GA, although there is no effect of vertical distribution) (Jamieson and Semenov, 2000) which declines to 1.0%N at senescence (Sinclair and Amir, 1992)
ii. whole stem (sheath and true stem) has a minimum requirement of 0.5% N, but may store excess N (from root uptake or leaf cycling) up to 1.5%, and declines to 0.3% on senescence (Jamieson and Semenov, 2000). Sinclair and Amir (1992) included a minimum requirement of the true stem of 1.2% N, also decreasing to 0.3% N on senescence)
iii. grain has a minimum of 1.5%N (Sinclair and Amir, 1992), but may have a higher concentration depending on the N availability from excess stem N, remobilisation from vegetative tissues, and some continued root uptake (Jamieson and Semenov, 2000).

Importantly, the revision of the SIRIUS model by Jamieson and Semenov (2000) allowed for excess N uptake and storage in the non-green (i.e. stem) tissue. This 'reserve N pool' confers several functions on the model stem; if root N uptake is limited during growth then the stem reserve N allows continued leaf expansion, the stem reserve capacity can allow continued uptake of excess available N when leaf demand is already satisfied, the stem reserve N supplies grain N demand during grain filling in preference to releasing N by leaf senescence, and the increased N supply from stem N reserves can increase the final grain protein content (due to the grain N content being source limited). Semenov *et al*. (2007) found that theoretical manipulation of crop N *storage* capacity had an effect on NUE; when N was limiting - decreasing storage which increased the N available for leaf growth, had a positive effect on NUE (through increased grain yield), whilst when N was not limiting - adequate N storage, which was translocated to the grain at a later stage, reduced potential losses from leaching and also increased NUE.

The more mechanistic approach of SIRIUS is supported by the literature reviewed in this study, which has shown that the N requirement of the component organs varies substantially, and that it is necessary to consider the function of the N within each organ. The inclusion of a dynamic reserve N pool in the canopy model has important physiological consequences, and affects both the N uptake and utilisation efficiency of the crop. However, despite including the capacity for storage of 'excess N' in the new version of SIRIUS (Jamieson and Semenov, 2000), the model is still somewhat limited by a lack of data on the functioning, location and capacity of this canopy trait and its response to N supply, and by the fact that these parameters in the model are not genotype specific. Quantitative knowledge, based on biological reality, is necessary to improve model performance.

2.7 OBJECTIVES AND HYPOTHESES

Wheat leaf lamina has been shown to require levels of 1 to 2 g N m^{-2} of green area for photosynthesis, depending on the light intensity. Analysis of the canopies of optimally fertilised UK wheat showed that leaf laminae tend to match these levels (Critchley,

2001), and as a result about half of the crop N is in the lamina at anthesis. However, evidence shows that the N content of the lamina, particularly that of the upper leaves, may increase significantly above the photosynthetic requirement when excess N is available to crop (Grindlay *et al*., 1993; Grindlay, 1997).

The remaining half of the crop N at anthesis is divided between the leaf sheath, true stem and ear. Although these organs also have a photosynthetic function, a significant proportion of the N which they contain is used for structural, metabolic, transport and storage functions. Critchley (2001) found that a substantial amount of crop N was in the true stem at anthesis (20-25% of the total) and genetic variation exists for N accumulation in the true stem (Triboi and Ollier, 1991). Despite a small amount of photosynthetic function in the exposed part of the true stem, the major functions of the true stem are as part of the structural and transport system of the plant. It is proposed that the high N content of the true stem at anthesis of crops grown at optimal N levels may represent labile N accumulation beyond essential storage of N for grain filling as a result of luxury consumption, and that this provides a potential candidate for reducing crop fertiliser requirements through breeding.

However, there has been very little research on the *accumulation* and *storage* of N in wheat canopies; its location and functioning, the response to N availability, and the existence of genotypic variation. It is clear that the high demand for N during grain filling necessitates significant mobilisation from vegetative tissues, and that N *storage* in the canopy would potentially confer significant benefits to the plant: (i) by reducing the rate of N relocation from photosynthetic tissues so delaying canopy senescence (i.e. increasing yield of low grain N for feed wheats), or (ii) increasing overall grain N supply (i.e. increasing grain protein concentration for bread-making wheats). Yet, the accumulation of N in the canopy without specific function (labile N accumulation) would cause an increase the crop N requirement without an increase in grain yield, and consequently reduce the crop UTE. There may therefore be the scope to reduce surplus N accumulation and to optimise N *storage* through breeding as a means of increasing crop NUE. New cultivars would have lowered N fertiliser requirements using canopy traits

identified in the present study, whilst maintaining yields of low N grain (i.e. feed/biscuit/biofuel wheats) by lowering grain gliadin concentrations using quality traits identified in the wider GREEN grain project.

This study will therefore identify physiological canopy traits underlying environmental and genetic variation in UTE of winter wheat through detailed physiological analyses in field experiments. Emphasis is placed on the accumulation, use, and remobilisation of N within the canopy in order to provide candidate traits for breeding to produce new cultivars with an increased NUE.

The two main study objectives are to:

(i) quantify how components of the N accumulation and remobilisation in wheat canopies respond to changes in crop N supply in temperate environments; and,

(ii) examine whether identifiable genetic variation exists in canopy N *storage* and remobilisation and whether there are interactions with N supply, and thus test whether the N fertiliser requirements of wheat cultivars are positively and quantitatively related to their capacity for N *storage* and/or remobilisation.

The following specific hypotheses are proposed:

1. That there is genetic variation in NUE linked to UTE (via both HI and BPE) amongst elite UK feed winter wheat varieties, and this is associated with differences in the optimum amount of applied N.
2. That N accumulates in the plant organs of wheat canopies at anthesis, which is in excess of that required for structural and photosynthetic uses.
3. That excess N accumulation responds disproportionately to the availability of N, and occurs particularly at high (i.e. supra-optimum) N availabilities.
4. That there is genetic variation in the partitioning of N and the amount of excess N accumulated in the plant organs of wheat canopies and their responses to N supply.

5. That RUE increases linearly with increasing SLN to the maximum RUE, and there are genetic differences in lamina SLN required for photosynthetic function and hence differences in RUE between varieties.
6. That crop components differ in their accumulation of reserve N, and the true stem has a more significant role in the accumulation of RN at anthesis than other crop components.
7. That *storage* N has an important physiological role in wheat crops, especially true stem *storage* N at low N availabilities.
8. That *accumulation* N creates inefficiencies in crop N use by reducing NRE and increasing straw N content, especially at high N availabilities.
9. That *storage* N provides a buffer against premature redistribution of photosynthetic N and hence canopy senescence.
10. That there are genetic differences in N remobilisation efficiencies of the plant organs (leaf laminae, leaf sheath and true stem) and their responses to N linked to patterns of senescence.
11. That remobilisation of N to the grain is source driven, and that source-sink manipulation treatments (through defoliation and de-graining) can promote the use of canopy RN. And,
12. That it is possible to identify a combination of physiological traits associated with N accumulation and partitioning in wheat canopies which have significant heritability and offer scope to markedly increase NUE.

2.8 THESIS STRUCTURE

This thesis is based on an experimental investigation of crop N partitioning between the component organs through loading (vegetative phase) and unloading (reproductive phase) of N during crop growth. Chapter 3 describes the materials and methodologies of the three field experiments in this investigation, conducted at two sites, with four cultivars over a range of N treatments.

The results of these experiments are presented in chapters 4, 5, 6, and 7. Chapter 4 presents the meteorological data and general crop growth results (grain yields, biomass accumulation, N uptake, yield and NUE components). Chapter 5 and 6 present the data from the vegetative (canopy loading) and reproductive (canopy unloading) growth phases, respectively. And chapter 7 presents the results from the source-sink manipulation treatments imposed in the experiments. Each results chapter concludes with a discussion relating to the specific findings in relation to the study hypotheses and the results of previous studies. Chapter 8 discusses the experimental results in the context of the objectives and hypotheses being tested, and concludes the thesis by relating the findings to wider perspectives for the application of physiology for the genetic improvement of NUE in future years.

3 MATERIALS AND METHODS

3.1 INTRODUCTION

In this chapter the materials and methodologies used in the field experiments of this study are described. Three field experiments were established during a period of 18 months; the first (sown October 2005) and third (sown October 2006) were at ADAS Terrington, Norfolk, UK (henceforth referred to as 'TT06' and 'TT07', respectively) and the second (sown June 2006) was established at the Institute for Crop and Food Research, Lincoln, New Zealand (henceforth referred to as 'LC07'). In each experiment a range of N treatments were applied to winter wheat cultivars.

3.2 EXPERIMENTAL SITES

ADAS Terrington is located near King's Lynn, Lincolnshire UK, (latitude 52^{o} 44' N; longitude 0^{o} 17' E). Winter wheat is typically sown in mid-October and harvested in early August (about 42 weeks). The Institute for Crop and Food Research is located near Lincoln, on the south island of New Zealand (latitude 43^{o} 39' S; longitude 172^{o} 29' E). Winter wheat is typically sown in early June and harvested in mid-February (about 36 weeks).

Both sites have a maritime temperate climate. The mean annual rainfall for Lincoln and Terrington are similar at 577 mm and 599 mm respectively, with no distinct wet or dry seasons. Lincoln receives 54% more incident solar radiation on average per year than Terrington at 4927 and 3193 MJ m^{-2}, respectively; and on average is warmer than Terrington with mean annual air temperatures of 11.5 and 9.7^{o}C, respectively.

The field sites were chosen for their low residual soil N. Samples for soil mineral N (SMN) testing were taken before sowing and/or during early establishment. Soil cores were taken to 90 cm, and divided into 30 cm (TT06 and TT07) or 20 cm (LC07) horizons, allowing the total SMN for the top 90 cm to be calculated. Despite the

geographical separation, the Lincoln site was well suited to growing UK-bred winter wheats.

Table 3-1 Sowing and emergence dates, soil description and previous cropping in TT06, TT07 and LC07.

	TT06	TT07	LC07
Sowing date	11 October 2005	30 October 2006	08 June 2006
Emergence	22 October 2005	11 November 2006	16 July 2006 ‡
Soil series	Agney series	Agney series	Wakanui silt loam
Soil texture	Clay silty loam to 40cm then silty loam	Clay silty loam to 40cm then silty loam	Typically 25% clay, 25% sand
Drainage	Well drained	Well drained	Slightly impeded
% Organic matter	2.1%	2.1%	3%
SMN to 90cm (kg ha^{-1})	103.3	95.1	59.7
Previous cropping			
One year previous	Winter OSR	Winter OSR	Mown ryegrass
Two years previous	Winter wheat	Winter wheat	with no grazing

† TT06 and TT07 were established on adjacent fields; 'Far west' (TT06) and 'Hatchett' (TT07).

‡ LC07 was sown 2 days prior to a significant snow fall (of around 10 cm) which caused a slow emergence.

3.3 EXPERIMENTAL DESIGN AND TREATMENTS

3.3.1 Terrington experiments: TT06 and TT07

The two experiments at Terrington were established in a randomised split-plot design with three replicates (detailed experimental plans are in Appendix II). Six fertiliser N treatments were randomised on the main plots and four winter wheat varieties were randomised on the sub-plots. There were a total of 72 sub-plots which were 1.68 m wide (14 rows spaced 12 cm apart) and 10 m long. Each sub-plot was duplicated to provide material for growth analysis during the season and combine harvest.

3.3.1.1 Nitrogen treatments

An 'optimal' N treatment was predicted in each experiment, with N treatments of increasing deficiency below (sub-optimal) or surplus (supra-optimal) allocated around this optimum. It should be noted that in the three experiments different amounts of N were applied (kg ha^{-1}) at the optimum and maximum N treatments.

The optimum N rate was estimated according to previous knowledge of the Terrington site taking into account soil N availability and yield potential using the guidelines in DEFRA booklet RB209 (DEFRA, 2000). The six N treatments were applied as ammonium nitrate prill (34.5%N) by hand to each sub-plot. N applications were split into three smaller amounts: (1) a small application at tillering (GS23) of 40 kg N ha^{-1}, with the remaining N was divided into two equal amounts applied at (2) the beginning of stem extension (GS30), and (3) the appearance of the second node (GS32). The N treatment rates are set out in Table 3-2, and application dates in Appendix I.

Table 3-2 Amount of fertiliser N applied (kg ha^{-1}) in N treatments in TT06 and TT07.

Exp.	Zero	Sub-optimal		Optimum	Supra-optimal	
TT06	0	70	150	220	290	370
TT07	0	60	120	180	260	340

3.3.2 Variety treatment

Four winter wheat varieties: Atlanta, Claire, Istabraq and Savannah, were chosen for the study. The varieties chosen were all modern, semi-dwarf, feed/biscuit wheats (National Association of British and Irish Millers; NABIM, Group 4) (see Table 3-3), as the target ideotype for improved UTE included low grain N%. Previous data from the DEFRA-LINK Project (LK0959) GREEN Grain project trial in 2003-4 at Terrington (henceforth referred to as 'GGTT04') testing 37 trial varieties at 160 kg ha^{-1} of applied N for whole 'stem' N partitioning at anthesis (i.e. leaf sheath plus true stem) showed these to be

contrasting lines for whole stem N content (Figure 3.1). Istabraq was chosen to be the principal variety throughout the three experiments as whole stem N was closest to the mean of the four varieties.

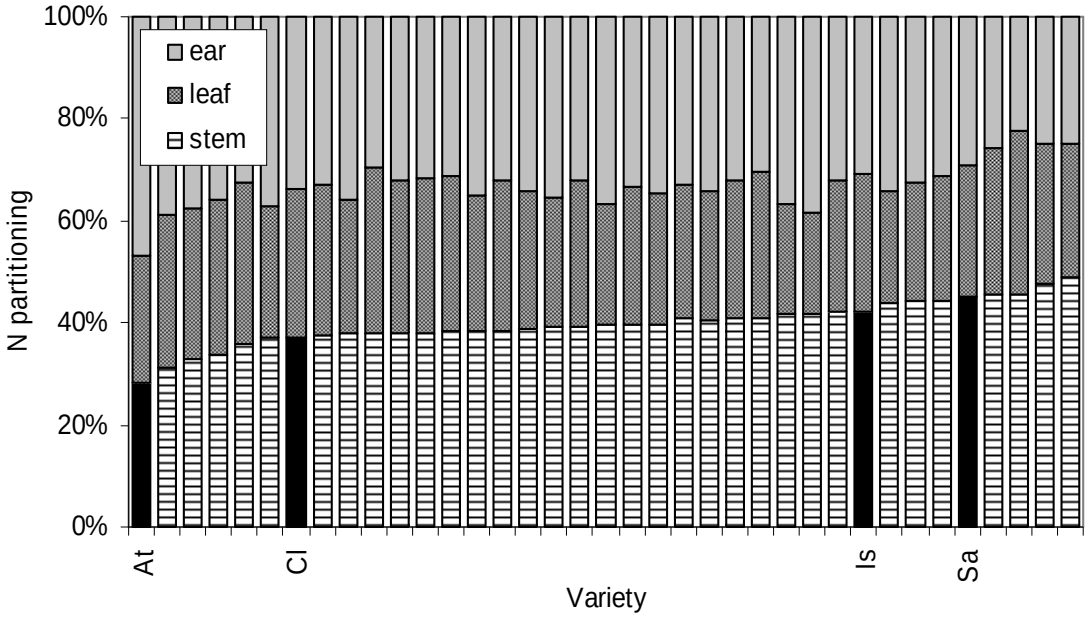


Figure 3.1 Choice of four study varieties: Atlanta (At), Claire (Cl), Istabraq (Is) and Savannah (Sa). N partitioning at anthesis for GGTT04 - observed values for 37 varieties at 160 kg ha^{-1} of applied N for three crop components (whole stem, leaf lamina and ear: in ascending order with whole stem nearest x axis).

Table 3-3 Variety characteristics. Source : HGCA recommended list 2006/07, and HGCA winter wheat trails 2004 - indicated by *.

	Atlanta	Claire	Istabraq	Savannah
Cultivar characteristics				
Parentage	(94-32 x Consort) x Krakatoa	Wasp x Flame	Consort x Claire	Riband x Brigadier
Breeder	KWS Ltd., Herts., UK.	Nickerson, Lincs., UK	Nickerson, Lincs., UK	Advanta Seeds UK, Norfolk
Year released	Estimated at 1999 †	1999	2004	1998
Group	3/4	3	4	4
Grain quality	Biscuit/distilling	Biscuit/distilling	Distilling	Biscuit/distilling
Agronomic characteristics				
Yield (85%DW, t ha^{-1}) *	10.47	10.02	10.38	10.05
Grain protein content (DW, %) *	12.1	11.8	11.1	11.1
Endosperm texture	-	Soft	Soft	Hard
Grain protein (%)	-	11.7	11.1	11.3
TGW (g)	-	47.7	49.1	55.9
Resistance to lodging (with PGR)	-	6 (moderate)	6 (moderate)	8 (stiff)
Height without PGR (cm*)	Short-medium (77)	91 (moderately long)	95 (long)	88 (moderate)
Ripening (days to Claire)	+1 *	0	+3 (late)	+2 (moderately late)
Resistance to sprouting	as Savannah *	5	5	6
Wall thickness (20cm below ear)	Thin	Thin	Thin	Thin
Distilling (LA/t) ‡	452.5	453.8	455.5	447.0

† note that Atlanta was not released as a recommended variety.

‡ distilling data supplied by Daniel Kindred, ADAS, UK.

3.3.3 Lincoln experiment: LC07

The experiment at Lincoln was established in a randomized block design with six replicates (detailed experimental plans are in Appendix I). Six fertiliser N treatments were randomized on replicates for one variety (Istabraq). There were a total of 36 plots which were 1.35 m wide (9 rows spaced 15 cm apart) and 12 m long. Duplicate plots for growth analysis sampling and combine harvest were not established due to space constraints, and instead a duplicate quadrat sample was taken at harvest.

3.3.3.1 Nitrogen treatments

The optimum N rate was predicted using the wheat simulation model SIRIUS (version 2006). The six N treatments were applied as granular urea (47%N) by hand to each plot. N applications were split into two equal quantities and applied at: (1) tillering (GS23), and (2) the appearance of the second node (GS32). The N treatment rates are set out in Table 3-4, and the application dates in Appendix I.

Table 3-4 Amount of fertiliser N applied (kg ha^{-1}) in N treatments in LC07.

Exp.	Zero	Sub-optimal		Optimum	Supra-optimal	
LC07	0	70	150	300	400	500

3.4 CROP HUSBANDRY

All three experiments were sown at a conventional farm seed rate (TT06, TT07 and LC07 at 250, 300 and 320 seeds m^{-2}, respectively) to achieve a plant population of approximately 200 m^{-2}. The N main-plots or plots were separated by buffer plots of unfertilised winter wheat to reduce inter-plot effects. Similar farm management techniques were used during crop cultivation at both sites, as set out below.

3.4.1 Herbicide, fungicide and pesticide applications

In each experiment a prophylactic programme was applied to control pests, diseases and weed infestations to minimum levels, and accordingly there were no significant incidences in all three experiments. In the Lincoln experiment, a minor infection (<1% of shoots) of Barley Yellow Dwarf Virus (BYDV) was noted in the crop after mid-grain filling, and where possible affected areas were excluded from sampling. Full details of the fungicides, pesticide and herbicides applied are provided in Appendix I.

3.4.2 Irrigation

At LC07 overhead irrigation was applied in order to avoid crop water stress and/or to wash N into the soil after application. The crop was irrigated three times: 6 October (5 mm), 6 November (20 mm), and 24 November (25 mm). Neither Terrington experiment was irrigated.

3.5 CROP MEASUREMENTS

3.5.1 Crop development

The date at which the crop reached a growth stage (GS) was assessed according to the decimal code of Zadok's growth stages (Zadoks *et al.*, 1974; Tottman and Broad, 1987). A growth stage was assigned when more than 50% of the main shoots were at the stage at GS31, and more than 50% of all shoots thereafter. Crop maturity was taken as the date when the entire green lamina area had senesced and <10% of stem green area remained. In the period leading up to anthesis, in each sub-plot or plot growth stage was assessed every 2-3 days in order to accurately record the exact date of anthesis.

3.5.2 Sampling timing

In each experiment destructive plant samples were taken at defined growth stages, with particular emphasis on anthesis and harvest (Table 3-5). The principal variety (Istabraq) was sampled more intensively than the other three varieties in all three experiments. In

the Terrington experiments, all varieties were sampled on the same calendar date at a given growth stage. Dates of sampling for treatments combinations are outlined in 5.3.1 and 6.3.1.

Table 3-5 Sampling regime with the growth stages indicated for TT06, TT07 and TT07; 'All' indicates all varieties sampled, 'Is' indicates only Istabraq sampled, with sampling date presented in brackets.

Exp.	GS31	GS39	GS61	GS75	GS92
TT06	All (20 Apr)	Is (23 May)	All (12 Jun)	Is (08 Jul)	All (12 Aug)
TT07	All (22 Apr)	-	All (08 Jun)	Is (06 Jul)	All (07 Aug)
LC07	Is (18 Oct)	Is (21 Nov)	Is (07 Dec)	Is (29 Dec)	Is (08-20 Feb)*

* In LC07, the sub-optimal N treatments were harvested first; 70N (08 Feb), 0N and 150 N (14 Feb); then the 300N, 400N and 500N treatments (20 Feb).

3.5.3 Sample area and sampling

In the Terrington experiments, the sampling positions were chosen randomly in each sub-plot at tillering and marked to avoid areas of poor establishment; quadrat samples were 8 rows (0.96 m) wide and 0.60 m long, giving an area of 0.576 m^2. In the Lincoln experiment, samples were selected systematically from alternating ends of the plot to make efficient use of the smaller plot areas; quadrat samples were 5 rows (0.75 m) wide and 0.67 m long, giving an area of 0.503 m^2. The outer rows were not sampled to avoid 'edge' effects, and a buffer of at least 50 cm (Terrington) or 33 cm (Lincoln) was left between successive sample areas and the plot ends to avoid 'near-neighbour' effects.

Plant samples were either uprooted (at GS31) or cut at ground level using a bread knife (GS39 onwards) and were placed directly into a labelled, large, clean plastic bag in the

field. Once cut, the material was rapidly transferred for storage in a cool room (at about 5^{o}C) until physiological analysis to prevent the deterioration of the sample, especially the lamina surfaces. All samples were analysed within a maximum of 4 days of collection.

3.5.4 Plant population density and shoot number

The plant material collected in each quadrat sample was weighed fresh (FW Q), and a 25% sub-sample randomly selected (SS1) which was then returned to cool storage. At GS31, the plants in SS1 were counted, the roots were then removed and discarded, and the remaining plant material used for physiological analysis. At all samplings, the number of shoots were counted in the SS1 sample, and allocated to one of two categories: (i) fertile shoot (healthy green shoot or those with an ear after ear emergence stage), or (ii) infertile shoot (shoot complete senesced (dead), newest fully expanded yellow (dying) or those without an ear after ear emergence stage). At anthesis and harvest (Terrington experiments) and at all sample stages (Lincoln experiment) the total number of fertile shoots was counted in the whole quadrat sample. From these measurements the shoot number per m^2 was calculated.

3.5.5 Green area and crop dry weight prior to harvest

For samples at GS31, all shoots in SS1 were used for physiological analysis. The fresh weight was recorded (FW SS1), and shoots were partitioned into leaf lamina (green and non-green) and pseudo-stem. For samples at GS39 onwards, twenty fertile shoots were selected at random from the SS1 for physiological analysis (SS2). The fresh weight of the twenty shoots was recorded (FW SS2), and each shoot was measured for height (from the base of stem to ear collar), partitioned into leaf lamina (green and non-green), leaf sheath (green and non-green), true stem (green and non-green) and ear.

The planar area for each green component was measured (in cm^2) using a leaf area meter. For TT06 and LC07 a Li-Cor 3100 model (Li-Cor Inc., Lincoln, Nebraska, USA) was used, and for TT07 an automatic planimeter model (Delta-T Devices, Cambridge, UK)

was used. These green area data allowed calculation of the leaf area index (LAI) and green area index (GAI; defined as the green canopy area per unit ground area).

The plant material was then dried in a force-ventilated oven at 80^{o}C until constant weight, generally about 72 hrs, and weighed. The dry weight (DW) of each component was then calculated per m^2 of ground area as;

$$DW\ (g\ m^2) = DW\ SS2 * 1/(SS1/SS2) * 1/(FWQ/SS1) * 1/(Q) \qquad \textbf{Equation 3-1}$$

Canopy senescence was scored non-destructively by visual assessment from about GS85 approximately every three days until complete canopy senescence. In all three experiments each sub-plot or plot was scored visually for whole canopy '% green' (from 100% - no senescence, to 0% - fully senesced) at three random positions along the plot/sub-plot and an average taken. Additionally in LC07 ten shoots were randomly chosen from the inner rows of the plot (excluding any with obvious diseases), and the '% green' of each leaf (flag, L2, L3, L4) and the number of green leaves on each shoot was scored.

3.5.6 Crop dry weight, grain yield and yield components at harvest

At harvest, the quadrat samples were placed inverted into clean, dry paper sacks in the field to avoid grain loss during transport. In the laboratory, the FW of the quadrat sample was determined, the ears were cut off at the ear collar, counted (to determine the ears m^{-2}), and dried to constant weight (DW ear). Then a sub-sample of 20 fertile shoots (minus ears) (SS2) was taken for physiological analysis as described for samples at GS39 onwards in 3.5.5.

The dry ears were threshed to remove the grain; in the Terrington experiments a Wintersteiger thresher was used (Wintersteiger, Austria), and in the Lincoln experiment a Kurt Pelz thresher was used (Kurt Pelz, Germany). Any remaining rachis or chaff in the

grain sample was carefully removed by hand, and the grain was re-dried and weighed (DW grain). The grain weight (i.e. yield, Y) is expressed either as dry weight (DW; t ha^{-1}) or adjusted to a standard 15% moisture content (85% DW; t ha^{-1}). The chaff weight (DW chaff) was determined by subtracting the DW grain from the DW ear.

The individual grain weight (IGW; mg) was determined using a Numigral seed counter (Tecator, Bristol, UK) to count the number of grains in a 50g sub-sample of the dried grain (any broken grains were removed), and thousand grain weight (TGW; g) calculated. The number of grains per ear (GPE) and harvest index (HI; yield/AGDM) were calculated from these data. In the Terrington experiments a combine yield was taken from the duplicate sub-plots using a Wintersteiger trial plot combine (Wintersteiger, Austria). Before combine harvest the actual length and width of the duplicate sub-plots were measured and any damaged areas measured and removed.

3.5.7 Determination of crop nitrogen content

The dried samples were milled and analysed for total N% by LECO analysis. The dried sample material was first milled to a fine powder (where necessary a sub-sample was taken) and then sealed in an air-tight plastic container to avoid moisture re-absorption. The mill was thoroughly cleaned between samples to avoid cross contamination, and where possible, the samples were ground in order of component type and N treatment (from zero N to maximum N). In the LECO Automatic Combustion Analyser (Carlo Erba Instruments analyser, model NA 2000), a small quantity (about 0.02 g) of the re-dried powdered sample was weighed into a ceramic dish and entered into the machine. The sample is combusted in pure oxygen at >1000^{o}C and the gaseous products of the combustion are passed over a hot copper catalyst to convert the oxides of N to N gas. Carbon dioxide and water are removed, and the amount of N in the sample is determined by the change in thermal conductivity in the sample stream (N_2 gas in helium carrier gas) compared to a reference cell.

The results are expressed as N% of the sample dry weight from which the total amount of N in the original sample can be calculated as:

$$N\ (g\ m^2) = (N\%/100) * DW\ (g\ m^2) \quad \textbf{Equation 3-2}$$

However, the significant cost of LECO analysis placed constraints on the N analysis of samples. The N analysis regime was therefore designed mainly to provide a detailed study of one variety (Istabraq) over three site-seasons, and study varietal effects at the key growth stages of anthesis and harvest. In each experiment, N% data for Istabraq are available at all six N treatments at all sample stages, whilst in the Terrington experiments N% data are available for all varieties at 6 N treatments at anthesis in TT06, and at 3 N treatments (zero, optimum and maximum) at anthesis in TT07 and harvest in both experiments.

The N harvest index (NHI; the proportion of N in total plant which is in the grain at harvest) was calculated from these data as:

$$NHI = \text{grain N}\ (kg\ ha^{-1}) / AGN_{harvest}\ (kg\ ha^{-1}) \quad \textbf{Equation 3-3}$$

3.5.8 Calculation of fertiliser N recovery

For crop N uptake, there are two sources of N for field-grown wheat: soil mineralisation and applied fertiliser N. The recovery of each of these sources is quantified by:

- Soil N: as the total soil mineral N is difficult to measure accurately over the period of crop growth, it is assumed that the N zero-trt (unfertilised) takes up all of the available soil N and has an AGN representative of the available soil mineral N at harvest.

- Fertiliser N: the 'apparent fertiliser recovery' (AFR) is the proportion of the applied fertiliser N which is recovered by the crop at harvest in comparison with the N zero-

trt (Bloom *et al.*, 1988). This can be calculated by the 'difference' or 'N balance' method, as described by Foulkes *et al*. (1998);

$$\text{AFRopt} = \frac{(\text{AGN N opt - trt} - \text{AGN N zero - trt})}{\text{N opt}} * 100$$ **Equation 3-4**

Where 'AGN N opt-trt' and 'AGN N zero-trt' are the crop N contents of an optimally fertilised and unfertilised crop respectively, and 'N opt' is the optimum amount of fertiliser N application.

3.5.9 Problems with plant establishment

In both Terrington experiments areas of poor plant establishment were observed. In TT06 problems appeared as distinct strips which were noticeable on the ends of some rows. These were likely caused by slug damage and associated with the organic debris from the previous crop, and these areas were avoided in the sampling. In TT07 problems appeared as patches within or across some sub-plots. These were likely caused by poor weather conditions at sowing which made drilling difficult. However the worst affected areas avoided in the sampling.

3.5.10 Lodging

All experiments were regularly assessed for lodging (defined as between 45^{o} and 90^{o} from the vertical). Lodging occurred only in TT07, caused by a period of poor weather (high rainfall and wind) after the mid-grain filling stage. Plots were scored for severity of leaning from vertical and percentage of plot affected at GS85 (24 July 2007). Details of incidence of lodging are presented in Table 4-3.

3.6 ENVIRONMENTAL MEASUREMENTS

3.6.1 Meteorological data

Data on sun-hours, rainfall, and maximum and minimum air temperatures were recorded manually on a daily basis at Terrington or by an automatic weather station at Lincoln. At both sites the total incident solar radiation (SR) throughout the study period (from tillering to harvest) was recorded: at Terrington using a dome solarimeter linked to a data-logger which was placed within the crop (checked and downloaded regularly), and at Lincoln SR using an automatic weather station. The PAR was calculated as SR/2 (Monteith, 1972).

For a brief period (15 April to 15 May 2007) the data-logger at Terrington malfunctioned and SR data was provided by the MetOffice weather station at Holbeach. Regression analysis of SR data from Terrington and Holbeach between 16 May and 29 May 2007 gave a strong correlation ($r^2 = 0.90$) and validated the use of these data.

3.6.2 Canopy light interception and radiation-use efficiency

Photosynthetically active radiation (PAR; μmol m^{-2} s^{-1}) in the canopy was measured as photosynthetic photon flux density (PPFD) using a Sunfleck Ceptometer (Delta-T Devices, Cambridge, UK). Measurements were taken at GS39 (TT06 only) and at anthesis (all experiments), between 11am and 3pm with a clouded sky (to give diffuse light conditions). Readings were taken at defined levels within the crop canopy (from top downwards); (i) at the ear collar, (ii) at the flag leaf ligule, (iii) at the leaf 2 ligule, and (iv) at soil level. At each level, three readings were taken diagonally across rows at random points in the crop and a mean value calculated. Total incident PAR was measured at the same time by a free standing dome solarimeter linked to the ceptometer.

From these data the fractional PAR interception (f) of the whole canopy, or any point in the canopy, can be calculated as:

$$\boldsymbol{f} = 1 - (I / Io) \qquad \textbf{Equation 3-5}$$

Where I_o is the incident PAR above the crop canopy and I is the PAR at a point within the canopy.

The light interception of the whole canopy was related to its total green area (L) through the extinction coefficient (K). K for PAR (K_{PAR}) was determined by plotting the natural log of the proportion of above canopy PAR remaining beneath each leaf layer of the canopy against the cumulative GAI, with the slope of the regression plotted through the origin:

$$K_{PAR} = (-\ln (1- \boldsymbol{f}) / L \qquad \textbf{Equation 3-6}$$

Light measurements were taken in the quadrat sample before it was destructively sampled in order to increase the accuracy of calculating K_{PAR}. In TT06 light measurements were also taken at GS39 and GS61 to test whether K_{PAR} was consistent through pre-anthesis growth, as was found by Thorne *et al*. (1988).

The PAR intercepted by the crop on a daily basis was calculated from the fractional interception at anthesis multiplied by the total daily PAR. Over the growing season (GS31 to anthesis) cumulative intercepted PAR was calculated by multiplying the total daily radiation above the crop by the fraction of incident light intercepted by the canopy (assuming a linear rate of GAI increase with calendar time between samplings). Radiation-use efficiency (g MJ^{-1}) was calculated for each plot by dividing the cumulative biomass by the cumulative PAR intercepted (MJ m^{-2} d^{-1}) over the same period:

$$\text{RUE} = \frac{(\text{DW t2} - \text{DW t1})}{(\text{MJ t2} - \text{MJ t1})} \qquad \textbf{Equation 3-7}$$

Where DW is the cumulative crop dry weight and MJ is the cumulative PAR intercepted at the first (t1) or the second (t2) samplings.

RUE can also be estimated by plotting a linear regression between accumulated PAR and accumulated crop DW, fitting the intercept to zero, and the slope of the line gives an estimate of RUE (Sinclair and Muchow, 1999).

3.7 STATISTICAL ANALYSIS

Data were entered into EXCEL 2003 (Microsoft Corporation) spreadsheets. Statistical analysis of data was carried out using GENSTAT version 9.1 (Lawes Agricultural Trust, Rothamsted Experimental Station; 2006). Standard analysis of variance (ANOVA) procedures were used to calculate treatment means, standard errors and significant differences between treatments. Linear regression analysis was used to determine relationships and correlations between crop and plant variables. Examples of GENSTAT outputs are given in Appendix III.

3.7.1 ANOVA and regression analysis

The significance of the treatment effects was determined by ANOVA where the variation due to the main effects ('N treatment' and/or 'variety'), and their interactions (in the Terrington experiments) were compared with the residual variation within the treatments between replicates (blocks).

Where there was a significant effect of N, polynomial regressions were fitted across N treatments to test whether the form of the function for the variation observed was significant as linear, quadratic or cubic. A probability value of 0.05 or less ($P \leq 0.05$) was taken to be significant, although consistent values between 0.05 and 0.10 may receive comment in the text. Where the effect of variety was statistically significant, regression analyses (linear and non-linear) were fitted to all data sets according to the ANOVA output to test the responses of the cultivars (except for the grain yield and AGN; see 3.7.2 and 3.7.3, respectively).

Parallel regression analysis was applied to determine the most parsimonious line fit for each regression, either; (i) a common line, (ii) separate lines for each variety using a common intercept, or (iii) separate lines allowing both slope coefficients and intercepts to vary for each variety. The sum of squares was calculated at each stage, and improvements tested against the residual mean square to determine their significance.

3.7.2 Grain yield N response curves

Experimentation across a broad range of N treatments, including zero N (unfertilised), allowed the fitting of a yield response curve. For each cultivar in each site season, the grain yield response to applied N was estimated by fitting a linear plus exponential (LEXP) function to grain yield data following the method described by Sylvester-Bradley *et al*. (1984), using the model:

$$Y = a + br^N + cN \qquad \textbf{Equation 3-8}$$

Where Y is the DW grain yield (t ha^{-1}), N is the total applied fertiliser N (kg ha^{-1}), and ***a***, ***b***, ***c*** and ***r*** are parameters of the model whose values are found by a least squares fit for each site) as described by George (1984) and Dampney *et al.* (2006). An example GENSTAT output for grain yield analysis including ANOVA and fitting of LEXP is given in Appendix III.

Figure 3.2 illustrates a typical yield response curve to N supply for winter wheat with the four parameters of the curve indicated, where; ***a*** indicates the maximum yield, ***b*** indicates the range of yield, and therefore (***a*** – ***b***) gives the unfertilised yield, ***c*** can have a negative function to allow for reduced yields at high N supply, and ***r*** gives the slope of the curve.

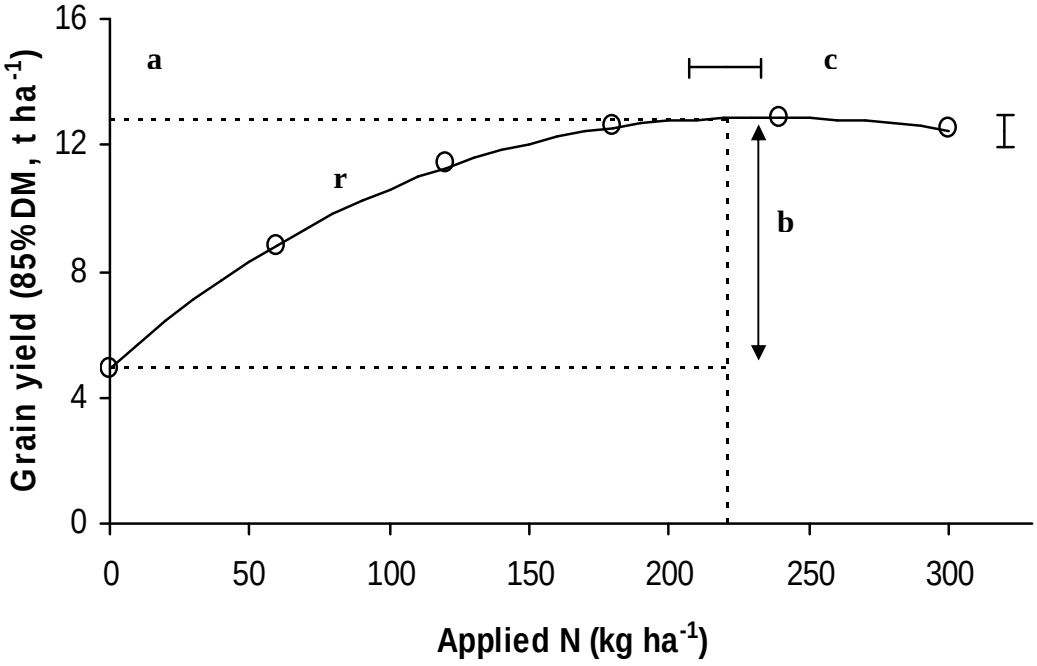


Figure 3.2 Typical yield response curve to N fertiliser supply for winter wheat indicating curve parameters from linear plus exponential function, and SED N bar. The 'economic optimum yield' at the 'economic fertiliser N amount' is shown as ●, and SE bar (N opt) above.

The shape of the yield response curve to applied N is generally quite stable (George, 1984). However, typically two variables show considerable variation: (i) the intercept which is set by the yield of the unfertilised crop from the supply of soil mineral N or the 'yield potential' of the variety, and (ii) the asymptote of the curve which is set by the growing conditions of the site and season (Sylvester-Bradley *et al.,* 1995). Generally the soil mineral N supply is sufficient for production of about half of the potential yield, the first half of the normal N requirement provides about 90% of the potential yield whilst the second half appears to be used less efficiently and typically provides for less than 10% of the potential yield (Stokes *et al*., 1997).

Using the parameters of the grain yield N response curve, the value of the interpolated 'economic optimum fertiliser N amount' which gives the 'economic optimum yield' is found from the expression described by Foulkes *et al*. (1998);

$$N_{opt} = \frac{\ln(k - c) - \ln(b \times \ln(r))}{\ln(r)}$$ **Equation 3-9**

Where k is the pre-determined constant set according to the breakeven ratio for economic optimum N applications; in this study, k is set as 0.003 (i.e. as t ha^{-1}), or 3 kg of grain per kg of applied N. At this 'economic optimum fertiliser N amount' the corresponding 'economic optimum yield' can be estimated using Equation 3-8.

However, several instances of problems with the estimation of the economic optimum amount of applied N have been reported (Foulkes *et al*., 1998). Estimated N optima should be treated with reserve where the data are very variable, the rate of change of slope is small in the regions of N optimum, or the N optimum lies close to the ends of the range of N levels tested. The majority of N response experiments therefore concentrate on N treatments in the sub-optimal N levels to increase the reliability of 'r' parameter (i.e. the shape of the curve), with few N levels above the estimated optimum.

3.7.3 Crop N uptake

The above-ground crop N content (AGN) response to applied N was fitted to a two line ('bi-linear') function ('broken stick model') using GENSTAT, following the method of Bloom *et al*. (1988):

$$AGN = [(N<t).\{a-b(t-N)\}+(N>t).\{a+c(N-t)\}]$$ **Equation 3-10**

Where N is the amount of applied N, and a, b, c and t are parameters estimated by maximum likelihood from each data set using numerical optimisation.

This divides the data into two linear sets with a distinct 'break point'. The slope of the line (1) with N less than that of the break point is greater than the slope of the line (2) following the break point (Foulkes *et al*., 1998). The break point is not necessarily related

to the optimum amount of applied N, but gives the point at which further N fertilisation is in excess to crop uptake and there is little or no effect of increasing fertiliser N on AGN. The slope of line 1 gives an estimate of the efficiency of applied N uptake by the crop (see 3.5.8). However, AGN data are required over a range of N treatments to fit the model, and these data were only available for Istabraq (all three experiments at six N treatments), with insufficient N data being available for the other three other varieties in the Terrington experiments to fit this model.

4 GENERAL CROP GROWTH

4.1 INTRODUCTION

This chapter describes the grain yield and yield components, N-use efficiency and its components, biomass production, N uptake, HI and biomass production efficiency at harvest for the three experiments. Meteorological data for the three site-seasons are presented for the most important variables affecting development, crop growth and yield: incident radiation, rainfall, and mean air temperature.

Results at harvest are presented and discussed. Comparison of Istabraq data between the site-seasons tests for common trends in physiological traits in response to N supply across different growing conditions (i.e. environmental effects), and comparison of data for the four varieties in the Terrington experiments tests for genetic differences in responses to N. The grain yield data are used to calculate the 'economic optimum N amount' (N opt) and to identify the individual N treatment level corresponding most closely to the 'optimum N amount' from curve-fitting analysis for each variety in each site-season (N opt-trt).

The aims of this chapter are firstly to describe results of the productivity at harvest for each variety in each site-season. Statistical analysis of data is applied to test responses to N supply of the variety or varieties in each experiment, and whether the pattern of responses to N is consistent or different between environments. These analyses of the results at harvest are important in providing the basis for the testing of the specific hypothesis: (1) 'that there is genetic variation in NUE linked to UTE (via both HI and BPE) amongst elite UK feed winter wheat varieties, and this is associated with differences in the optimum amount of applied N', and relating to the detailed crop physiological processes determining NUE which are examined in the following results chapters. The chapter concludes with a discussion of the findings and links to the hypotheses to be examined in the next results chapter.

4.2 GROWING CONDITIONS IN THE EXPERIMENTAL SITE-SEASONS

The prevailing weather varied greatly between site-seasons giving the potential for environmental effects on crop growth and yield. The meteorological data for the Terrington, UK experiments (TT06 and TT07) and Lincoln, New Zealand experiment (LC07) are presented in Figure 4.1 and Figure 4.2 respectively, together with the long-term means (LTM).

4.2.1 Mean air temperature

In the Terrington experiments, the mean air temperature from sowing (October) to harvest (August) for TT06 was 10.7^{o}C (0.9^{o}C > LTM (1951 to 2005)), and for TT07 was 11.6^{o}C (1.7^{o}C > LTM). The warmer than average TT06 was associated with high temperatures from the start of stem extension (May) to the end of grain filling (July) (2.3 ^{o}C > LTM). The warmer than average TT07 was associated with high temperatures from sowing (October) until anthesis (June) (2.1^{o}C > LTM). However, TT07 was slightly cooler than average from the end of anthesis until harvest (July and August) (0.3 ^{o}C < LTM).

In LC07, the mean air temperature from sowing (June) until harvest (February) was 10.8^{o}C (0.5^{o}C < LTM (1981 to 2005)). This was 0.6 ^{o}C warmer and 0.2 ^{o}C cooler than TT06 and TT07 from sowing to harvest, respectively. The cooler than average temperature at LC07 was accounted for by the grain filling period (December and January) (2.1 ^{o}C < LTM). This was 4.2 ^{o}C cooler and 1.8 ^{o}C cooler than TT06 and TT07 during the grain-filling period, respectively.

4.2.2 Solar radiation

In the Terrington experiments, the total incident solar radiation from sowing to harvest for TT06 was 3315 MJ m^{-2} (4% > LTM (1971 to 2000)), and for TT07 was similar to the LTM (3193 MJ m^{-2} and 3185 MJ m^{-2}, respectively). The higher than average solar

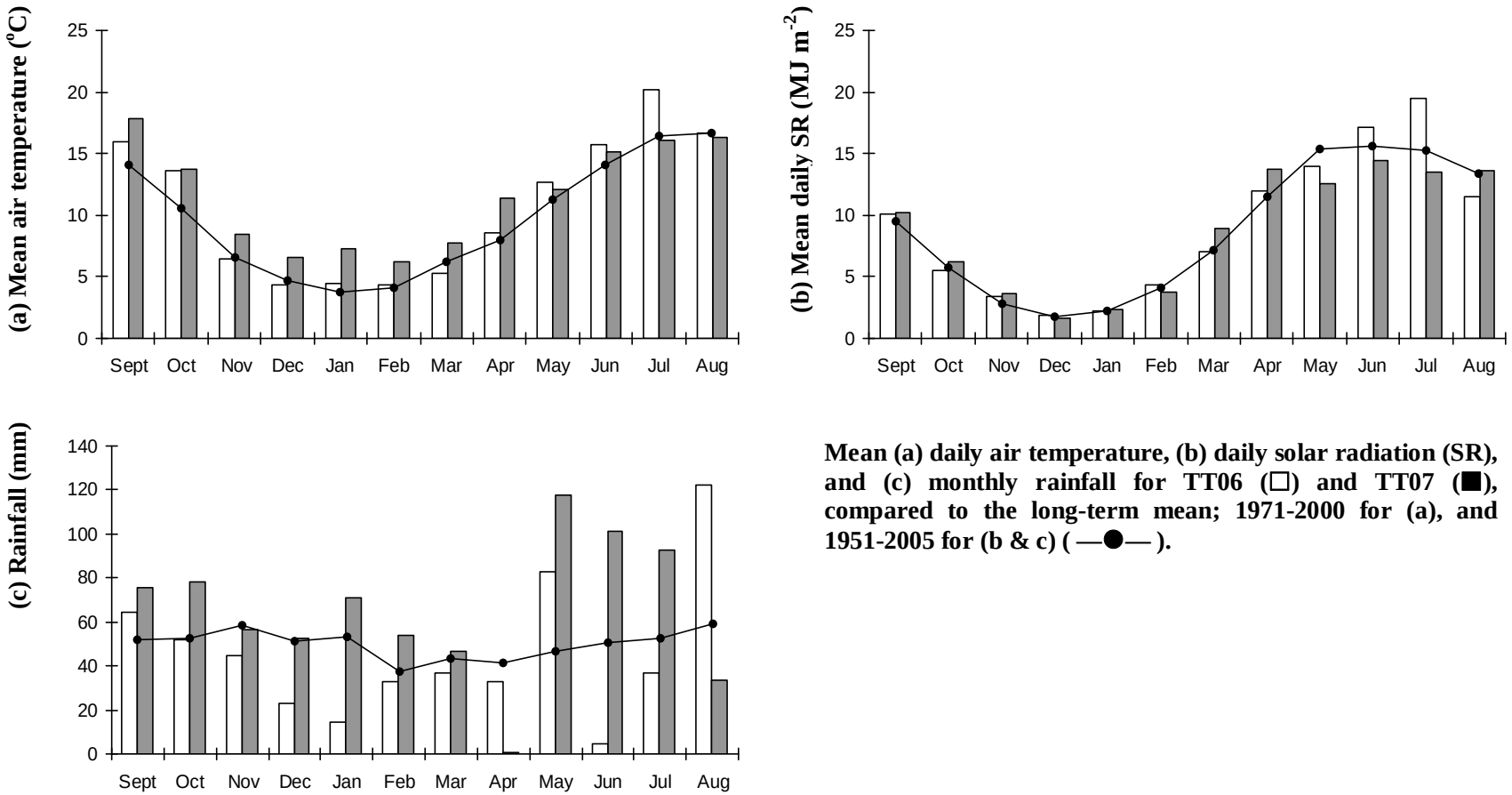
radiation in TT06 related to conditions from the end of booting to the end of grain filling (June and July; 1120 MJ m^{-2} cf. LTM of 941 MJ m^{-2}). In TT07, conditions were brighter than average during tillering (March and April; 687 MJ m^{-2} cf. LTM of 568 MJ m^{-2}). However there was less than average incident solar radiation from the end of stem extension to late grain filling (May, June and July; 1241 MJ m^{-2} cf. LTM of 1417 MJ m^{-2}).

In LC07, the total incident solar radiation received from sowing to harvest was 3824 MJ m^{-2} (4% < LTM (1981 to 2005)); but 814 MJ m^{-2} and 938 MJ m^{-2} more than TT06 and TT07 from sowing to harvest, respectively. LC07 received 12% less than LTM incident solar radiation from anthesis to late grain filling (December and January), but 114 MJ m^{-2} and 382 MJ m^{-2} more than TT06 and TT07 during June and July, respectively.

4.2.3 Rainfall

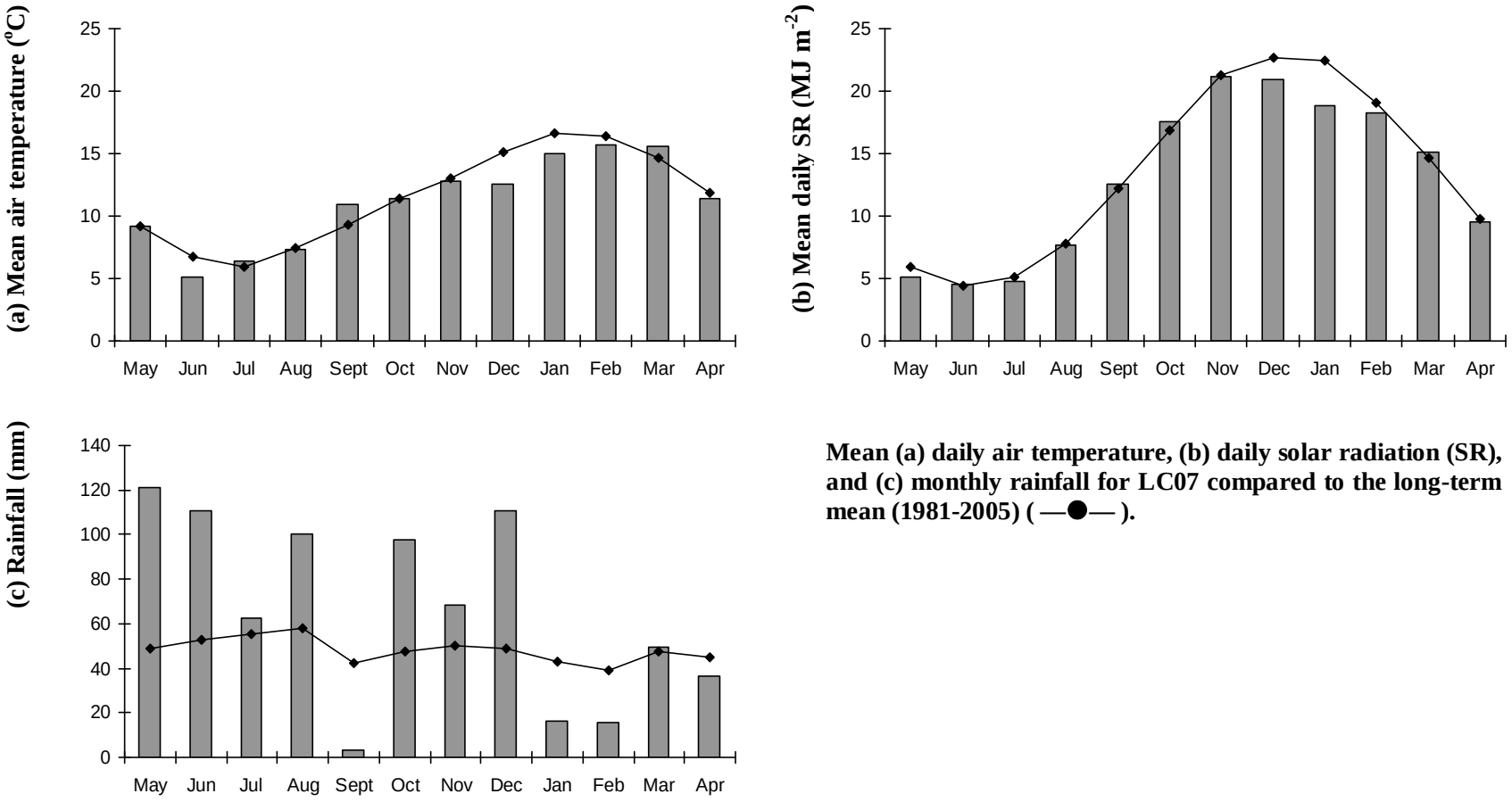
The total rainfall from sowing to harvest for TT06 was 547 mm (12% < LTM (1951 to 2005)), and for TT07 was 780 mm (30% > LTM). The lower than average rainfall in TT06 related to conditions during the end of grain filling (June and July) which was especially dry (42 mm cf. LTM of 103 mm). However, during late stem extension and early booting (May), TT06 received more rainfall than the LTM (83 mm cf. LTM of 47 mm) with rainfall in August mostly after harvest. The higher than average rainfall in TT07 related to conditions from the end of stem extension (May) to the end of grain filling (July) (312 mm cf. LTM of 150 mm). TT07 also experienced a very dry month in April (0.7 mm cf. LTM of 41 mm).

In LC07, the rainfall from sowing to harvest was 585 mm (34% more than the LTM (1981 to 2005)); but 102 mm more and 119 mm less than TT06 and TT07 from sowing to harvest, respectively. The higher than average rainfall related to conditions from the end of tillering (October) to mid-grain filling (December) (277 mm cf. LTM of 147mm), but from mid-grain filling until harvest conditions were drier than average (32 mm cf. LTM of 82 mm).



Mean (a) daily air temperature, (b) daily solar radiation (SR), and (c) monthly rainfall for TT06 (□) and TT07 (■), compared to the long-term mean; 1971-2000 for (a), and 1951-2005 for (b & c) (—●—).

Figure 4.1 (a, b & c) Meteorological data for Terrington, UK experiments.



Mean (a) daily air temperature, (b) daily solar radiation (SR), and (c) monthly rainfall for LC07 compared to the long-term mean (1981-2005) (—●—).

Figure 4.2 (a, b & c) Meteorological data for Lincoln, New Zealand experiment.

4.3 METHODOLOGY

4.3.1 Treatment combinations and statistical analysis

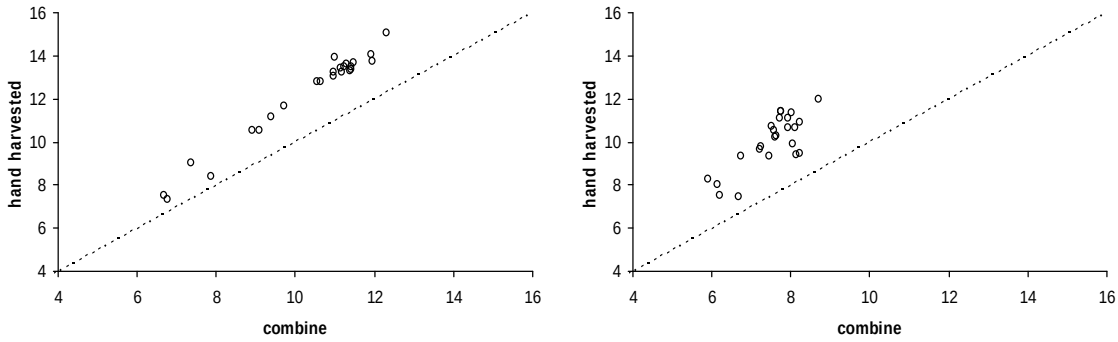
In the experiments at Terrington, Istabraq was sampled more frequently than the other three varieties. The effect of N supply for Istabraq is analysed in all three experiments at harvest at six N treatments. Unless otherwise specified, results for the effect of N supply and variety are presented for all four varieties in the Terrington experiments at harvest at six (TT06) or three (TT07; zero, optimum and maximum) N treatments, and at the three N treatments (TT06 and TT07) for results requiring N% data. ANOVAs for the effect of N supply for Istabraq are at six N treatments in all three experiments, and for all four varieties at six (TT06) or three (TT07) N treatments, or three (TT06 and TT07) N treatments for results requiring N% data. SEDs from ANOVAs are shown as error bars in figures, and model parameters for fitted curves are found in Appendix III.

4.4 RESULTS

4.4.1 Grain yield

4.4.1.1 Combine and hand-harvested grain yield

In the Terrington experiments, both a combine and a hand-harvested measurement of grain yield (hereafter referred to as 'yield'; Y (85% DM; t ha^{-1})) was taken. Linear regression analysis between the two estimates showed that the hand-harvested yields were higher in both experiments for all N treatments; hand-harvested means were 18% higher in TT06, and 33% higher in TT07 (Figure 4.3). In both experiments, the difference between the combine and hand-harvest yields was affected by N treatment (the difference increasing with N supply), but was not affected by variety.



(a) TT06 (b) TT07

Figure 4.3 (a & b) Terrington experiments, regression of hand-harvested and combine-harvested grain yield (85% DM; t ha^{-1}). Data plotted for N treatment means; dashed line represents a 1:1 ratio.

In this thesis, the hand-harvested yield data are presented from quadrat samples for all experiments, unless specified otherwise. These yield data are derived from the same samples as the crop growth data at harvest (i.e. dry matter and N%).

4.4.1.2 Estimating the 'economic optimum fertiliser N amount' and the 'economic optimum yield'

Using the curve-fitting method described in 3.7.2, values were estimated for the 'economic optimum N amount' (N opt; defined as the amount of fertiliser N below which 1 kg N ha^{-1} elicited a response in grain yield of more than 3 kg ha^{-1}) and the 'economic optimum grain yield' (Y opt) for each variety in each site season (Table 4-1; Figure 4.1).

Table 4-1 The 'economic optimum N amount' and the corresponding grain yield ('economic optimum yield') for the experiments at TT06, TT07 and LC07.

Exp.	Variety	Economic optimum N amount (kg ha^{-1})	Economic optimum yield (85%DM; t ha^{-1})
TT06	Istabraq	236	13.50
	Atlanta	236	14.41
	Claire	236	13.20
	Savannah	236	13.95
	SE	13 (df = 65)	
TT07	Istabraq	140	10.58
	Claire	140	10.58
	Savannah	140	10.58
	SE	29 (df = 50)	
	Atlanta	221	11.93
	SE	124 (df = 14)	
LC07	Istabraq	312	15.18
	SE	13.6 (df = 32)	

Parallel regression analysis of the grain yield response to applied N in TT07 showed that the response of Atlanta was different to the other three varieties. Therefore, Atlanta yield data were analysed separately in the calculation of the economic optimum amount of applied N. The reasons and consequences for this difference are discussed in 4.4.1.4.

4.4.1.3 Estimating the optimum fertiliser N treatment level

The 'optimum fertiliser N treatment level' (N opt-trt) from the range of N treatments used in each experiment was determined as the N treatment closest to the estimated N opt from the curve fitting (Table 4-2). N opt-trt will be used and referred to as such throughout the following analysis.

Table 4-2 The economic N optimum amount (N opt) and the corresponding 'optimum fertiliser N treatment level' (N opt-trt) for the variety or varieties in the experiments at TT06, TT07 and LC07.

Exp.	Variety	N opt (kg ha^{-1})	N opt-trt (kg ha^{-1})
TT06	Istabraq, Atlanta, Claire & Savannah	236	220
TT07	Istabraq, Claire & Savannah	140	180*
TT07	Atlanta	221	180*
LC07	Istabraq	311	300

* Despite different estimated values of N opt for Istabraq, Claire and Savannah (140 kg N ha^{-1}) and Atlanta (221 kg ha^{-1}) in TT07, the N opt-trt is taken in this study as 180 kg ha^{-1} for all four varieties. The 180 kg ha^{-1} N treatment was considered sufficiently close to both of the estimated values of N opt, and had been sampled more intensively during the experiment than either the 120 kg ha^{-1} or 260 kg ha^{-1} N treatment levels.

4.4.1.4 Responses of yield to fertiliser N and variety

The yield for Istabraq was affected by N treatment in all three experiments ($P<0.001$) (Figure 4.4). Averaging across N treatments, LC07 had the highest yield at 12.2 t ha^{-1}, then TT06 at 11.4 t ha^{-1}, and TT07 at 9.6 t ha^{-1}. Yield increased with N supply between the N zero-trt and the N opt-trt; thereafter yield continued to increase slightly in TT06, but decreased in TT07 and LC07. LC07 had the lowest yield at the N zero-trt at 6.7 t ha^{-1}; cf. TT06 and TT07 which were similar at 7.5 t ha^{-1}. However LC07 had the highest yield at the N opt-trt at 15.5 t ha^{-1} (cf. TT06 at 13.3 t ha^{-1}, and TT07 at 10.7 t ha^{-1}), and the greatest response to N supply was observed at this site-season with a range of 8.8 t ha^{-1} between N treatments, then TT06 at 5.8 t ha^{-1}, and the least response in TT07 at 3.2 t ha^{-1}.

In both of the Terrington experiments, the yield for all varieties was affected by N treatment ($P<0.001$), and by variety in TT06 ($P<0.001$) but not in TT07; the interaction was only significant in TT07 ($P<0.01$). Averaging across N treatments, in TT06 Atlanta

had the highest yield (12.3 t ha^{-1}), then Savannah (11.9 t ha^{-1}), Istabraq (11.4 t ha^{-1}), and Claire (11.1 t ha^{-1}). In TT07 although differences were not significant, the varietal pattern was generally similar in the range 9.4 to 10.2 t ha^{-1}. Thus, Atlanta had the highest yield at the N zero-trt in TT06 at 9.0 t ha^{-1}, and had the highest yield at the N opt-trt in both TT06 and TT07 at 14.1 ha^{-1} and 11.4 ha^{-1}, respectively.

Linear plus exponential (LEXP; Equation 3-8) curves were fitted to the yield response to N supply in all three experiments (model parameters shown in Table 4-4). In LC07 a single LEXP curve was fitted to the data for Istabraq and explained 94% of the variation. In TT06 and TT07 LEXP curves were fitted according to the parallel regression analysis. In TT06 the parallel regression analysis fitted LEXP curves to the data with only the 'a' parameter (i.e. y axis intercept) differing between varieties. Therefore the yield potential of the four varieties differed, but the pattern of response to N availability was the same – the estimated N opt was the same for all varieties but the Y opt differed (Table 4-1).

In TT07 parallel regression analysis fitted LEXP curves to the data giving the most parsimonious fit to be separate lines with all curve parameters varying due to the N treatment x variety interaction. However, this result must be treated with some caution because when Atlanta data were excluded from the analysis, a single linear plus exponential curve fitted the remaining three varieties. Therefore the pattern of the N responses for Istabraq, Claire and Savannah appeared to differ from Atlanta. Atlanta showed a sharp decrease in the grain yield beyond the N opt at the N max-trt (associated with poor establishment and low shoot number at this one treatment combination) causing the ***r*** parameter of the model to exceed 1. This made a LEXP curve more difficult to fit and reduced the accuracy of the estimation of the N opt with a much larger SE.

The basis of difference in yield response of Atlanta compared to the other three varieties in TT07 was also related to the wet weather conditions during grain filling which caused crop leaning and/or lodging at the highest N treatments in Istabraq, Claire and Savannah, but not in Atlanta (shorter straw length) (Table 4-3). This explained the higher N opt for Atlanta. In summary, taking account of the factors explained above it was decided that

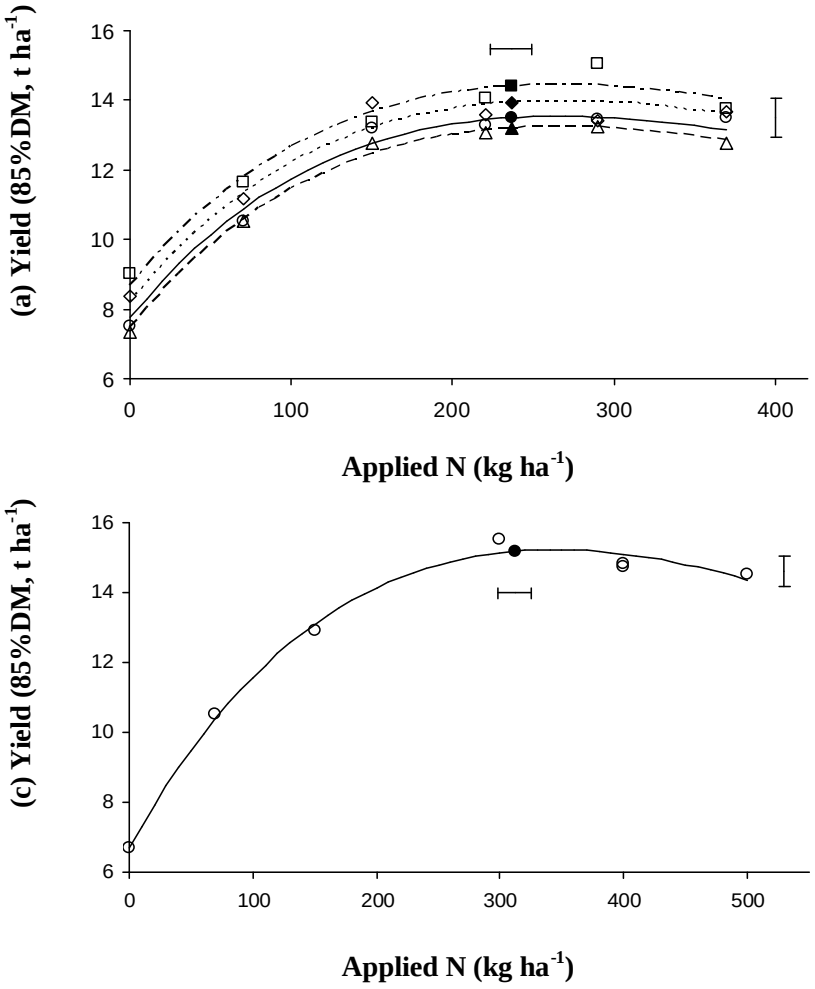
the most reliable explanation of the yield responses related to one LEXP curve fitted to Istabraq, Claire and Savannah and a separate LEXP curve fitted for Atlanta (which differed in all parameters; Table 4-4) – the N opt and Y opt for the three varieties was therefore the same, but different to that of Atlanta (Table 4-1).

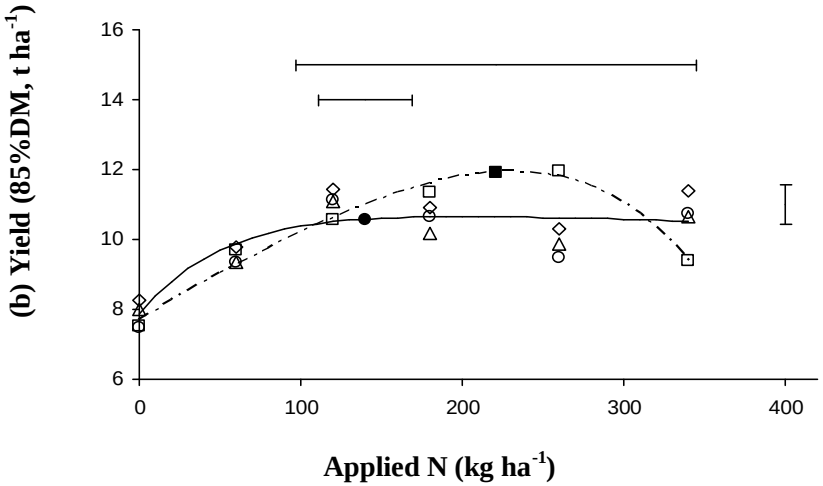
Table 4-3 TT07 – Crop leaning and lodging at GS85 (24 July 2007); where, a '*lean*ing' crop is between 0° and 44°, a '*lodg*ing' crop is between 45° and 89°, and a 'flat' crop is at 90°. displaced from the vertical. Numerical score refers to mean plot area affected; where, (1) is 0-25%, (2) is 26-50%, (3) is 51-75%, and (4) is 76-100%.

N rate	Istabraq	Atlanta	Claire	Savannah
0	Nil	Nil	Nil	Nil
60	lean (1)	Nil	Lean (1)	Nil
120	lean (2)	Nil	Lean (2)	lean (1)
180	lodge (3)	Lean (1)	lodge (3)	lean (2)
260	lodge (3)	Lean (1)	lodge (4)	lean (2)
340	lodge (3)	Lean (1)	lodge (3)	lodge (2)

Table 4-4 Model parameters for fitted linear plus exponential curves (Equation 3-8) for grain yields.

Exp.	Variety	A	B	C	R	% Variance accounted (SE)
TT06	Istabraq	20.64	-12.89	-0.01608	0.9943	85.7 (0.862)
	Atlanta	21.55	-12.89	-0.01608	0.9943	
	Claire	20.34	-12.89	-0.01608	0.9943	
	Savannah	21.09	-12.89	-0.01608	0.9943	
TT07	Istabraq, Claire & Savannah	11.03	-3.17	-0.00148	0.9818	58.7 (0.825)
	Atlanta	8.05	-0.34	0.0306	1.0097	83.2 (0.654)
LC07	Istabraq	28.20	-21.50	-0.0218	0.9960	93.9 (0.788)





Mean values for each treatment combination (open symbols) with fitted linear plus exponential curves and SED bar (vertical; from ANOVA). N opt and Y opt plotted (solid symbols) with SE bars (horizontal; from regression analysis). Model parameters for curves are presented in Table 4-4.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; in TT06 - Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------) with SED N x V bar (df = 36); in TT07 - Istabraq, Claire and Savannah (———) and Atlanta (— – —); with SED N x V bar (df = 36).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 25).

Figure 4.4 (a, b & c) Effect of applied N and variety on the yield (@ 85% DM, t ha^{-1}) in TT06, TT07 and LC07.

4.4.2 Responses of yield components to N and variety

The yield is the product of three components: the ear population density, the grains per ear, and the grain weight. The number of grains m^{-2} is the product of the ear population density and the number of grains ear^{-1}, and represents the 'sink' to be filled by photo-assimilates.

4.4.2.1 Ear population density

The ear population density (ears m^{-2}) for Istabraq increased with N supply in all three experiments ($P<0.01$) (Figure 4.5). Averaging across N treatments, TT06 had the highest ear population (486), then LC07 (439) and TT07 (407 ears m^{-2}). LC07 had the lowest ear population density at the N zero-trt but the highest ear population density at the N opt-trt, giving the largest response to N supply of the three experiments at 308; cf. TT06 at 142, and TT07 at 133 ears m^{-2}.

In both the Terrington experiments, ears m^{-2} for all varieties was affected by N treatment and variety ($P<0.05$); and the interaction was significant in TT07 ($P<0.05$). Averaging across N treatments, in TT06 the varieties differed in the range 486 to 531, and in TT07 in the range 373 to 437. The two seasons showed different varietal patterns; however, in both years Claire had the highest ear population density. In TT07, as with the yield results, the effect of variety and the interaction was due to a difference between the response of Atlanta and the other three varieties. All varieties had a similar ear population at the zero and optimum N treatments (range 310-360, and 427-457, respectively), however at the supra-optimal N treatments the ear population of the three varieties (Istabraq, Claire and Savannah) continued to increase slightly whereas the ear population of Atlanta decreased sharply. Regression analysis fitted a curve to the data in LC07, parallel curves to the data for each variety in TT06, and separate curves to Atlanta, and to the other three varieties in TT07.

4.4.2.2 Grains per ear

The grains ear^{-1} (GPE) for Istabraq was affected by N treatment in TT06 ($P<0.01$), with a trend for higher GPE with N supply in TT07 and LC07 (Figure 4.6). Averaging across N treatments, GPE was higher in LC07 (53.9) than TT07 (52.5) and TT06 (45.1). The N zero-trt always had fewest GPE and there was generally a positive response to N supply, particularly in TT06. Generally LC07 had the highest GPE for each of the zero, optimum and maximum N treatments, with on average at the N opt-trt 6 more grains ear^{-1} than TT06, and a slightly higher number compared with TT07.

In the Terrington experiments, GPE was affected by N treatment ($P<0.05$) and variety ($P<0.001$); there was no interaction in either experiment. Averaging across N treatments, in TT06 Atlanta had more grains ear^{-1} at 50.1, than Istabraq at 45.1, Claire at 42.1, and Savannah at 41.7. In TT06 and TT07, Atlanta again had the highest GPE (57.7) and Claire the lowest GPE (52.5). Again in TT07 the effect of variety was due to a difference between the response of Atlanta and the other three varieties. Atlanta had more grains ear^{-1} than the other three varieties at all N treatments (except 70 kg ha^{-1}), and especially at the supra-optimum N treatments. Parallel regression analysis fitted parallel curves for each variety in TT06, and separate curves to Atlanta and the other varieties in TT07.

4.4.2.3 Individual grain weight

The individual grain weight (GW; mg) for Istabraq was affected by N treatment in LC07 ($P<0.05$), but not in TT06 and TT07 (Figure 4.7). Averaging across N treatments, GW was highest in TT06 (44.0 mg), then LC07 (42.8 mg), and TT07 (38.6 mg). In each site-season grain weight decreased with N supply from the N zero-trt to the N max-trt; in LC07 in the range 43.4 to 41.5 mg, and showed a trend for decreasing grain weight with N supply in TT06 and TT07 in the ranges 46.0 to 42.4 mg and 41.2 to 37.5 mg, respectively.

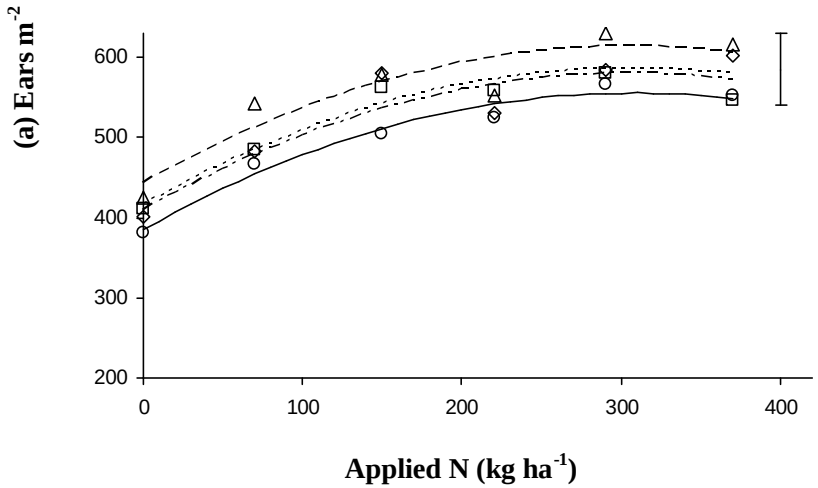
In the Terrington experiments, GW was affected by N treatment and variety ($P<0.01$), and the interaction was significant in TT07 ($P<0.01$). Averaging across N treatments, in

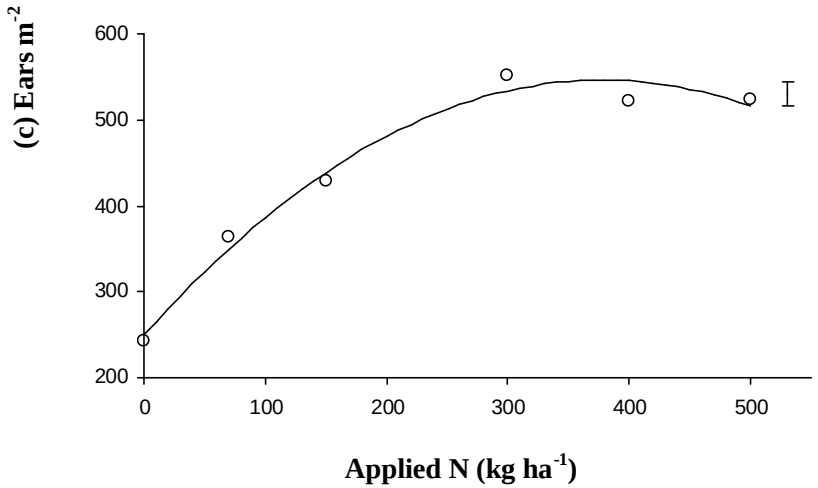
TT06 Savannah had the heaviest grains (47.4 mg), then Istabraq (44.0 mg), Claire (42.2 mg), and Atlanta (41.2 mg); cf. TT07 where the grain weight was lower than the previous season but showed a similar varietal pattern with Savannah highest (40.7 mg), Istabraq (38.6 mg), Atlanta (37.2 mg) and Claire (36.2 mg). Parallel regression analysis fitted a negative linear line to the data in LC07, and parallel negative linear lines to each variety in TT06. In TT07 each variety responded differently to N supply, and separate curves were fitted to each variety.

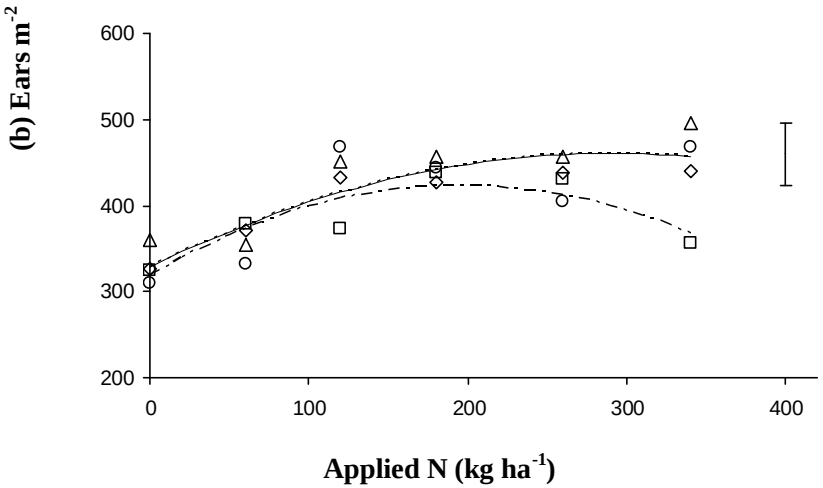
4.4.2.4 Yield components summary

For Istabraq in each experiment the yield increased with N supply while the grain weight decreased. The increase in yield was mainly a consequence of more ears m^{-2} which, averaged across all three experiments, increased by 38% between the zero and optimum N treatments; cf. GPE increased by 13%. The crops in both TT06 and LC07 produced more than 500 ears m^{-2} at the N opt-trt providing for sufficient sink capacity to accommodate a high-yielding crop close to the maximum attainable yield (i.e. best yield achieved through skilful use of the best available technology) for the respective sites. In TT07, the lower ears m^{-2} may have limited actual yields to slightly below the attainable yield for the site.

There was a negative relationship between grains m^{-2} and grain weight for Istabraq in each experiment, as increased competition for assimilates (source) amongst grains likely limited grain weight. However, overall for Istabraq the decrease in grain weight between the zero and optimum N treatments was small (5%), whilst the decrease between the optimum and maximum N treatments experiments was smaller still (2%). Averaging across N treatments, the high yield in LC07 was mainly the result of high GPE. In the Terrington experiments, Atlanta produced the highest yield response to N supply of the four varieties due to increased grains m^{-2} (although this lowered grain weight). In TT07 the N x variety interaction for yield was principally due a different response by Atlanta at the supra-optimal N treatments. At the N max-trt the ear population of Atlanta decreased sharply, and although the grains ear^{-1} was relatively high, grain weight was low causing low yield compared to the other three varieties.





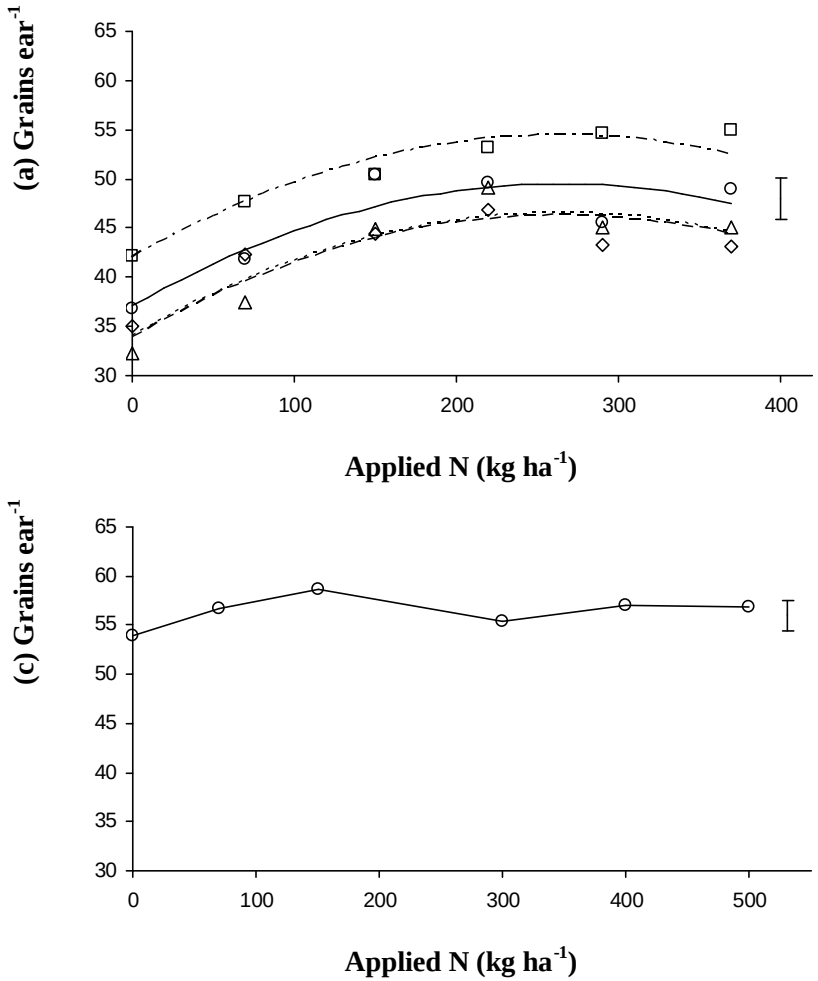


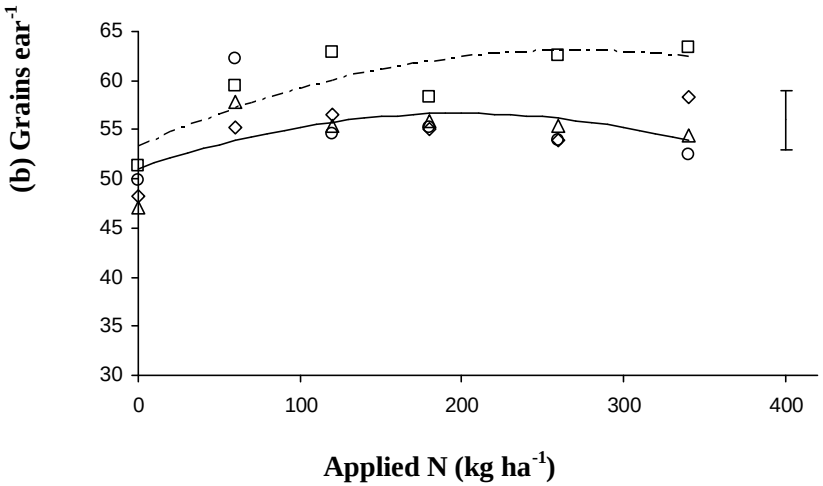
Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; in TT06 - Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------) with SED N x V bar (df = 36); in TT07 - Istabraq, Claire and Savannah (———) and Atlanta (— – —); with SED N x V bar (df = 36).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 25).

Figure 4.5 (a, b & c) Effect of applied N and variety on the ear population density in TT06, TT07 and LC07.



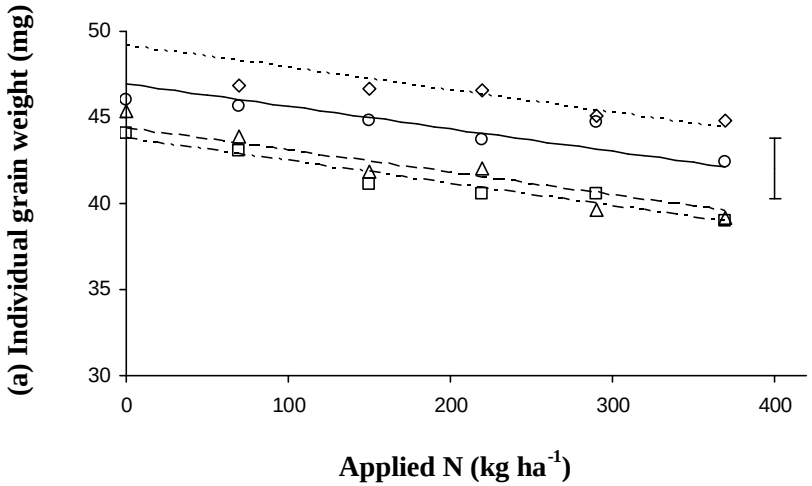


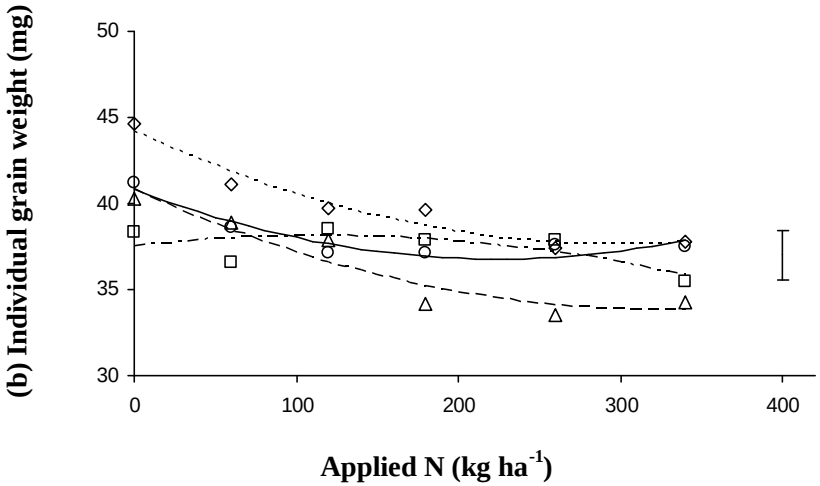
Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

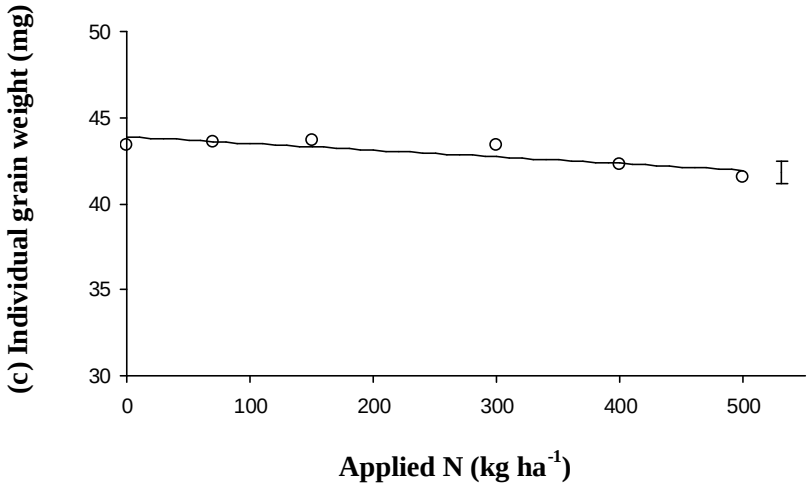
(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; in TT06 - Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------) with SED N x V bar (df = 36); in TT07 - Istabraq, Claire and Savannah (———) and Atlanta (— – —); with SED N x V bar (df = 36).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○), with SED N bar (df = 25).

Figure 4.6 (a, b & c) Effect of applied N and variety on the grains per ear in TT06, TT07 and LC07.







Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------); with SED N x V bar (df = 36 (TT06) and 36 (TT07)).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 25).

Figure 4.7 (a, b & c) Effect of applied N and variety on the individual grain weight in TT06, TT07 and LC07.

4.4.3 Responses of NUE and components to N and variety

Due to the constraints on the N analysis regime, dry matter samples at harvest were not submitted for N analysis for all treatment combinations. In each experiment N% data for Istabraq are available at all six N treatments, whilst in the Terrington experiments N% data are available for all varieties at 3 N treatments (zero, optimum and maximum). As a consequence, regression analysis describing the relationship between UPE and UTE and fertiliser N amount is performed for Istabraq data only. Data for the other three varieties are plotted on the same figures for the relevant N treatment levels.

4.4.3.1 N-use efficiency

The NUE for Istabraq decreased with N supply in all three site-seasons ($P<0.001$) (Figure 4.8). Averaging across N treatments, LC07 had higher NUE (51.4) than TT06 and TT07 (both 40.3). In LC07 the NUE ranged from 95 at the N zero-trt to 22 at the N max-trt; cf. TT06 at 62 to 24, and TT07 at 67 to 21, respectively. LC07 had the highest NUE at the N zero-trt compared with TT06 and TT07 whilst all three experiments had similar NUE at the optimum and maximum N treatments (range 33-37 and 21-24, respectively).

In both the Terrington experiments the NUE was affected by N treatment ($P<0.001$), and by variety in TT06 ($P<0.001$); there was no interaction in either experiment. Averaging across N treatments, in TT06 Atlanta had the highest NUE (45.3), then Savannah (43.1), Istabraq (40.3), and Claire (39.2); cf. TT07 with varieties in the range 40.2 to 43.2. Regression analysis fitted a curve to the data in LC07, parallel curves to the data for each variety in TT06, and a single curve for all varieties in TT07.

4.4.3.2 N-uptake efficiency

The UPE for Istabraq was affected by N treatment in all three experiments ($P<0.01$) (Figure 4.9). UPE was always highest at the N zero-trt, and decreased with increasing applied N. Averaging across N treatments, all three site-seasons had similar UPE at 1.07 (LC07), 1.05 (TT06) and 1.00 (TT07). At the N zero-trt UPE was higher at Lincoln

(1.60) than Terrington (range 1.31-1.40) possibly due to increased N supply associated with high soil N mineralization, but similar at the optimum and maximum N treatments (in the range 0.94-1.08 and 0.61-0.77, respectively). The regression analysis of UPE on applied N for Istabraq fitted a negative linear regression in TT06 and TT07, and a curve in LC07.

In each of the Terrington experiments, the UPE for all varieties was affected by N treatment ($P<0.05$), and by variety in TT07 ($P<0.05$); there was no interaction in either experiment. Averaging across N treatments, in TT06 varieties were in the range 1.04 to 1.08; whereas in TT07 differences were statistically significant with Claire having the highest UPE at 1.18, then Savannah at 1.13, Atlanta at 1.10, and Istabraq at 1.00. The varietal effect was therefore different between the two experiments.

4.4.3.3 N-utilisation efficiency

The UTE for Istabraq was affected by N treatment in all three experiments ($P<0.001$) (Figure 4.10); UTE decreased with N supply. Averaging across N treatments, LC07 had the highest UTE at 43.3, then TT07 at 38.4, and TT06 at 37.1. In LC07 the UTE decreased from 58 at the N zero-trt to 33 at the N max-trt; cf. TT07 at 48 to 35, and TT06 at 48 to 31, respectively. Thus LC07 had the highest UTE at the zero and optimum N treatments, but all experiments had similar UTE at the maximum N treatment (range 31-35). Overall UTE decreased by 32% between the zero and optimum N treatments (i.e. the amount of grain produced per unit of canopy N was reduced by about a third), whereas UTE was relatively unchanged (decreased by 5%) between the optimum and maximum N treatments.

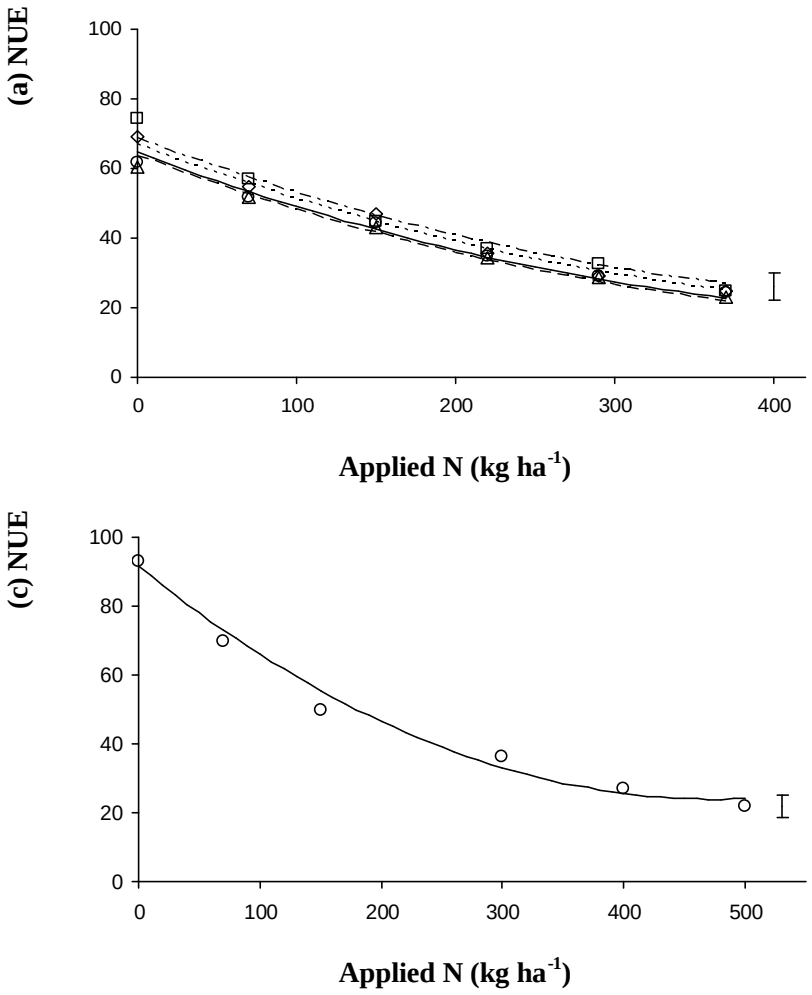
In each of the Terrington experiments UTE was affected by N treatment and variety ($P<0.01$); there was no interaction in either experiment. Averaging across N treatments, in TT06 Atlanta had the highest UTE 40.1, then Savannah at 38.7, Istabraq at 37.1, and Claire had the lowest at 36.6; cf. TT07 which had a different varietal pattern with Istabraq at 38.4, then Savannah at 36.3, Atlanta at 34.7, and Claire at 33.5. Although the

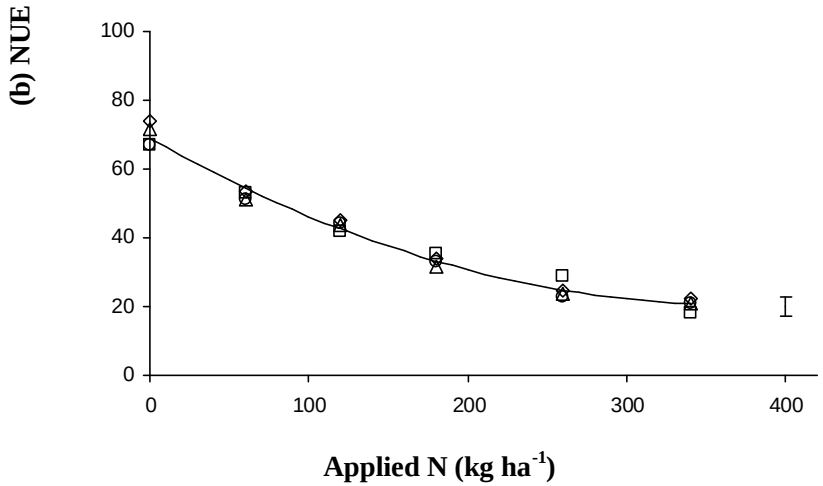
varietal pattern was different between the two experiments, Claire had the lowest UTE in both seasons.

4.4.3.4 NUE and NUE components summary

Averaged across all three experiments, NUE was reduced by 52% between the zero and optimum N treatments (i.e. the amount of grain produced per unit of N available was halved) and this was the result of an equal reduction in both the UPE and UTE (by 30 and 32%, respectively). Therefore, at the sub-optimal and optimal N treatments the association between decreasing NUE and N supply was the result of effects of both UPE and UTE. Overall NUE declined by 36% between the optimum and maximum N treatment. This was associated with a decrease in UPE (by 32%) whilst UTE declined only slightly (by 5%) and was apparently not associated with N supply. Therefore, the NUE at supra-optimal N treatments was determined more by ability to take up N than by ability to utilise it.

In the Terrington experiments, there was an effect of variety on NUE in TT06, on UPE in TT07, and on UTE in TT06 and TT07. Regression analysis for NUE in TT06 fitted parallel curves, i.e. varieties responded similarly to N supply, and a single curve was fitted to all varieties in TT07. There was a small varietal effect on UPE in TT07. There was a strong varietal effect on UTE in both experiments, and Claire consistently had the lowest UTE although other variety differences were not consistent across seasons. Regression analysis for UPE and UTE for Istabraq fitted similar responses for all varieties in both experiments, and Istabraq can therefore be assumed to demonstrate the physiological trends for all varieties.



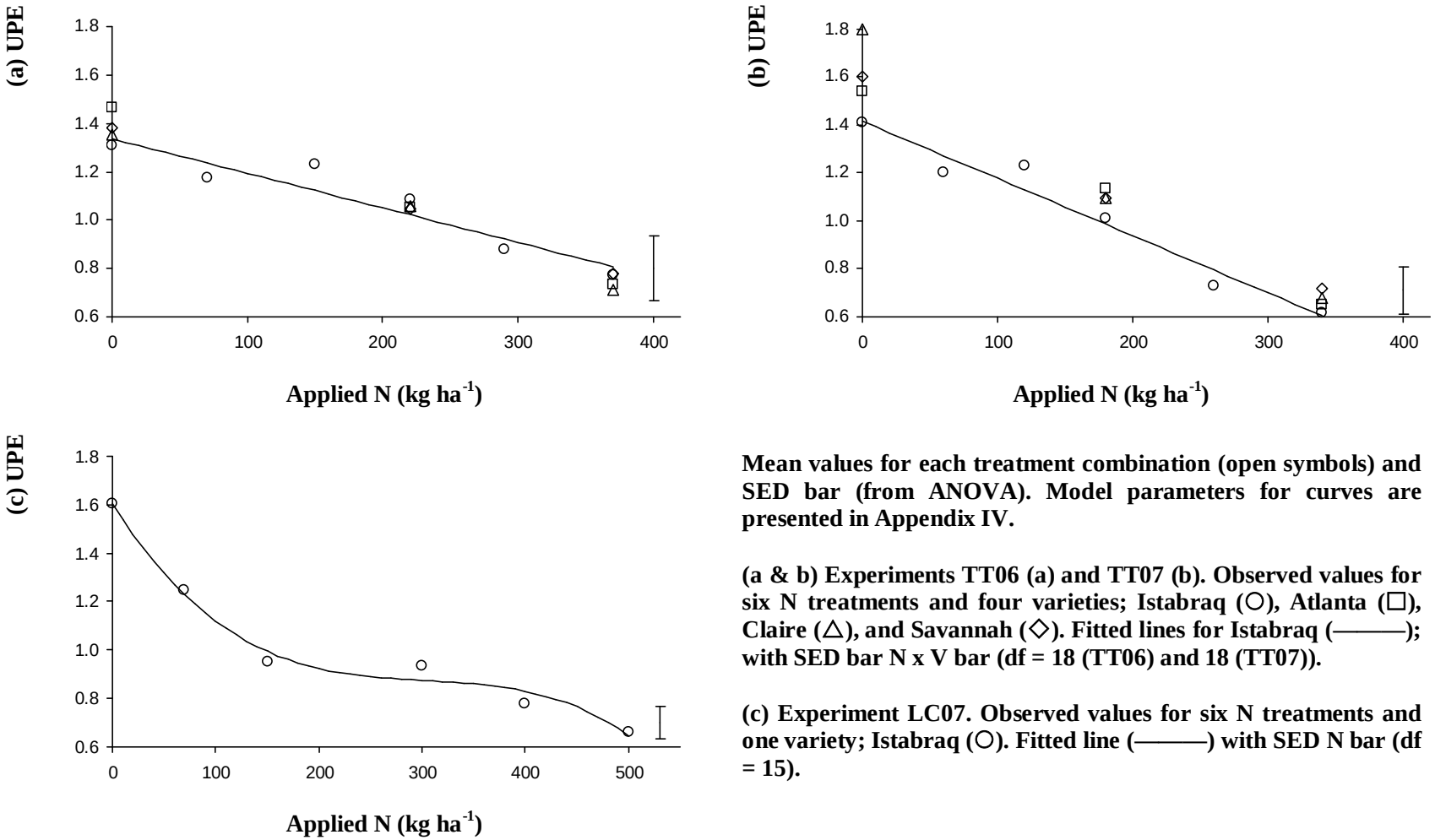


Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; in TT06 - Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------); in TT07 – all varieties (———); with SED bar N x V bar (df = 36 (TT06) and 36 (TT07)).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 25).

Figure 4.8 (a, b & c) Effect of applied N and variety on the N-use efficiency (NUE) in TT06, TT07 and LC07.

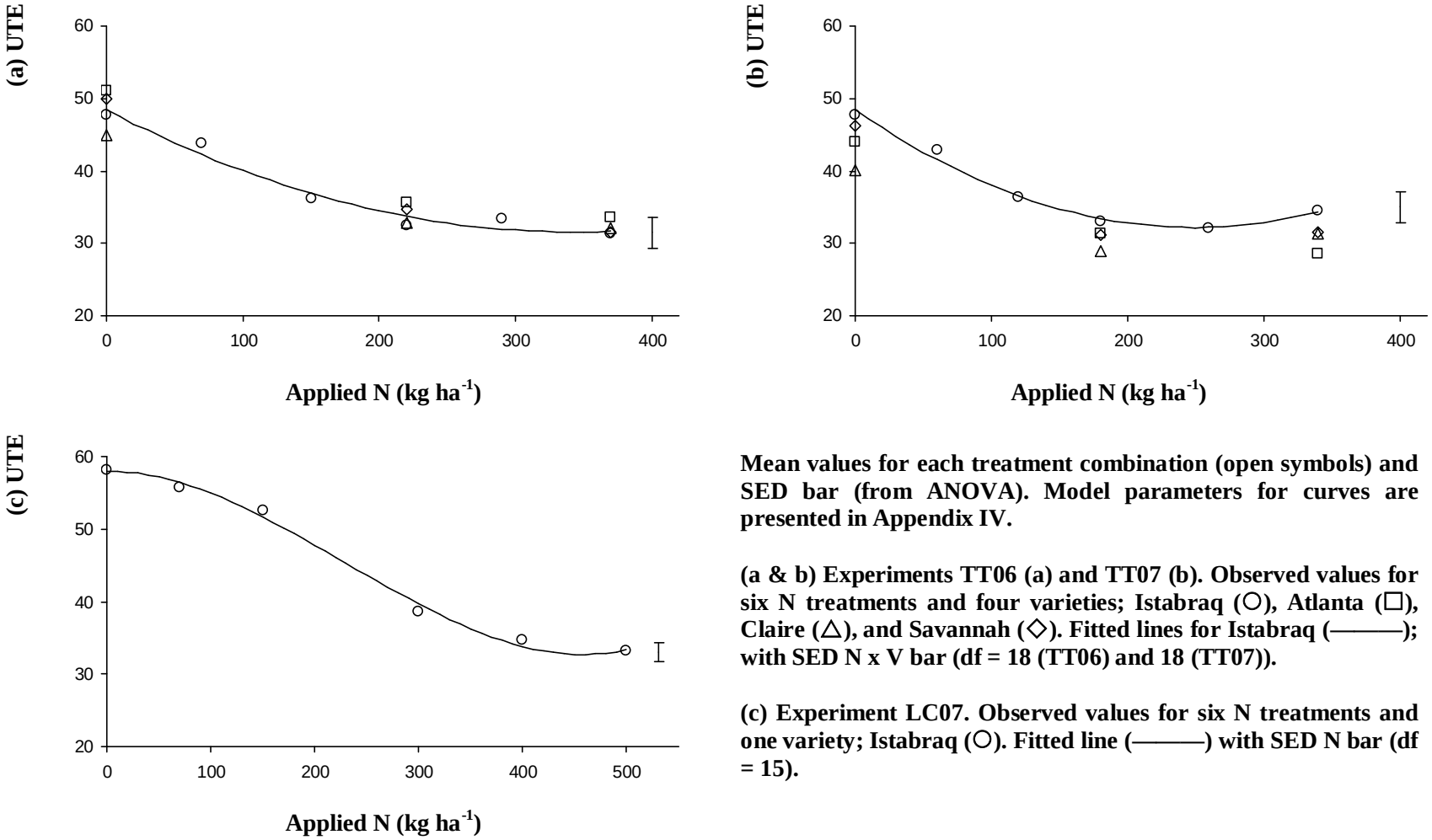


Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for Istabraq (———); with SED bar N x V bar (df = 18 (TT06) and 18 (TT07)).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

Figure 4.9 (a, b & c) Effect of applied N and variety on the N-uptake efficiency (UPE) in TT06, TT07 and LC07.



Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for Istabraq (———); with SED N x V bar (df = 18 (TT06) and 18 (TT07)).

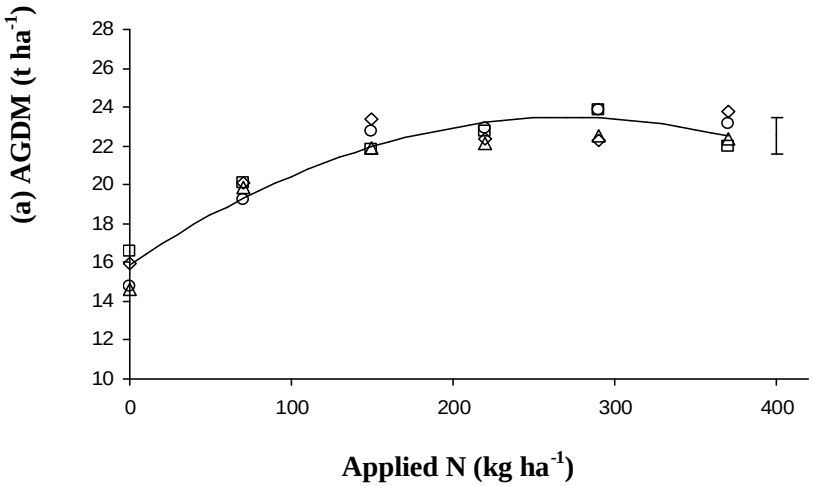
(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

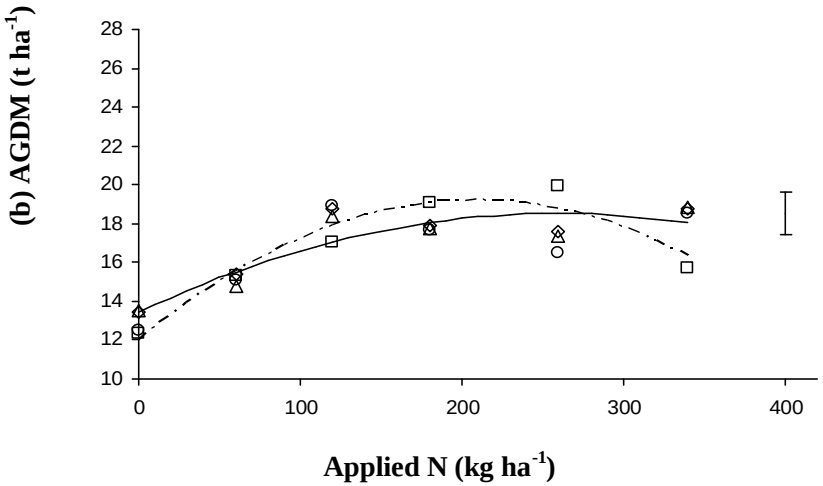
Figure 4.10 (a, b & c) Effect of applied N and variety on the N-utilisation efficiency (UTE) in TT06, TT07 and LC07.

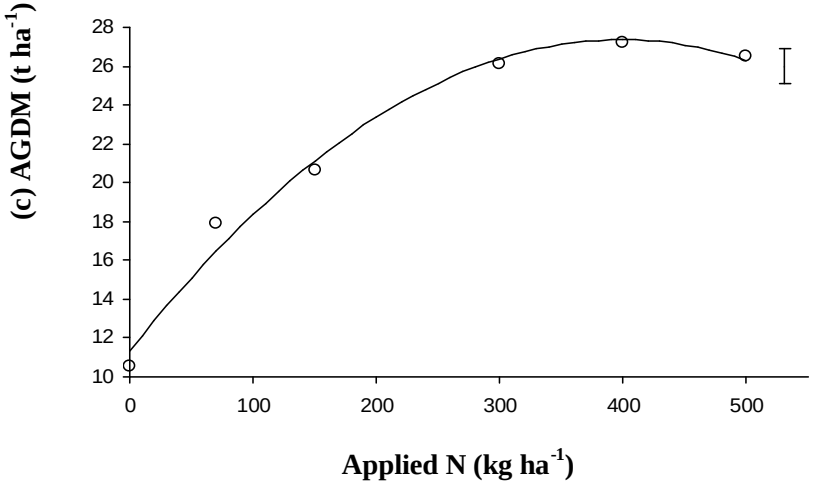
4.4.4 Response of above-ground dry mass to N and variety

Above-ground dry mass (AGDM; t ha^{-1}) for Istabraq was affected by N treatment in all three experiments ($P<0.001$) (Figure 4.11). Averaging across N treatments, LC07 produced the highest AGDM (21.1 t ha^{-1}), then TT06 (20.3 t ha^{-1}), and TT07 produced the lowest (16.2 t ha^{-1}). LC07 had the lowest AGDM at the N zero-trt at 10.5 t ha^{-1}; cf. TT06 at 14.8 t ha^{-1}, and TT07 at 12.5 t ha^{-1}, but had the highest AGDM at the N opt-trt at 26.1 t ha^{-1}; cf. TT06 at 22.9 t ha^{-1}, and TT07 at 17.6 t ha^{-1}; thereafter remaining unchanged at the N max-trt. LC07 therefore had the greatest response to N supply of the three experiments with a range of 15.6 t ha^{-1} between N treatments; cf. TT06 at 8.1 t ha^{-1}, and TT07 at 5.1 t ha^{-1}.

In both the Terrington experiments, AGDM was affected by N treatment ($P<0.001$), but not by variety; and the interaction was not significant in either experiment. Averaging across N treatments, AGDM in TT06 was in the range of 19.7-20.7 t ha^{-1}, and in TT07 in the range 15.7-16.7 t ha^{-1}. However, from the regression analysis in TT07 Atlanta data showed a different response to N treatment to the other three varieties for the same reasons described in the yield analysis. Atlanta showed a similar AGDM to the other three varieties at the zero and optimum N treatments (12.4 and 19.1 t ha^{-1}, respectively), but decreased sharply at the N max-trt to 15.8 t ha^{-1}. Again, the differences in the AGDM response to N between varieties were likely related to effects of lodging and poor establishment for Atlanta at the supra-optimal N treatments. Parallel regression analysis fitted a curve to the data in LC07, and a single curve to all varieties in TT06, and in TT07 a single curve to three varieties Istabraq, Claire and Savannah, and a separate curve to Atlanta (all curve parameters varying).







Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; in TT06 – all varieties (———) with SED N x V bar (df = 36); in TT07 - Istabraq, Claire and Savannah (———) and Atlanta (— – —); with SED N x V bar (df = 36).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 25).

Figure 4.11 (a, b & c) Effect of applied N and variety on the above-ground DM (AGDM) in TT06, TT07 and LC07.

4.4.5 Response of Crop N uptake to N and variety

As a consequence of the constraints on the N analysis regime only Istabraq data can be fitted to the 'bi-linear' model (as described in 3.7.3), describing the relationship between AGN and fertiliser N amount. Data for the other three varieties are plotted on the same figures for the relevant N treatment levels.

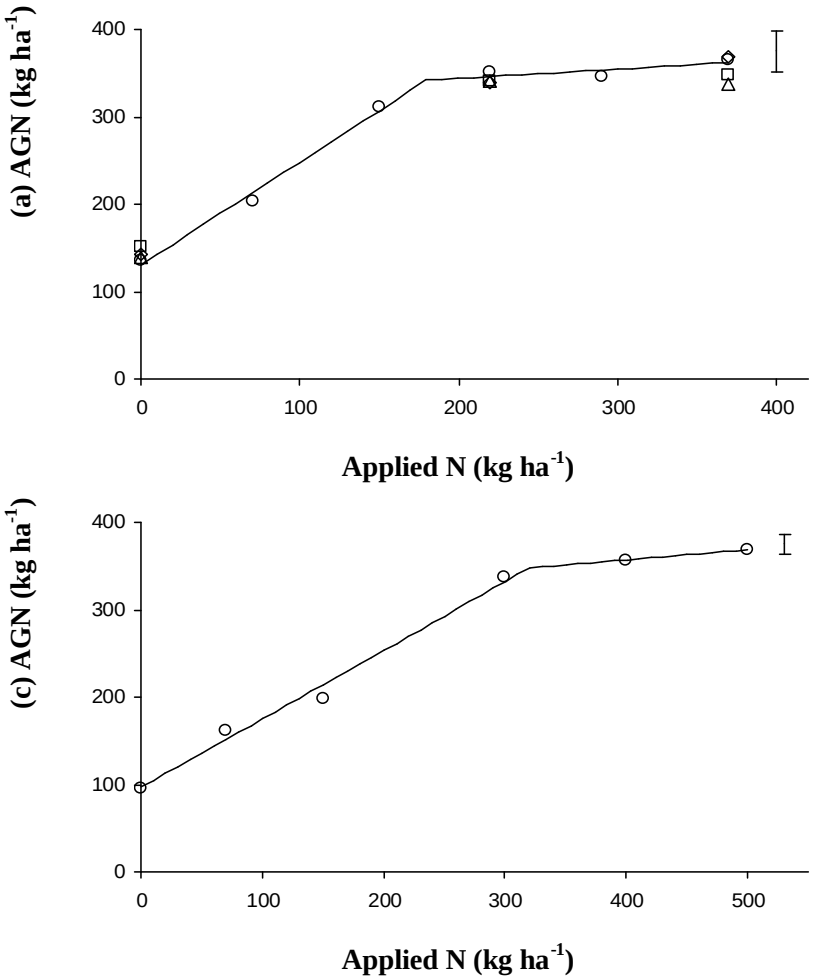
The AGN for Istabraq was affected by N treatment in all three experiments ($P<0.001$) (Figure 4.12). Averaging across N treatments, TT06 had the highest AGN (284 kg ha^{-1}), then LC07 (265 kg ha^{-1}), and TT07 (224 kg ha^{-1}). In each experiment AGN increased with N supply to the N opt-trt then increased more slowly. The Terrington experiments had a higher unfertilised AGN (by an average of 41 kg ha^{-1}) than Lincoln, associated with higher soil mineral N at Terrington. However, the AGN for Istabraq at TT07 was lower at both the N opt-trt and the N max-trt (275 and 264 kg ha^{-1}, respectively); than TT06 and LC07 (351 and 366 kg ha^{-1}, and 331 and 369 kg ha^{-1}, respectively). There was no effect of variety on AGN in either of the Terrington experiments; averaging across N treatments, AGN in TT06 varied in the range 273 to 284 kg ha^{-1}, and in TT07 in the range 224 to 254 kg ha^{-1}.

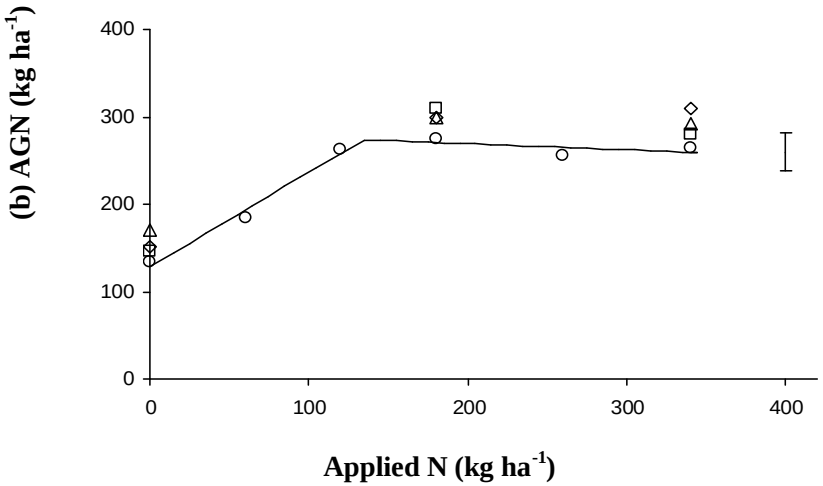
Fitting the bi-linear model to Istabraq data gave the breakpoint and slopes of phase 1 (before the breakpoint) and phase 2 (after the breakpoint) (Table 4-5). Model fitting in TT06 and TT07 gave a breakpoints at 180 kg ha^{-1} and 135 kg ha^{-1} of applied N respectively, about 40 to 45 kg ha^{-1} less than the N opt-trt applied N. The analysis showed a much higher rate of N uptake with N supply before the breakpoint, as the slope for phase 1 increased AGN more rapidly than fertiliser N supply (i.e. in TT06 AGN increased by 1.37 kg for every 1 kg ha^{-1} fertiliser N applied). After the breakpoint the slope for phase 2 showed there was only a small increase in AGN with increasing applied N at the optimum and supra-optimum N treatments in TT06, and in TT07 there was actually a small decrease in AGN with increasing applied N; AGN decreased by 0.07 kg for every 1 kg ha^{-1} of fertiliser N applied.

Model fitting in LC07 gave the breakpoint at 320 kg ha^{-1} of applied N, 20 kg ha^{-1} more than the N opt-trt. The slope of phase 1 showed that AGN increased by 0.79 kg per kg N applied. After the breakpoint there was only a small increase in AGN with increasing applied N at the supra-optimum N treatments. The fitted models show a broadly similar AGN at the breakpoint for Istabraq in all three experiments in the range 274 to 348 kg ha^{-1}. However, the amount of applied N at the breakpoint differed significantly between site-seasons in the range 135 to 320 kg ha^{-1} of applied N, associated with lower soil mineral N at sowing, possibly lower N mineralisation during the growing season, and higher yield potential at Lincoln than Terrington.

Table 4-5 Model parameters for fitted bi-linear curve for the relationship between above-ground N and fertiliser N applied for Istabraq in TT06, TT07 and LC07.

Exp.	Slope 1	Slope 2	Breakpoint (SE)		% Variance accounted (SE)
			Applied N	AGN	
TT06	1.178	0.105	179.7 (24.9)	342.0 (21.4)	90.9 (27.6)
TT07	1.075	-0.070	134.9 (21.5)	273.9 (17.7)	80.0 (26.1)
LC07	0.7855	0.116	320.4 (27.2)	348.0 (19.2)	97.7 (16.6)





Mean values for each treatment combination (open symbols) with fitted 'bi-linear' model to Istabraq data and SED bar (from ANOVA). Model parameters for curves are presented in Table 4-5.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments for Istabraq (○), and 3 N treatments for Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for Istabraq (———) with SED N x V bar (df = 18 (TT06) and 18 (TT07)).

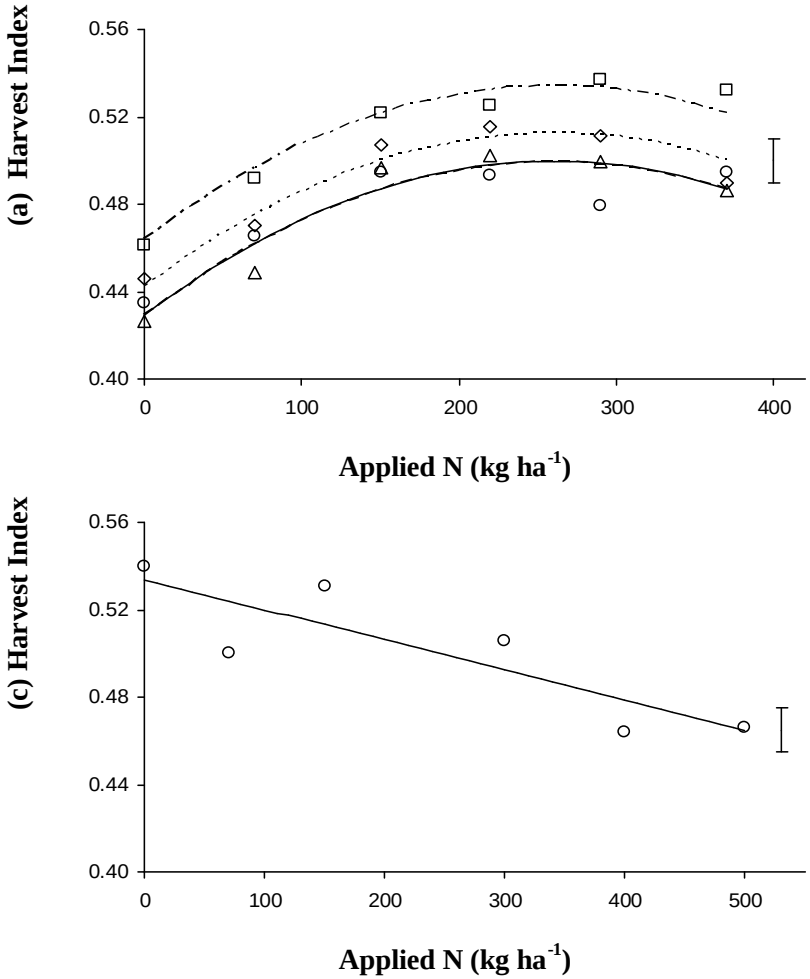
(c) Experiment LC07. Data for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

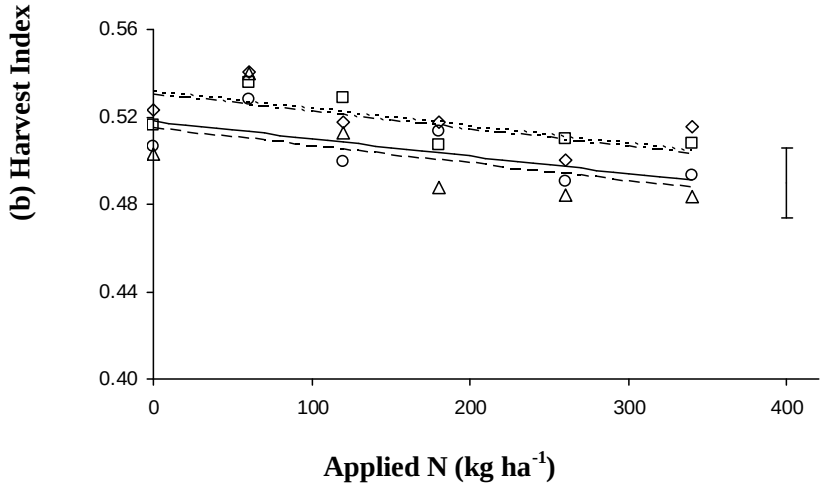
Figure 4.12 (a, b & c) Effect of applied N and variety on the above-ground N (AGN) in TT06, TT07 and LC07.

4.4.6 Response of Harvest Index to N and variety

Harvest index (HI; Grain DM / AGDM) for Istabraq was affected by N treatment in TT06 and LC07 ($P<0.01$), but not in TT07 (Figure 4.13). Averaging across N treatments, LC07 and TT07 had the highest HI (both 0.50), and TT06 had the lowest HI (0.47). However the response of HI to N supply differed between site-seasons; in TT06 HI increased from 0.43 at the N zero-trt to 0.49 at the N max-trt and was positively correlated with fertiliser N amount ($r^2 = 0.96$); whereas in LC07 HI decreased with N supply from 0.54 to 0.47 respectively, and was negatively correlated with fertiliser N amount ($r^2 = -0.74$); values at TT07 remained unchanged at 0.51 to 0.49.

In both the Terrington experiments, HI was affected by N treatment and variety ($P<0.05$); the interaction was not significant in either experiment. Averaging across N treatments, in TT06 Atlanta had the highest HI (0.51), then Savannah (0.48), Istabraq and Claire (both 0.47); cf. TT07 which showed a similar varietal pattern with Savannah the highest HI (0.52), then Atlanta (0.51), Istabraq (0.50), and Claire (0.49). Regression analysis fitted a negative linear regression to the data ($r^2 = 0.54$) in LC07, and TT07 (with separate lines for varieties), and fitted parallel curves to the data in TT06 with the 'y' axis intercept different between varieties.





Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------) with SED N x V bar (df = 36 (TT06) and 36 (TT07)).

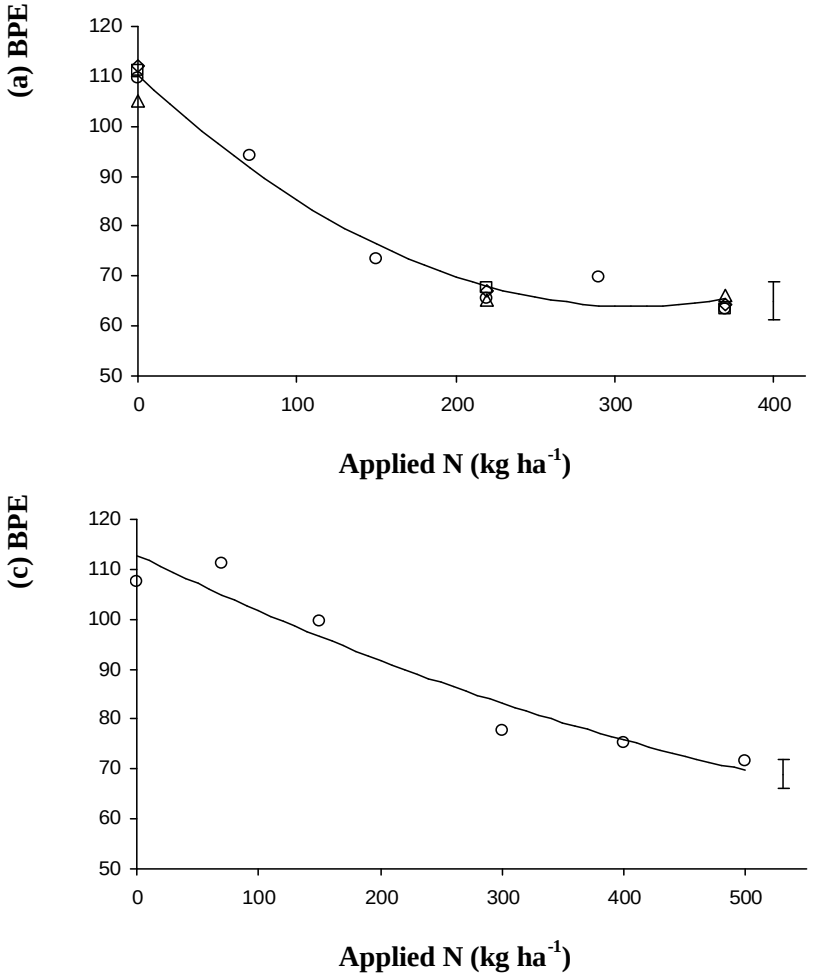
(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 25).

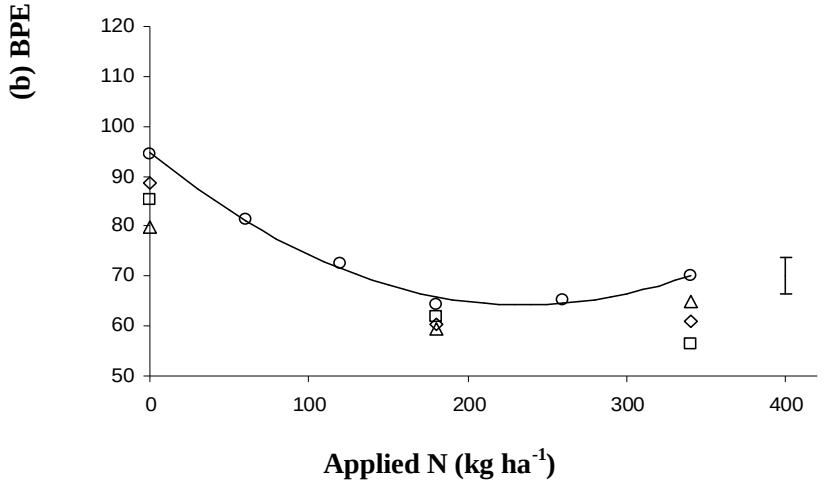
Figure 4.13 (a, b & c) Effect of applied N and variety on the harvest index in TT06, TT07 and LC07.

4.4.7 Response of Biomass Production Efficiency to N and variety

'Biomass production efficiency' (BPE) (i.e. AGDM / AGN; g DM g N^{-1}) for Istabraq was affected by N treatment in all three experiments ($P<0.001$) (Figure 4.14). Averaging across N treatments, LC07 had the highest BPE (85.6), then TT06 (79.5), and TT07 had the lowest BPE (76.3). On average, the crop at Lincoln was more productive than Terrington in producing AGDM from crop N, possibly due to the higher solar radiation availability. In all three experiments, BPE decreased with N supply, mainly between the zero and optimum N treatments thereafter decreasing only slowly at the supra-optimum N treatments. In LC07 BPE decreased from 108 at the N zero-trt to 72 at N max-trt; cf. TT06 from 110 to 63, and TT07 from 94 to 70, respectively. All three experiments showed a similar BPE at the N zero-trt (range 94-110), but TT06 gave the largest response to N supply to the N opt-trt at -44; cf. TT07 and LC07 both at -30. Regression analysis for the data for Istabraq only fitted curves to all three experiments (although in LC07 a negative linear regression fitted slightly better).

In the Terrington experiments, the BPE was affected by N treatment in TT06 and TT07 ($P<0.001$), and by variety in TT07 ($P<0.01$) but not in TT06; there was no interaction in either experiment. Averaging across N treatments in TT06 BPE for all varieties were in the range 78.9 to 81.2; cf. TT07 which showed significant varietal difference with Istabraq the highest (76.3), then Savannah (70.0), Claire (68.0), and Atlanta (67.8). Therefore the varietal effect was different between seasons.





Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Data for six N treatments and four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for Istabraq (———); with SED N x V bar (df = 18 (TT06) and 18 (TT07)).

(c) Experiment LC07. Data for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

Figure 4.14 (a, b and c) Effect of applied N and variety on the biomass production efficiency (BPE) in TT06, TT07 and LC07.

4.5 DISCUSSION

This discussion of results considers the response to N supply by Istabraq in the three site-seasons and the variety responses in the Terrington experiments; of the grain yield, numerical and physiological components of yield, N optima, and NUE and its components, with a comparison of the UK and NZ environments. The conclusion discusses the hypotheses addressed in this chapter in relation to the results.

4.5.1 Grain yield and N optima

Yields increased significantly with N supply in all three experiments; yields were almost doubled by the application of fertiliser N, but showed a diminishing response to increasing amounts of applied N. Averaging across experiments, about half the yield (57%) of the N opt-trt was achieved at the N zero-trt, and 90% of the yield at the N opt-trt was achieved at about half (47%) of the N applied at the N opt-trt. These findings are consistent with previous N response studies in winter wheat (Scott *et al.*, 1994; Stokes *et al.*, 1997; Foulkes *et al.*, 1998; Sylvester-Bradley and Kindred, 2009). Yields in experiments were higher than average combine harvested 'on-farm' yield in N.W. Europe which are typically about 4 to 5 t ha^{-1} and 8 to 9 t ha^{-1} for unfertilised and well fertilised crops respectively (Sylvester-Bradley *et al.*, 1997; Austin, 1999; Sylvester-Bradley *et al.*, 2005). This was due both to hand harvesting of grain (raised yield by around 26%) and good, well managed growing conditions.

The unfertilised yield was the same in the two Terrington experiments but lower at Lincoln associated with the lower SMN of approximately 41 kg ha^{-1}, whilst the optimally fertilised yield was highest in LC07, then TT06, and lowest in TT07; the yield response to N supply was therefore largest in LC07. Results at the non N-limiting treatments indicated that Lincoln was a higher yielding environment than Terrington, associated with both the higher incident solar radiation and longer post-anthesis period (N opt-trt at 75 days and 57 days (averaged over TT06 and TT07), respectively. Comparing between the two seasons at Terrington at the N opt-trt showed that TT06 was higher yielding (by

2.6 t ha^{-1}, or +20%) which was associated with TT06 experiencing relatively sunnier (higher incident solar radiation) conditions from the end of stem extension to harvest compared with TT07 which experienced wet and dull conditions, likely reducing yield potential through reduced floret survival in spikelets, accumulation of stem soluble carbohydrates, and mobilisation of biomass to the grain during grain-filling.

Yield response to fertiliser N was fitted to LEXP curves in all three experiments. The rate of the exponential increase in yield with applied N as indicated by the shape of the curve is generally quite stable (George, 1984; determined from the r parameter from the LEXP function) and was similar in TT06 and LC07 (0.994 and 0.996 respectively), but shallower in TT07 (0.981) due to relatively low yields at the optimum and supra-optimum treatments. The estimated N and Y optima for Istabraq showed large cross site-season differences, and Sylvester-Bradley *et al*. (1984) noted that N opt vary widely between trials and should be treated with reserve, particularly where the shape of the curve is very flat as in TT07. N opt was higher in Lincoln than in Terrington related to differences in SMN and yield potential. However, when the difference in applied N between the N opt at LC07 and TT06 was multiplied by the AFR at the N opt-trt (0.80 this study overall mean; Scott *et al*., 1994) then the adjusted 'N optima' were similar. The low N opt for TT07 compared with TT06 and LC07 was caused by the smaller yield potential of this crop response to N supply reducing the applied N required to reach the optimum.

In TT06 results showed that all varieties had the same response to N supply but showed different yield potentials, averaging across N treatments, Atlanta had the highest yield, then Savannah, Istabraq and Claire. The N opt was the same for all varieties at 236 kg ha^{-1} of applied N, within the varietal range of previous studies on modern cultivars in UK conditions (Sylvester-Bradley, 1993; Sylvester-Bradley *et al*., 2001; Foulkes *et al*., 2005), but the yield at the N opt differed (range 13.2-14.4 t ha^{-1}). There was no varietal effect on yield in TT07, although there was a significant interaction, where the N opt and Y opt were the same for Claire, Istabraq and Savannah but not for Atlanta. At the high N rates in TT07 negative effects on yield for all varieties were apparent through lodging/leaning,

but Atlanta had lowest yield at the N max-trt due to low establishment. At the N opt-trt both experiments had the same varietal pattern for yield; Atlanta had the highest yield, then Savannah, Istabraq and Claire. This indicates genetic determination of yield potential, and predicts a varietal difference in NUE. However, the genetic range in yields was relatively small which was partly related to the similar dates of release (range 1998-2004), height and flowering time, and end-use for the four varieties.

4.5.2 Basis of yield response to N: numerical components

Yield can be expressed as a product of grains m^{-2} (i.e. ears m^{-2} x grains ear^{-1}) and grain weight. The number of grains m^{-2} determines the 'sink size' of the crop, and is typically more closely related to yield under optimal conditions (Satorre and Slafer, 1999; Shearman *et al*., 2005) than grain weight (Fischer, 1985; Savin and Slafer, 1991). The three components are developed sequentially during the development of the crop; first ears m^{-2}, then grains ear^{-2}, and finally grain weight. Among the yield components, the grains m^{-2} was observed to be the best indicator of response to N application. However, yield components frequently mutually compensate and high yields are often attainable by diametrically opposite routes.

Results showed that the increase in yield with N supply was due to an increase in both ears m^{-2} and grains ear^{-1}, whilst grain weight decreased. The negative relationship between grains m^{-2} and individual grain weight is commonly observed (Slafer *et al*., 1996) as the sink size capacity exceeds the source supply limiting the grain yield and additional grains are located in more distal florets and/or spikelets with lower grain weight potential. Averaging across experiments between the zero and optimum N treatments, ears m^{-2} increased (69%), grains ear^{-1} increased (16%), and grain weight decreased (5%). Averaging across experiments, grains m^{-2} increased from 14,186 at the N zero-trt to 26,990 at the N opt-trt; and thus at the N opt-trt all three experiments had sufficient grain sink size to accommodate a high yielding crop, at 30,457 (LC07), 25,786 (TT06), and 24,449 (TT07) grains m^{-2}.

Ears m^{-2} typically shows the greatest response to N supply (Blacklow and Incoll, 1981), and is the product of plants m^{-2} and ears $plant^{-1}$. Establishment (plants m^{-2}) is a function of the seed rate and quality and over winter survival, and was not found to be significantly different between site seasons. The number of ears $plant^{-1}$ is determined by tiller production (to GS31) and tiller survival (between GS31 and harvest) which are both susceptible to agronomic influences, particularly N availability. Around 3 to 5 tillers were produced per plant by GS31 (i.e. producing a tiller population of around 600-1000 m^{-2}) and between 50 to 100% of these survived to anthesis depending on site season. High over winter N availability from SMN increased tiller production in the Terrington experiments, whilst deficiency during tillering may have reduced the tiller production in LC07 at the low N treatments. However early and/or high fertiliser N application increased tiller survival between GS31 and anthesis (Spiertz *et al*., 1984) particularly in LC07 at the optimum and maximum N treatments. Excessive ear proliferation can occur in response to supra-optimal N applications and can reduce yield (Pearman *et al*., 1978), but was not observed in this study.

Grains ear^{-1} is determined by the product of spikelets ear^{-1} and fertile florets $spikelet^{-1}$ in an approximately 20 to 30 day period prior to anthesis (Fischer, 1985). There was a trend for increased grains ear^{-1} with N supply in all three experiments. High N availability presumably increased grains ear^{-1} through factors which increase photo-assimilate supply during that time, such as high leaf area index and a high leaf photosynthetic rate (Satorre and Slaffer, 1999). N deficiency during stem elongation, booting, and/or spike initiation, which corresponds to the rapid increase in N demand, reduces both vegetative development and ear formation causing reduced floret formation and increased floret abortion thereby reducing grains ear^{-1} (Frederick and Camberato, 1995), and N deficiency at flowering time may reduce seed setting (Satorre and Slaffer, 1999). Averaged across N treatments, grains ear^{-1} was highest in LC07 (due to high fertiliser N rates), then TT07 (possibly compensating for low ears m^{-2}), and lowest in TT06.

Finally, grain weight is determined during the grain filling period according to assimilate supply per grain (i.e. total assimilate supply divided by the number of grains). Grain

weight is normally the most conservative yield component (Gallagher *et al*., 1975) and is typically either not affected or decreases with N supply (Waddington *et al*., 1986; Siddique *et al*., 1989). All three experiments showed a decrease in grain weight with N supply although there was only a small effect on yield. Photo-assimilate production during grain fill affects grain weight given that 70 to 90% of the grain dry weight comes from photo-assimilate production during the grain filling period (Austin *et al*., 1977) and higher grain set per spikelet increases the number of grains competing for assimilates within a single vascular supply, thereby reducing assimilate supply per grain. In addition, higher grains set m^{-2} increases the proportion of grains in distal positions or on distal spikelets as a proportion of the total, and this reduces the potential weight per grain. A combination of these factors may partly explain the low grain weight in TT07 cf. TT06 and LC07.

There were varietal effects on all yield components in both Terrington experiments. Grains m^{-2} was the main driver for yield increase with N supply, while grain weight decreased in both experiments. The different yield potentials between varieties in TT06 were obtained by different combinations of grains m^{-2} and grain weight. Averaging across N treatments, Atlanta produced the highest yield from the highest grains m^{-2} (25,604) despite having the lowest grain weight, whilst Savannah had the second highest yield with the highest grain weight but the lowest grains m^{-2} (21,623). In TT07 Savannah again had the highest grain weight and low grains m^{-2} (21,608) and achieved the highest yield, however Atlanta had the lowest yield as a result of both low grain weight and low grains m^{-2} (due to low ears^{-2} at the N max-trt despite having the highest grains ear^{-1}). This resulted in a significant N treatment x variety interaction for yield between Atlanta and the other three varieties. Overall comparing the yield components at the N opt-trt to explain the observed varietal pattern in yields; Atlanta, was the highest yielding variety with the highest grains m^{-2} as a consequence of high grains ear^{-1} yet had low or lowest grain weight in both seasons, whilst the other three varieties had higher grain weights but lower grains ear^{-2}.

4.5.3 Basis of yield response to N: physiological components

Yield can also be expressed as a product of biomass production (i.e. AGDM; where AGDM is the product of radiation interception and RUE) and the partitioning of biomass to the grain yield at harvest (i.e. HI) (Reynolds *et al*., 2005). Examination of AGDM and HI for Istabraq in the three experiments showed that the increase in yield with N supply up to the optimum N treatment mainly resulted from increased biomass production. Overall between the zero and optimum N treatments AGDM increased by 76% whilst HI increased by 2%, whereas between the optimum and maximum N treatments there was only a small increase in AGDM (3%) and HI decreased (4%). Averaging across N treatments, LC07 produced the highest yield from a high AGDM and a high HI. In TT06 yield was lower associated with lower AGDM and similar HI; and TT07 produced the lowest yield through low AGDM despite similar HI to LC07.

Biomass production generally paralleled that of yield, with the exceptions that the slope of the linear growth phase of the relationship between AGDM and applied N was higher, the plateau was reached at higher N rates, and at the supra-optimal N rates the yield decreased before the AGDM. Biomass production is linearly related to intercepted solar radiation (Monteith, 1994). Assuming neutral effects of N supply on RUE (which will be further commented on in chapter 5), the increase in growth with N supply can be attributed to increased interception of solar radiation by the canopy associated with more fertile shoots and/or more or larger leaves that stay green longer. Large cross site-season differences in biomass production seem likely to be mainly a consequence of differences in the amount of solar radiation intercepted. Averaging across N treatments, the crop at LC07 produced the most biomass by harvest, and received the most incident radiation, suggesting that biomass production in TT06 and TT07 may have been limited mainly by radiation availability. TT07 benefitted from brighter conditions between emergence and anthesis than the LTM and the high soil mineral N content encouraged the establishment of a high GAI at GS31; the same period for TT06 was duller on average, and LC07 had low SMN. TT07 had a longer post-anthesis period than TT06 giving longer duration for light interception (52 and 61 days respectively). However TT06 was dry during June and

July, and TT07 was significantly wetter than LTM during May, June and July; both factors possibly temporarily checking biomass growth.

HI response to N supply was inconsistent between the three experiments; TT06 increased, TT07 remained unchanged, and LC07 decreased. HI is generally found to decrease with N supply at higher N rates as yield declines before AGDM (Satorre and Slafer, 1999), although by less in proportion to the increase in biomass (Austin, 1982). Averaged across experiments, between the zero and optimum N treatments, HI increased slightly from 0.494 to 0.504, but decreased to 0.485 at the N max-trt. Observed values were generally similar between site-seasons, with HI at the N opt in the range 0.49 to 0.51; broadly consistent with published values for winter wheat in the UK at 0.47 (Sylvester-Bradley *et al*., 2008). It is not unusual for HI to vary between sites and seasons and has been observed in previous studies, e.g. Sylvester-Bradley *et al*. (1998) found HI varied from 0.35-0.55 for one variety of winter wheat studied over 18 site seasons.

With regard to the genetic differences in biomass and HI in the Terrington experiments; results in TT06 showed no varietal effect on AGDM but a varietal effect on HI. Therefore yield differences between varieties were due to HI; Atlanta had the highest yield with AGDM production similar to other varieties but partitioned more DM to the grain at harvest than the other three varieties (averaging across N treatments, HI at 0.51, and range 0.47-0.48, respectively). In TT07 the varietal difference in AGDM was between Atlanta and the other three varieties. Whilst there was a varietal difference in HI there was no N treatment x genotype effect for HI. The difference in yield response to N supply (significant N x variety interaction for yield) between Atlanta and the other three varieties was therefore due to lower AGDM production by Atlanta compared to the other varieties at high N supply rather than as a consequence of differences in HI (averaging across N treatments, HI at 0.51 and range 0.49-0.52, respectively).

4.5.4 NUE and NUE components

Because NUE is defined as the ratio of yield to N supplied from soil and fertiliser N, the diminishing response in yield to increasing amounts of applied N implies a decreasing efficiency in N use. However NUE is a complex trait, partly because the components (i.e. UPE and UTE) are not independent and that their relative contributions to genetic variation in NUE have been found to vary with environmental factors, including N availability (Ortiz-Monasterio *et al*., 1997; Le Gouis *et al*., 2000; Chamorro *et al*., 2002). Consistent with other studies (Simonis, 1987), results showed that the highest NUE was achieved at the N zero-trt and declined with N supply for Istabraq in all three experiments.

Overall NUE declined more between the zero and optimum N treatments than between the optimum and maximum N treatments (by 53% and 36%, respectively). Both Terrington experiments had similar NUE at the zero, optimum and maximum N treatments, although TT06 had higher UPE and lower UTE than TT07. Lincoln had a considerably higher NUE at the N zero-trt due to low SMN limiting uptake, but had similar NUE at both the optimum and maximum N treatments (averaging across N treatments for all three experiments at 33-37 and 21-24, respectively). For both Terrington experiments at the N opt-trt, the genetic ranges for NUE reported in the present study (34-37 in TT06, and 32-35 in TT07) were generally similar to those reported in the GREEN Grain data set growing under conditions at ADAS Terrington, UK in 2005/6 (Elite UK varieties, and some 'global' varieties) and 2006/7 (Elite UK varieties and some 'older' UK varieties) (30-44 in GGTT06, and 28-41 in GGTT07) (Kindred, personal communication).

Analysis of UPE and UTE patterns showed that their relative contribution to NUE differed with N treatment. Averaging across experiments between the zero and optimum N treatments the decrease in NUE was a consequence of approximately equal declines in UPE and UTE, whereas between the optimum and maximum N treatments the decrease in NUE was almost entirely due to the decline in UPE as N supply exceeded uptake capacity and UTE was relatively unchanged. These results indicate agreement with Le

Gouis *et al*. (2000) that UPE is a more important component of NUE at low N supply, whilst UTE is more important in determining NUE at high N supply, although Dhugga and Waines (1989) found UPE to be important at all N levels. Comparing between sites at the N opt-trt similar values for NUE were achieved through opposite routes; LC07 had low UPE but high UTE, whereas TT06 and TT07 had high UPE but low UTE. Comparison of the proportion of genetic variation in NUE accounted by UPE and UTE at N treatments showed only a trend for UTE explaining more of the variation at high N supply as reported by Ortiz-Monasterio *et al.* (1997), however analysis was limited by the small number of varieties in the present study.

NUE was affected by variety in TT06 related to the variety effect on yield. Since there was no varietal difference in N uptake and UPE, this variation in NUE was due to UTE. The contrasting yield response to N between Atlanta and the other three varieties in TT07 at the supra-optimal N treatments was associated with reduced UTE for Atlanta. Several studies have reported varietal differences in NUE in cereals (Ortiz-Monasterio *et al*., 1997). The majority of these studies have examined the relative changes in contributions UPE and UTE to improvements in NUE through breeding, concluding that genetic improvements have been mainly driven by the increase in UTE via HI. Early improvements in UTE were through reduced plant height and lodging which improved yields via improved HI (Ortiz *et al*., 1998), and Ortiz-Monasterio *et al*. (1997) showed that new wheat cultivars have an improved HI rather than BPE.

4.5.5 N-uptake efficiency

At the N zero-trt, all the crop N was derived from soil mineralization and/or fertiliser residues from the previous crop, and this available N was taken up and utilised efficiently by the crop leading to high NUE. In each experiment AGN was higher than SMN availability (tested during tillering pre-GS31) as additional mineralisation occurred during the growing season which increased UPE to >1. N uptake at the N zero-trt was therefore principally limited by ‘soil N supply’ rather than ‘crop N demand’, and the ability of the plant to recover N from the soil (i.e. UPE) can considered to be the

predominant component determining yield across environments rather than the ability to use N to produce grain (i.e. UTE).

In all three experiments UPE declined with fertiliser N supply indicating that fertiliser N is recovered less efficiently than SMN (typically at around 60% in UK climates; Scott *et al*., 1994). Averaging across experiments, UPE decreased slightly faster between the zero and optimum treatments than at the supra-optimum treatments. UPE at the N opt-trt differed between site seasons and was highest in TT06 and TT07 (which received the lowest N applications and had the highest soil mineralisation) and lowest in LC07 (which received the highest N applications and had the lowest soil mineralisation). The supra-optimal N treatments created a surplus of N availability and UPE for Istabraq at the N max-trt was in the range 0.61 to 0.77 across the three experiments, indicating that considerable quantities of available N were not taken up by the crop and were therefore potentially lost through leaching, denitrification or immobilisation. The amount of fertiliser N not taken up by the crop at the N max-trt can be estimated by 'excess fertiliser N = applied N – (AGN - SMN)' (assuming that the SMN is equal to the AGN at N zero-trt). Averaging across experiments the excess fertiliser N at the N max-trt was 157 kg ha^{-1} which indicates that almost all of the fertiliser applied above the optimum (i.e. 170 kg ha^{-1}) was not taken up by the crop, although excess fertiliser N differed between site seasons and was highest in LC07 at 193 kg ha^{-1}, then TT07 at 171 kg ha^{-1}, and lowest in TT06 at 107 kg ha^{-1}.

In TT06 there was no overall varietal difference in UPE (range 1.04-1.08), while in TT07 there was a small varietal difference in UPE (range 1.00-1.18). There was no interaction in either experiment and no varietal trend in UPE between seasons. Although studies have shown that genetic variability for UPE exists (Van Sanford and MacKnown, 1987, May *et al*., 1991, Foulkes *et al.* 1998) probably through differences in rooting characteristics, the four varieties in these experiments are all derived from current elite UK germplasm with similar release dates and parentage (see Table 3-3) and so would be expected to be more similar in UPE. Muurinen *et al*. (2006) on a study of Nordic barley

varieties noted that given the majority of current breeding takes place in high-yielding environments the ability of the plant to take up N is a trait that is not directly selected for.

4.5.6 N-utilisation efficiency

N-utilisation efficiency also declined with N supply in each experiment, although this decline with increasing N was much greater at the sub-optimal N treatments than at the supra-optimal N treatments. Averaging across experiments, UTE decreased by 32% between the zero and optimum N treatments but by only 5% between the optimum and maximum N treatments. A similar pattern of results was found by Delogu *et al.* (1998) studying Mediterranean wheat. Between the zero and optimum N treatments UTE declined despite the increase in both AGN and yield, as the efficiency of yield production per unit of canopy N reduced with N supply to the N opt-trt. However, between the optimum and maximum N treatments the UTE remained almost constant as there was no significant change in AGN or yield. UTE therefore became relatively more important in affecting increases in NUE with N increasing N supply, as N uptake became increasingly independent of the size of the root system. UTE at the N opt-trt differed between site-seasons demonstrating the environmental effect on the increased ability to turn N into grain, likely related to differences in the amount of solar radiation availability.

A significant effect of variety on UTE was observed in both experiments indicating a strong genetic component to UTE, and with Claire lowest in both site-seasons. Further the varietal pattern was the same as that for NUE in TT06, indicating the influence of UTE on NUE and the potential to improve NUE through breeding for higher UTE. Genotypic variation for UTE in wheat and barley has been demonstrated in previous studies (Cox *et al*., 1986; Van Sanford and MacKnown, 1987; May *et al*., 1991) and UTE appears to be the trait most affected by breeding (Slafer *et al*., 1996; Ortiz-Monasterio *et al*., 1997). HI has increased substantially through breeding (Austin *et al*., 1980) and these improvements have contributed substantially to improved UTE (Calderini *et al*., 1999). Examination of the components of UTE in both experiments showed that the effect of variety on HI was the main cause of the variety effect on UTE. The varietal pattern of HI

was the same as that for UTE in both experiments, with the exception of Istabraq and Savannah in TT07 which had lower HI at the supra-optimal N treatments that would be expected from the UTE possibly as a result of reduced grain filling through significant leaning and/or lodging.

Examination of the sub-components of UTE (i.e. HI and BPE) showed that the decline at the sub-optimal N treatments up to N opt-trt was principally due to a decline in BPE rather than HI which increased (averaged across experiments by -33% and +2%, respectively), whilst at the supra-optimal N treatments there was only a small decrease in UTE as both BPE and HI were relatively unchanged. The decrease in BPE could therefore be considered to be the major driver of the observed decrease in UTE with N supply. However the response of BPE to N supply differed between site seasons, possibly related to the amount of light intercepted. At the N zero-trt, BPE was highest in TT06, then LC07 (where low SMN may have been limited leaf laminae expansion), and lowest in TT07 (where dull weather from the end of stem extension to harvest reduced incident solar radiation). At the N opt-trt despite similar AGN in TT06 and LC07 (351 kg N ha^{-1} and 331 kg N ha^{-1}, respectively), BPE was higher in LC07 (78) than in the Terrington experiments (range 64-66) as there was considerably higher incident solar radiation at Lincoln thereby increasing the productivity of the canopy per unit N. The decrease in BPE between the zero and optimum N treatments was therefore greatest in TT06 (40%); cf. TT07 and LC07 (30%). However, BPE at LC07 continued to decrease to the N max-trt as the AGN continued to increase (by 38 kg ha^{-1}) whilst the AGDM increased only slightly (by 0.3 t ha^{-1}), perhaps indicating accumulation and/or storage in the components of N in the crop canopy.

These results imply that an increasing amount of N in the crop is not used for biomass production (i.e. functional N) with increasing N supply, and is potentially accumulated in the shoot tissues either as storage for later functional use or grain protein synthesis, or simply through luxury accumulation. Examination of the N content of the crop components with regards to their functional requirement at anthesis might be expected to show leaf lamina N accumulation above that required to maximise photosynthesis, and/or

true stem N accumulation above that require for structural requirements. Additionally, N taken up for storage might cause an increase in the yield quality at harvest through reducing the demand for remobilisation of functional N during grain filling (e.g. through stay-green effects), and/or through increased N availability to the grain.

There was no difference in BPE between varieties in TT06, and in TT07 the varietal effect on BPE was through Istabraq having a lower AGN than the other three varieties (averaged across N treatments at 224 kg ha^{-1} and range 245-254 kg ha^{-1}, respectively). There was a trend in both experiments for a varietal pattern in BPE averaged across N treatments; Savannah and Istabraq with the highest, then Atlanta and Claire with the lowest. This indicates that varietal differences in BPE likely exist amongst UK varieties, but they are small and further multi-site-season trials would be required to quantify the variety differences with greater statistical precision. Nevertheless, these differences could potentially be used to breed for increased UTE as a means of increasing NUE. Selection for increased BPE at the N opt-trt could therefore importantly increase biomass production, since future gains in yield will increasingly be dependent on achieving greater biomass production whilst maintaining HI. At the N opt-trt, the ability of the crop to utilise canopy N rather than the ability to take up more N is perhaps more important to drive increases in NUE as the canopy typically contains sufficient or excess N for the physiological canopy requirements of the crop.

4.5.7 Conclusions

Fertiliser N significantly increased yield, associated with an increase in grains m^{-2} and biomass production. However the yield response to N diminished with applied N and caused a reduction in NUE as the result of a decline in both UPE and UTE. The NUE component most limiting yield increases differed with N status, at low N availabilities UPE was the most limiting component whereas at high N availabilities UTE became more important. With applied N up to the N opt-trt, the HI and grain weight were relatively unchanged while the BPE decreased significantly demonstrating that canopy N was being used increasingly inefficiently in biomass production. This implies that

significant quantities of N are present in the crop of the N opt-trt which are not contributing to either biomass production or grain yield, and that the potential to improve UTE exists possibly through either more N efficient biomass production and/or the reduction of this excess crop N.

Varietal effects on yield in TT06 were explained by the difference in NUE due to UTE, and in TT07 similar trends were apparent. Examination of the sub-components of UTE in both experiments showed that the main cause of this variety effect on UTE in both experiments was the effect of variety on HI, whilst BPE was less affected. However the existence of statistically significant differences between varieties in TT07 and consistent varietal trends for BPE across years at the N opt-trt would indicate the possibility to breed for superior UTE through increased BPE. Present results would support the hypothesis: (1) 'that there is genetic variation in NUE linked to UTE (via both HI and BPE) amongst elite UK feed winter wheat varieties, and this is associated with differences in the optimum amount of applied N'. Improvements in grain production in optimally fertilised crops could therefore be achieved through the selection of traits associated with increased BPE while simultaneously maintaining HI. However this requires an improved understanding of the traits underlying BPE as well as eventually screening a wider range of wheat germplasm to identify the maximum genetic variability available for these traits in order to develop superior, N-efficient genotypes.

The following two chapters will investigate the canopy traits associated with N use for biomass production and grain yield in fertilised crops. Chapters 5 and 6 will examine the uptake, accumulation, partitioning and use of N in the pre- and post- anthesis growth phases, respectively. Examination of the physiological response of the crop to N supply of each of the canopy components, and the existence of differences in responses between varieties, will be tested with reference to the specific hypotheses proposed in chapter 2.

5 PRE-ANTHESIS GROWTH PHASE

5.1 INTRODUCTION

This chapter examines the physiological components of crop growth in the period from emergence to anthesis, and investigates the sequence of yield-forming processes to anthesis in relation to N supply and variety treatments.

Data are presented for crop development and growth between GS31 and anthesis for each variety in each site-season. The effects of N supply and variety on N uptake and partitioning between crop components, production of the green canopy area, interception and use of solar radiation, and dry matter production and partitioning between crop components are quantified. For each variety in each site-season, the canopy N content at anthesis is quantified according to the critical N concentration (Justes *et al*., 1994) and N nutrition index models (Lemaire *et al*., 1997) to identify crops with deficient, optimal or excess canopy N. Partitioning of N between the crop components at N supply above NNI of 1 at anthesis is examined to identify potential locations for canopy N accumulation and storage, affecting efficiency of crop N use.

The chapter concludes with a discussion of the findings in relation to testing the study hypotheses: (2) 'that N accumulates in the plant organs of wheat canopies at anthesis, which is in excess of that required for structural and photosynthetic uses'; (3) 'that excess N accumulation responds disproportionately to the availability of N, and occurs particularly at high (i.e. supra-optimum) N availabilities'; (4) 'that there is genetic variation in the partitioning of N and the amount of excess N accumulated in the plant organs of wheat canopies and their responses to N supply'; and (5) 'that RUE increases linearly with increasing SLN to the maximum RUE, and there are genetic differences in lamina SLN required for photosynthetic function and hence differences in RUE between varieties'.

5.2 METHODOLOGY

5.2.1 Treatment combinations and statistical analysis

The effect of N supply for Istabraq is analysed in all three experiments in the pre-anthesis period (GS31, GS39 and anthesis) and at anthesis at six N treatments. Unless otherwise specified, results for the effect of N supply and variety are presented for all four varieties in the Terrington experiments at anthesis at six (TT06) or three (TT07; zero, optimum and maximum) N treatments. ANOVAs for the effect of N supply for Istabraq are at six N treatments in all three experiments, and for all four varieties at six (TT06) or three (TT07) N treatments. SEDs from ANOVAs are shown as error bars in figures, and model parameters for fitted curves are found in Appendix IV.

5.3 RESULTS

5.3.1 Crop development

There was no effect of N treatment on the dates of GS31, GS39, and anthesis in all three site-seasons (Table 5-1). In the Terrington experiments, varieties reached GS31 on the same date in TT06 and TT07. It is unusual for varieties to have exactly the same date for growth stages, and this may have been the consequence of the limited frequency of assessment at GS31 (every 2-3 days). The date of GS39 in TT07 and the date of anthesis in TT06 and TT07 were affected by variety. In TT07 Atlanta reached GS39 three days earlier than the other three varieties, and in TT06 and TT07 Atlanta reached anthesis 3 to 4 days earlier than the other three varieties. There was no N treatment x variety interaction in either experiment at Terrington.

Table 5-1 Dates of growth stages (DAS, days after sowing) for N treatment x variety combinations in TT06, TT07 and LC07.

Exp.	Variety	31	39	61
TT06	Istabraq	20 April (191)	23 May (224)	12 June (244)
	Atlanta	-	-	09 June (241)
	Claire	-	-	12 June (244)
	Savannah	-	-	12 June (244)
TT07	Istabraq	22 April (174)	17 May (199)	08 June (221)
	Atlanta	-	14 May (196)	04 June (217)
	Claire	-	17 May (199)	08 June (221)
	Savannah	-	14 May (196)	08 June (221)
LC07	Istabraq	18 Oct (132)	21 Nov (166)	07 Dec (182)

In the Terrington experiments, sampling at anthesis for all varieties was on the same calendar date. As a consequence, Atlanta was sampled approximately 3-4 days after anthesis (GS61).

5.3.2 Crop establishment

5.3.2.1 Plant population at GS31

There was no effect of N treatment on the plant population density of Istabraq in all three site-seasons; averaging across N treatments (zero and optimum) the plant population density was 204 m^{-2} in TT06, 174 m^{-2} in LC07, and 172 m^{-2} in TT07. In the Terrington experiments, there was no effect of N treatment or variety on the plant population density and the interaction was not significant.

5.3.2.2 Shoot production

The maximum density of potentially fertile shoots (shoot density; m^{-2}) occurred at GS31 in all three experiments after which the fertile shoot density declined until anthesis (Figure 5.1). As the plant population remains relatively constant after GS31, the reduction in shoot density can be attributed to a reduction in the number of tillers $plant^{-1}$.

The shoot density for Istabraq at GS31 was not affected by N treatment in any of the three experiments. Averaging across N treatments, tiller production was highest in TT06 (902 m^{-2}), then TT07 (797 m^{-2}), and lowest in LC07 (548 m^{-2}). In the Terrington experiments, there was an effect of variety on shoot density in TT06 and TT07 ($P<0.01$) in the ranges 902 (Istabraq) to 1182 m^{-2} (Savannah), and 615 (Atlanta) to 915 m^{-2} (Savannah), respectively, and an interaction in TT07 ($P<0.05$), with Savannah increasing more at the N opt-trt than the other varieties.

At anthesis the shoot density for Istabraq was affected by N treatment in LC07 ($P<0.05$), but not in TT06 or TT07, although there was a trend for the N zero-trt to have the lowest shoot density (Figure 5.2). In LC07 shoot density increased with N supply from 247 m^{-2} at the N zero-trt to 521 m^{-2} at the N opt-trt, thereafter increasing only slightly to 546 m^{-2} at the N max-trt. Averaging across N treatments, the crop at TT06 had the highest shoot density (514 m^{-2}), then LC07 (438 m^{-2}), and TT07 (339 m^{-2}). In the Terrington experiments, shoot density was affected by variety in TT06 ($P<0.05$) but not in TT07; the interaction was not significant in either experiment. Averaging across N treatments, in TT06 Claire had more shoots (569 m^{-2}), than Atlanta (534 m^{-2}), Savannah (530 m^{-2}) and Istabraq (514 m^{-2}). In TT07 there was again a trend for Claire and Savannah (385 and 370 m^{-2}, respectively) to have more shoots than Istabraq and Atlanta (339 and 326 m^{-2}, respectively).

Tiller death between GS31 and anthesis was affected by N treatment in LC07 ($P<0.001$) but not in TT06 or TT07; decreasing with N supply in LC07 from 316 m^{-2} at the N zero-trt to 13 m^{-2} at the N max-trt. Overall, tiller death was greatest in TT07, then TT06, and lowest in LC07 representing a reduction of 458, 388, 110 shoots m^{-2} respectively. There was an effect of variety in TT06 and TT07 ($P<0.01$); with Savannah in each year having the highest tiller death (552 and 674 m^{-2}, respectively) and Atlanta having the lowest tiller death (293 and 383 m^{-2}, respectively). Higher shoot density m^{-2} at anthesis for Claire was therefore the result of high tiller survival compared with Istabraq and Savannah.

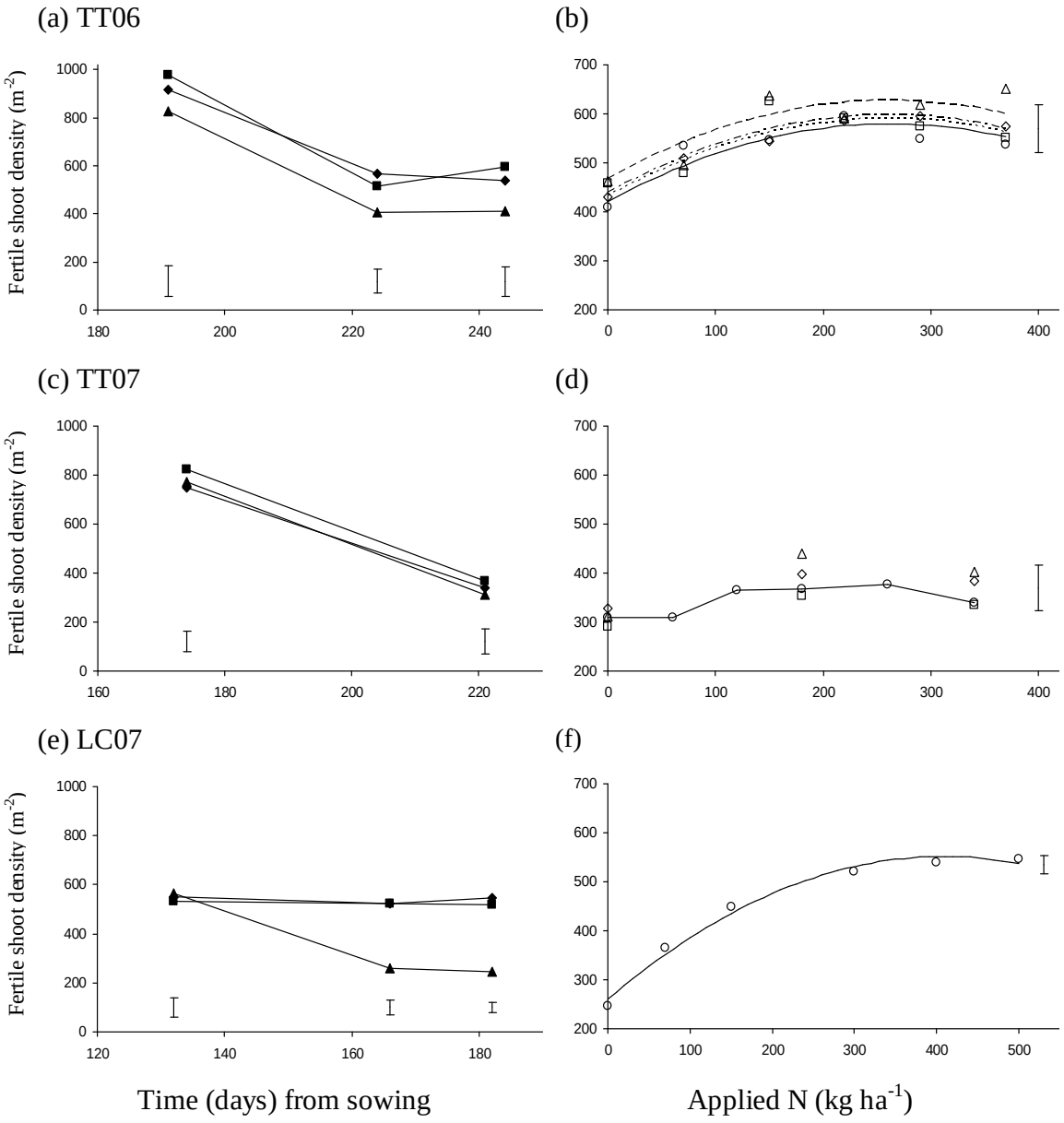


Figure 5.1 (a, c, e) Effect of applied N on fertile shoot density (m^{-2}) between GS31 and anthesis in TT06, TT07 and LC07. Observed values for Istabraq at three growth stages; 31, 39, 61 and three N treatments; zero (▲), optimum (■), and maximum (◆); with SED N bar at each growth stage (df = 2, 10 and 10 (TT06); 2 and 10 (TT07); 20, 25 and 25 (LC07), respectively).

Figure 5.2 (b, c, f) Effect of applied N and variety on fertile shoot density (m^{-2}) at anthesis in TT06, TT07 and LC07. Observed values at six N treatments for one (LC07) or four (TT06 and TT07) varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------); with SED bars for N (LC07; df = 25) and N x V (TT06 and TT07; df = 36 (TT06) and 18 (TT07)). Model parameters for curves are presented in Appendix IV.

5.3.2.3 Plant height at anthesis

The plant height for Istabraq at anthesis was affected by N treatment in all three experiments ($P<0.05$), being lower at the N zero-trt compared to the optimum and maximum N treatments by 100-150 mm . Averaging across N treatments, TT06 had the tallest shoots (747 mm), then TT07 (692 mm), and LC07 had the shortest (505 mm). In the Terrington experiments, plant height was affected by variety in TT06 and TT07 ($P<0.05$); the interaction was not significant in either experiment. Averaging across N treatments, in TT06 plant height varied from Istabraq (747 mm), Savannah (712 mm), Claire (697 mm) to Atlanta (637 mm). In TT07 the crop showed a slightly different varietal pattern, but again with Istabraq the tallest (692 mm) and Atlanta the shortest (623 mm) variety.

5.3.3 Crop N uptake

5.3.3.1 Above-ground N uptake

The above-ground N for Istabraq at GS31 was affected by N treatment in LC07 ($P<0.001$), but not in TT06 and TT07 although there was a trend for lower AGN at the zero than optimum N treatments (Figure 5.3). Averaging across N treatments (zero and optimum), the crop at TT06 took up more N (79 kg ha^{-1}), than at TT07 (67 kg ha^{-1}) or LC07 (31 kg ha^{-1}). In the Terrington experiments, AGN was affected by variety in TT06 ($P<0.05$) but not in TT07; the interaction was not significant in either experiment. AGN for Istabraq at GS39 was again affected by N treatment in TT07 and LC07 ($P<0.01$), increasing by 129 and 151 kg N ha^{-1} with N supply from the zero to the optimum, respectively.

Above-ground N for Istabraq at anthesis was affected by N treatment in all three site-seasons ($P<0.01$), increasing with N supply (Figure 5.4). Unfertilised Istabraq at Terrington had double the AGN of the crop at Lincoln (range 103-125 kg ha^{-1}, and 54 kg ha^{-1}, respectively) reflecting the higher SMN availability at the Terrington site. At the optimum N treatment, TT06 had the highest AGN (291 kg ha^{-1}), then LC07 (263 kg ha^{-1})

and TT07 (213 kg ha^{-1}). In the Terrington experiments, AGN was affected by variety in TT06 ($P<0.001$) but not in TT07; there was no interaction in either experiment. Averaging across N treatments, in TT06 Atlanta had higher AGN (271 kg ha^{-1}), than Istabraq, Claire and Savannah (range 236-240 kg ha^{-1}). In TT07 the varietal pattern was different, in the range Istabraq (169 kg ha^{-1}) to Savannah (197 kg ha^{-1}).

The response of AGN to N supply at anthesis for Istabraq was fitted to a bi-linear model in all three experiments (model parameters shown in Table 5-2). The slope of phase 1 showed that the AGN for the crop at TT06 increased twice as quickly than at TT07, giving TT06 an applied N breakpoint of 90 kg ha^{-1} cf. 170 kg ha^{-1} for TT07 (close to N opt-trt of 180 kg ha^{-1}). TT06 and LC07 showed a broadly similar pattern for recovery of applied N with the breakpoint on the x-axis occurring before the N opt-trt and N uptake continuing above the breakpoint reaching about 300 kg ha^{-1} at the N max-trt.

Table 5-2 Model parameters for fitted bi-linear lines for Istabraq for above-ground N (AGN) versus applied N at anthesis (see Figure 5.3 and Figure 5.4) in TT06, TT07 and LC07.

Exp.	Slope 1	Slope 2	Breakpoint (SE)		% Variance accounted (SE)
			Applied N	AGN	
TT06	1.328 ± 0.345	0.249 ± 0.105	89.7 (27.6)	244.4 (24.6)	81.9 (29.6)
TT07	0.654 ± 0.198	-0.147 ± 0.15	170.2 (36.2)	214.0 (14.2)	61.6 (29.1)
LC07	0.959 ± 0.077	0.174 ± 0.060	210.1 (21.2)	252.5 (14.8)	97.1 (16.4)

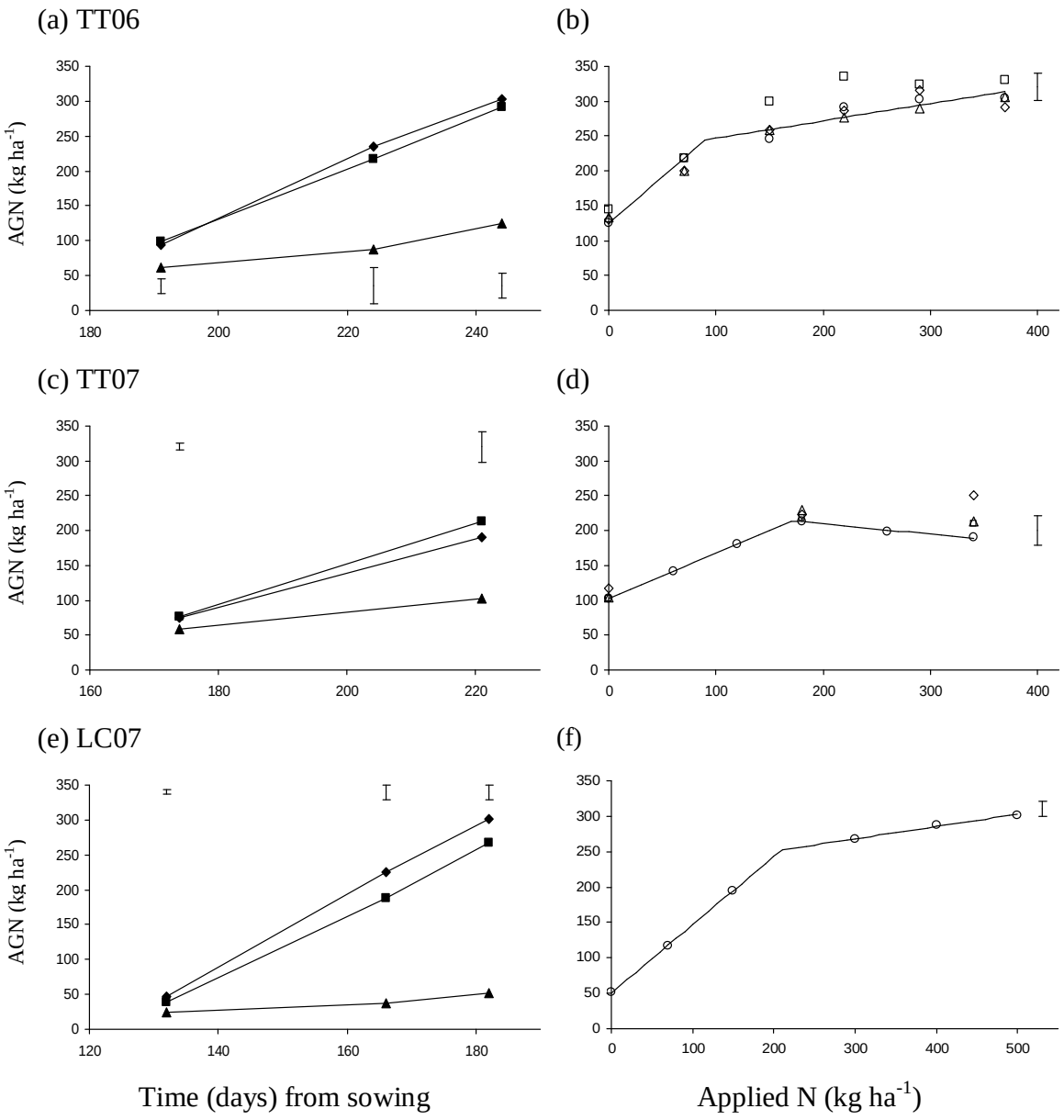


Figure 5.3 (a, c, e) Effect of applied N on above-ground N (AGN; kg ha^{-1}) between GS31 and anthesis in TT06, TT07 and LC07. Observed values for Istabraq at three growth stages; 31, 39 (except TT07), and 61 at three N treatments; zero (▲), optimum (■), and maximum (◆); with SED N bar at each growth stage (df = 2, 10 and 10 (TT06); 2 and 10 (TT07); 12, 15 and 15 (LC07), respectively).

Figure 5.4 (b, c, f) Effect of applied N and variety on the above-ground N (AGN; kg ha^{-1}) at anthesis in TT06, TT07 and LC07. Observed values at six N treatments for one (LC07) or four (TT06) varieties, or three N treatments for four varieties (TT07); Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted bi-linear lines for Istabraq (———); with SED for N (LC07: df = 15) and N x V (df = 36 (TT06) and 18 (TT07)). Model parameters for curves are presented in Table 5-2.

5.3.3.2 Apparent fertiliser recovery at anthesis

The proportion of applied fertiliser N recovered by the crop at anthesis (AFR; apparent fertiliser recovery) was calculated using Equation 3-4. Analysis was carried out for data at five N treatments (excluding N zero-trt) for Istabraq in all three experiments, and at two N treatments (optimum and maximum N; excluding N zero-trt) for all varieties in TT06 and TT07. AFR for Istabraq decreased with N supply in all three site-seasons ($P<0.07$) as a surplus of un-recovered soil N occurred at the high N treatments (i.e. as soil N supply exceeded AGN). Averaging across N treatments, TT06 and LC07 had similar AFR (0.62 and 0.61, respectively), whilst TT07 had the lowest (0.44). The low AFR in TT07 may have been accounted for by low fertiliser N uptake during dull conditions from the end of stem extension to anthesis. In the Terrington experiments, the AFR was affected by variety in TT06 ($P<0.05$) but not in TT07, the interaction was not significant in either experiment. In TT06 Atlanta overall had the highest AFR (0.68), then Istabraq (0.62), Savannah (0.57), and Claire the lowest (0.56); cf. TT07 which showed a different pattern in the range 0.44 (Istabraq) to 0.51 (Claire).

5.3.4 N partitioning between crop components

The total canopy AGN at anthesis was partitioned between the four crop components: true stem, leaf sheath, leaf lamina and ear. The effect of applied N and variety on the amount and proportion of AGN in each of these crop components was analysed.

5.3.4.1 Effects at GS39

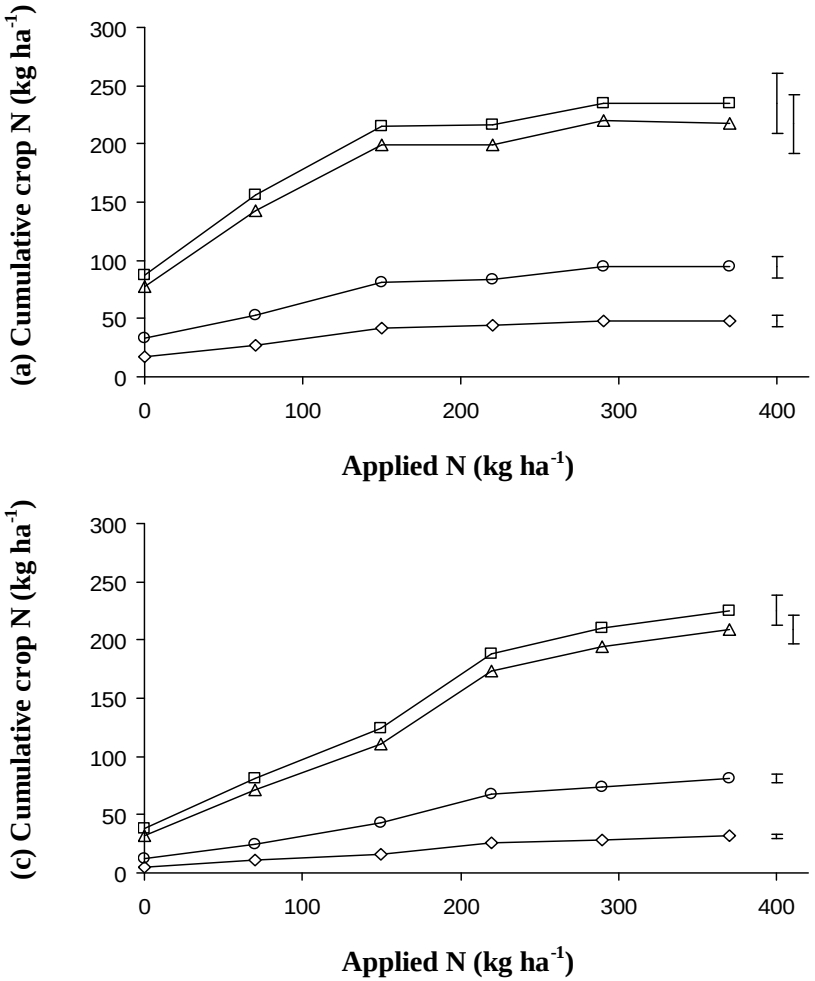
The amount of N in the crop components for Istabraq increased with N supply in TT06 and LC07 for all components ($P<0.01$) (Figure 5.5). Overall AGN was partitioned similarly between the crop components in both experiments; over half of the AGN was in the leaf lamina which responded positively to N supply. However, around 40% of the AGN was in the leaf sheath and true stem, and the remaining N was in the ear which responded negatively to N supply. The proportion of AGN in each canopy component was affected by N treatment in TT06 (ear $P<0.01$) and LC07 (lamina and ear $P<0.001$).

Therefore, as N supply increased, in both experiments there was a trend for a higher proportion of N in the leaf lamina, a slightly higher proportion in the leaf sheath and true stem, and a lower proportion of N in the ear.

5.3.4.2 Effects at anthesis

The amount of N in the crop components for Istabraq increased with N supply in all three site-seasons for all crop components ($P<0.05$) (Figure 5.6). The proportion of AGN in each crop component for Istabraq was affected by N treatment in LC07 ($P<0.01$), for the ear and leaf lamina in TT06 and the ear in TT07 ($P<0.05$). In all three experiments as N supply increased the proportion of N in the leaf lamina and leaf sheath increased. For the true stem, the results were not consistent (TT06 increased, TT07 was unchanged, and LC07 decreased with N supply), and the proportion of N in the ear decreased (all experiments). Averaging across N treatments, all three experiments had a similar proportion of AGN in the lamina (range 0.34-0.38) and sheath (range 0.15-0.19). However, TT06 and TT07 had a higher proportion of AGN in the true stem (0.28-0.29) and ear (0.21-0.22), than LC07 (0.21 and 0.17, respectively).

In the Terrington experiments (Figure 5.7), there was an effect of variety on the proportion of AGN in each crop component in TT06 (leaf sheath, true stem and ear $P<0.001$); the interaction was significant for sheath ($P<0.05$); and in TT07 (leaf sheath and ear $P<0.01$) but not the true stem ($P=0.064$); the interaction was significant for true stem ($P<0.05$). Overall, the proportion of AGN in the leaf lamina was similar for all varieties (0.34-0.35 in TT06, and 0.32-0.34 in TT07). For the leaf sheath in both experiments it was higher for in Istabraq and Claire than Savannah and Atlanta in the ranges 0.13 to 0.15 (TT06) and 0.17 to 0.19 (TT07). Whereas for the true stem in TT06 it was highest for Savannah (0.29), then Istabraq and Claire (both 0.28), and Atlanta lowest (0.25); cf. TT07 Savannah and Atlanta had the highest (both 0.31), and Istabraq and Claire had the lowest (both 0.29). The proportion of N in the ear in both experiments was highest in Atlanta, then Claire, and lowest in Istabraq and Savannah (TT06 at 0.27, 0.23, 0.21 and 0.21 respectively, and TT07 at 0.20, 0.18, 0.17 and 0.17 respectively).

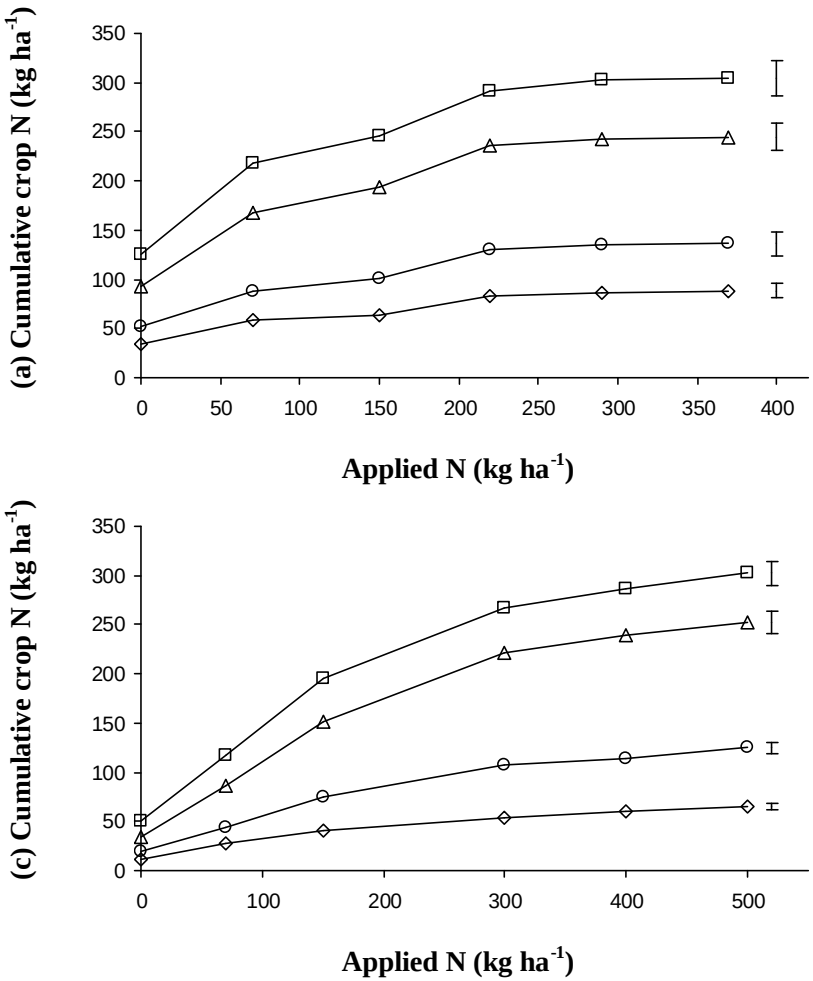


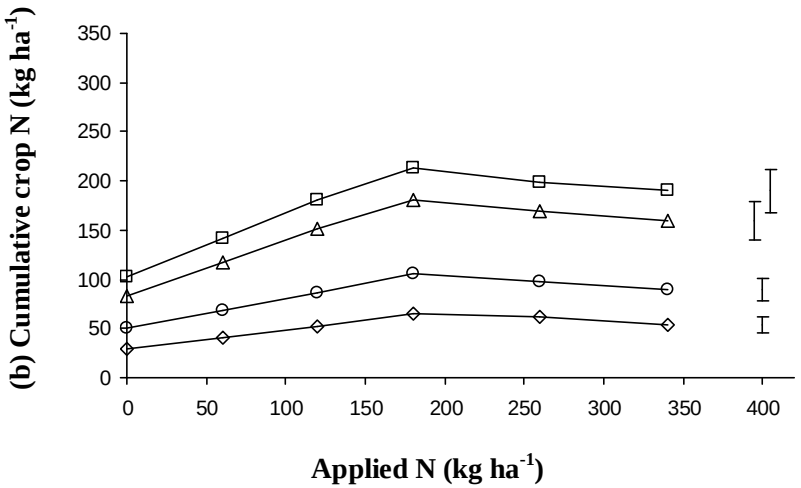
No data available for TT07

(a & c) Experiments TT06 (a) and LC07 (c).

Observed values for Istabraq at six N treatments cumulatively for four crop components; true stem (◇), sheath plus true stem (○), lamina plus true stem and sheath (△), and ear plus lamina, sheath and true stem (□); with SED N bar (df = 10 (TT06) and 15 (LC07)).

Figure 5.5 (a & c) Effect of applied N on the crop N cumulatively for four crop components at GS39 for Istabraq in TT06 and LC07.





(a, b & c) Experiments TT06 (a), TT07 (b), and LC07 (c).

Observed values for Istabraq, at six N treatments cumulatively for four crop components; true stem (◇), sheath plus true stem (○), lamina plus true stem and sheath (△), and ear plus lamina, sheath and true stem (□); with SED N bar (df = 10 (TT06), 10 (TT07) and 15 (LC07)).

Figure 5.6 (a, b & c) Effect of applied N on the crop N cumulatively for four crop components at anthesis for Istabraq in TT06, TT07 and LC07.

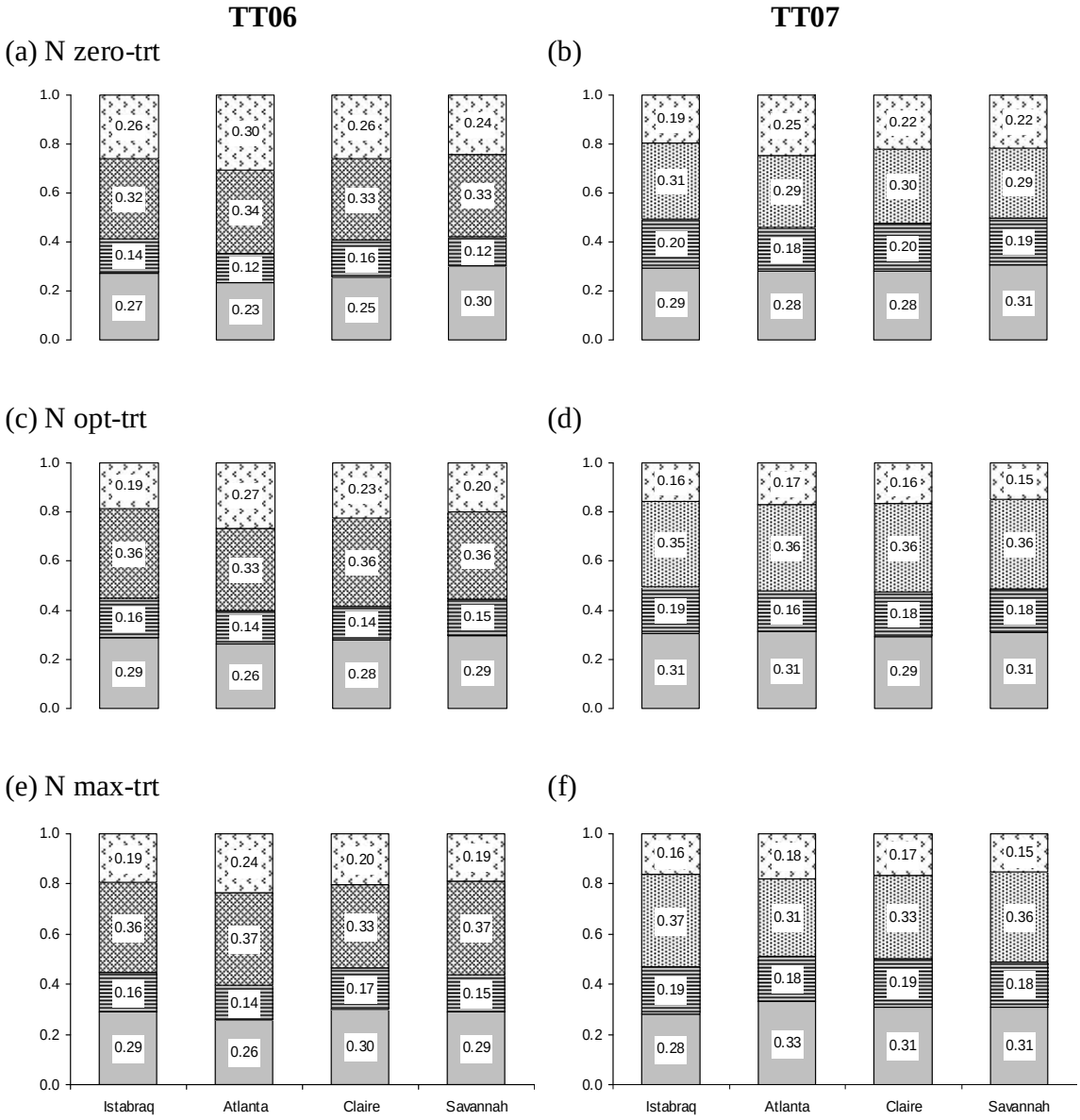


Figure 5.7 (TT06; a, c, e) (TT07; b, d, f) - Effect of applied N on the N partitioning at anthesis for the Terrington experiments. Observed values for four varieties, at three N treatments (zero, optimum and maximum), for four crop components (true stem, sheath, lamina, and ear: in ascending order with true stem nearest x axis). Values represent proportion of AGN in component.

5.3.5 Canopy Green Area

5.3.5.1 Green area index

During the stem-extension period GAI for Istabraq was affected by N treatment at GS31 (except TT07), GS39 (TT07 not sampled), and anthesis in all three experiments; GAI increased with N supply (Figure 5.8). Averaging across N treatments at anthesis TT06 had the highest GAI, then TT07, and LC07 had the lowest (5.9, 5.8, and 4.1, respectively). At anthesis GAI in the unfertilised Istabraq crop was similar in TT06 and TT07 (4.3 and 4.7, respectively) but much lower in LC07 (1.6), however at the optimal fertilised treatment GAI were similar in all three experiments (6.8, 6.4 and 5.1, respectively).

In the Terrington experiments, GAI at GS31 was affected by variety in TT06 and TT07 ($P<0.01$), and there was an interaction in TT07 ($P<0.05$) with Savannah increasing more than Istabraq with N supply. Averaging across N treatments both experiments showed the same varietal pattern, Atlanta had the highest GAI, then Savannah, Claire, and Istabraq had the lowest (TT06 at 2.9, 2.4, 2.3 and 2.2 respectively, and TT07 at 3.0, 2.7, 2.7 and 2.0, respectively). At anthesis GAI was affected by variety in TT06 ($P<0.001$) but not in TT07; the interaction was not significant in either experiment (Figure 5.9). Averaging across N treatments, in TT06 Atlanta had the highest GAI (6.9), then Claire (6.6), Savannah (6.2), and Istabraq (5.9); cf. TT07 which showed a different but not significant varietal pattern from Claire (5.1) to Istabraq (5.8). Regression analysis for the relationship of GAI to N supply fitted parallel curves to varieties in TT06, i.e. the effect of N on increasing GAI was the same for all four varieties. No curves were fitted to TT07 data as there was no significant effect of N treatment or variety.

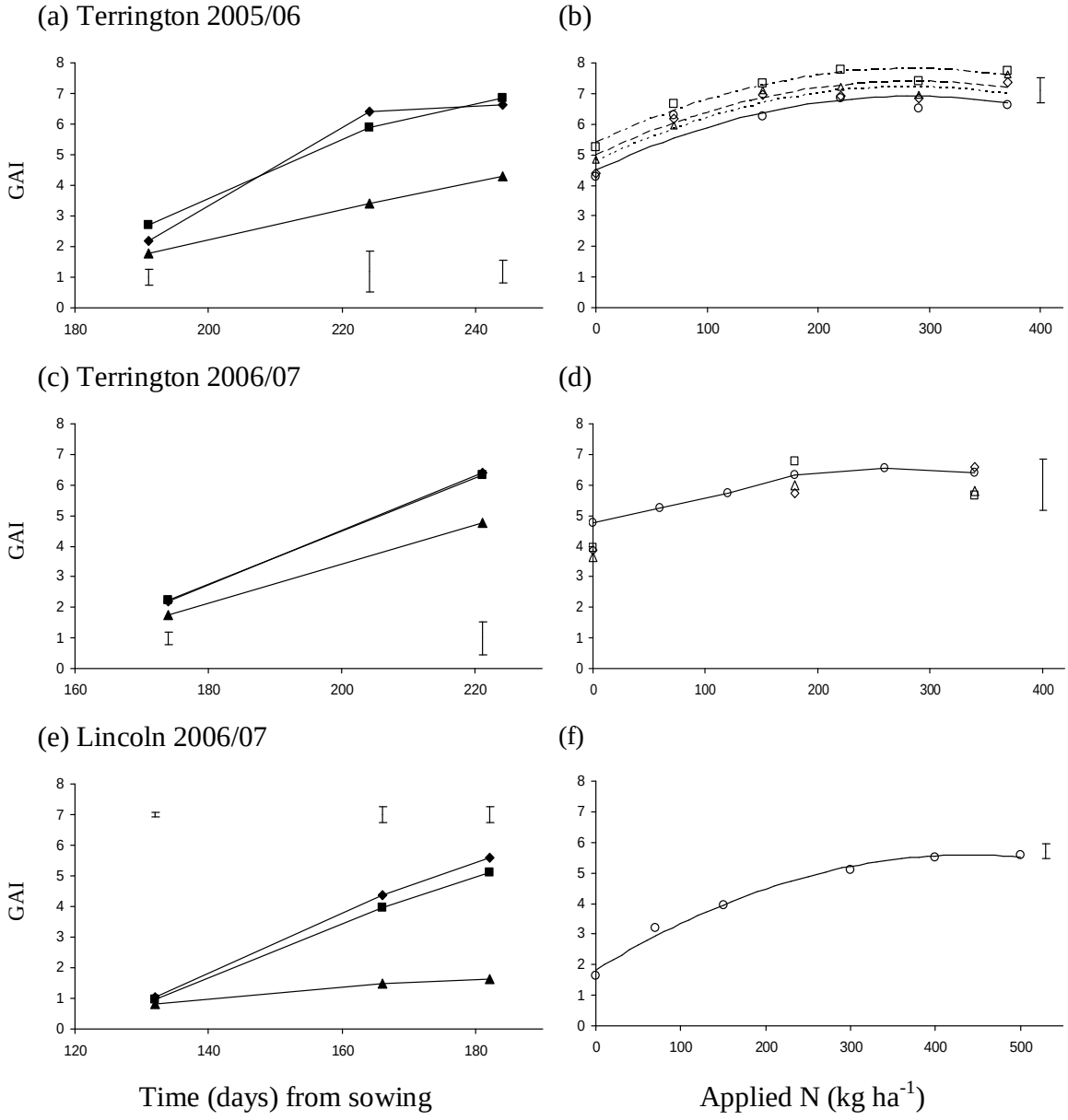
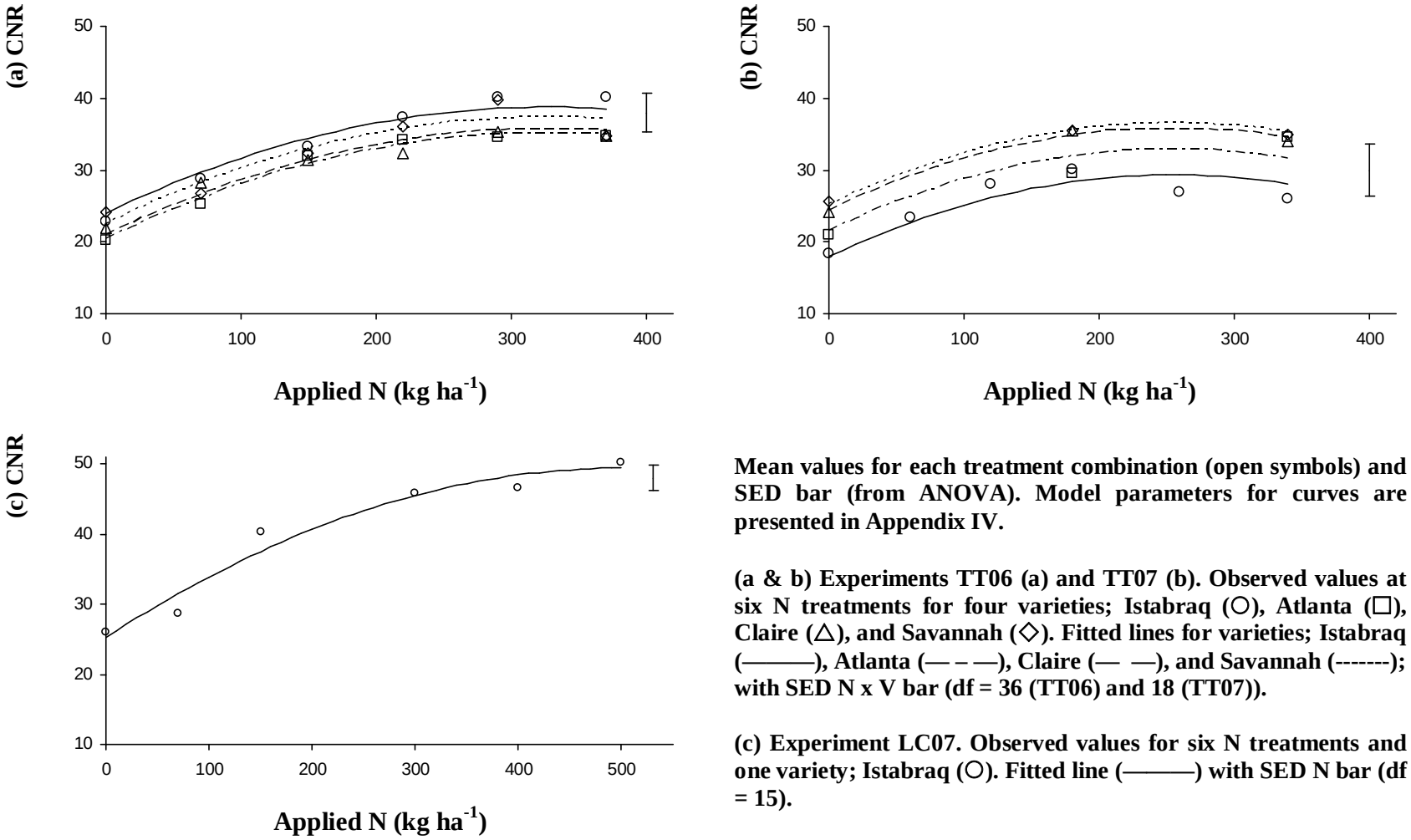


Figure 5.8 (a, c, e) Effect of applied N on the green area index (GAI) between GS31 and anthesis in TT06, TT07 and LC07. Observed values for Istabraq at three growth stages; 31, 39, 61 and at three N treatments; zero (▲), optimum (■), and maximum (◆); with SED N bar at each growth stage (df = 2, 10 and 10 (TT06); 2 and 10 (TT07); 20, 25 and 25 (LC07), respectively).

Figure 5.9 (b, d, f) Effect of applied N and variety on the green area index (GAI) at anthesis in TT06, TT07 and LC07. Observed values at six N treatments for one (LC07) or four (TT06 and TT07) varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------); with SED bars for N (LC07; df = 25) and N x V (df = 36 (TT06) and 18 (TT07)). Model parameters for curves are presented in Appendix IV.

5.3.6 Canopy Nitrogen Requirement

The canopy nitrogen requirement (CNR) is the amount of N (kg) in the shoot which corresponds to 1 ha of green area (excluding ears but including dead leaves). The CNR for Istabraq at anthesis was affected by N treatment in all three experiments ($P<0.001$); CNR increased with N supply (Figure 5.10). LC07 had the highest CNR at all N treatments, at the zero, optimum and maximum N treatments of 26.0, 45.8, and 50.3, respectively; cf. TT06 at 22.8, 37.3, and 40.1, respectively; and TT07 at 18.3, 30.0, and 26.0, respectively. In the Terrington experiments, CNR for all varieties was affected by variety in TT06 and TT07 ($P<0.05$), the interaction was not significant in either experiment. Averaging across N treatments, in TT06 Istabraq had the highest CNR (33.4), then Savannah (31.7), Atlanta and Claire had the lowest (29.7); cf. TT07 showed a different varietal pattern with Savannah (32.0), Claire (31.2), Atlanta (28.4), and Istabraq (24.8).



Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values at six N treatments for four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------); with SED N x V bar (df = 36 (TT06) and 18 (TT07)).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

Figure 5.10 (a, b & c) Effect of applied N and variety on the canopy N requirement (CNR) at anthesis in TT06, TT07 and LC07.

5.3.7 Specific leaf nitrogen at anthesis

5.3.7.1 Global SLN for all leaf layers

The specific leaf N (SLN; g N m^{-2}) for Istabraq was affected by N treatment in all three experiments ($P<0.001$) (Figure 5.11); SLN increased with N supply, in LC07 from 1.57 g N m^{-2} at the N zero-trt to 3.39 g N m^{-2} at the N max-trt; cf. TT06 at 1.40 to 2.61 g N m^{-2}, respectively, and TT07 at 0.96 to 1.52 g N m^{-2}, respectively. Averaging across N treatments, LC07 had the highest SLN (2.69 g N m^{-2}), then TT06 (2.17 g N m^{-2}), and TT07 had the lowest (1.42 g N m^{-2}). In the Terrington experiments SLN for all varieties was affected by variety in both site-seasons ($P<0.05$); the interaction was not significant in either experiment. Averaging across N treatments, in TT06 Istabraq had the highest SLN (2.17), then Savannah (2.06 g N m^{-2}), Atlanta and Claire (both 1.90 g N m^{-2}); cf. TT07 showed a different varietal pattern as Claire had the highest (1.94 g N m^{-2}) and Istabraq the lowest (1.42 g N m^{-2}).

5.3.7.2 SLN for individual leaf layers

The total leaf lamina for Istabraq at anthesis was separated into three leaf layers; the flag leaf, leaf two (penultimate leaf), and leaf three and below. The SLN of each of these leaf layers was affected by N treatment in all three experiments ($P<0.01$), with SLN of all leaf layers increasing with N supply to the N opt-trt, and thereafter continuing to increase or decreased slightly (Figure 5.12). Averaging across N treatments, LC07 had the highest SLN for all leaf layers, then, TT06 and TT07 had the lowest. The SLN of the three leaf layers were significantly different in all three experiments, overall the response was 2.51, 2.19 and 1.68 g N m^{-2} for the flag leaf, leaf two and leaf three and below, respectively ($P<0.01$), there was no interaction in either experiment. Differences amongst leaf layers showed a similar pattern in TT06 and LC07, but TT07 showed a less distinct separation between the three leaf layers with leaf two having a slightly higher SLN than the flag leaf, but higher than leaf three and below. Overall, TT07 had a much lower SLN in all leaf layers than the other two experiments.

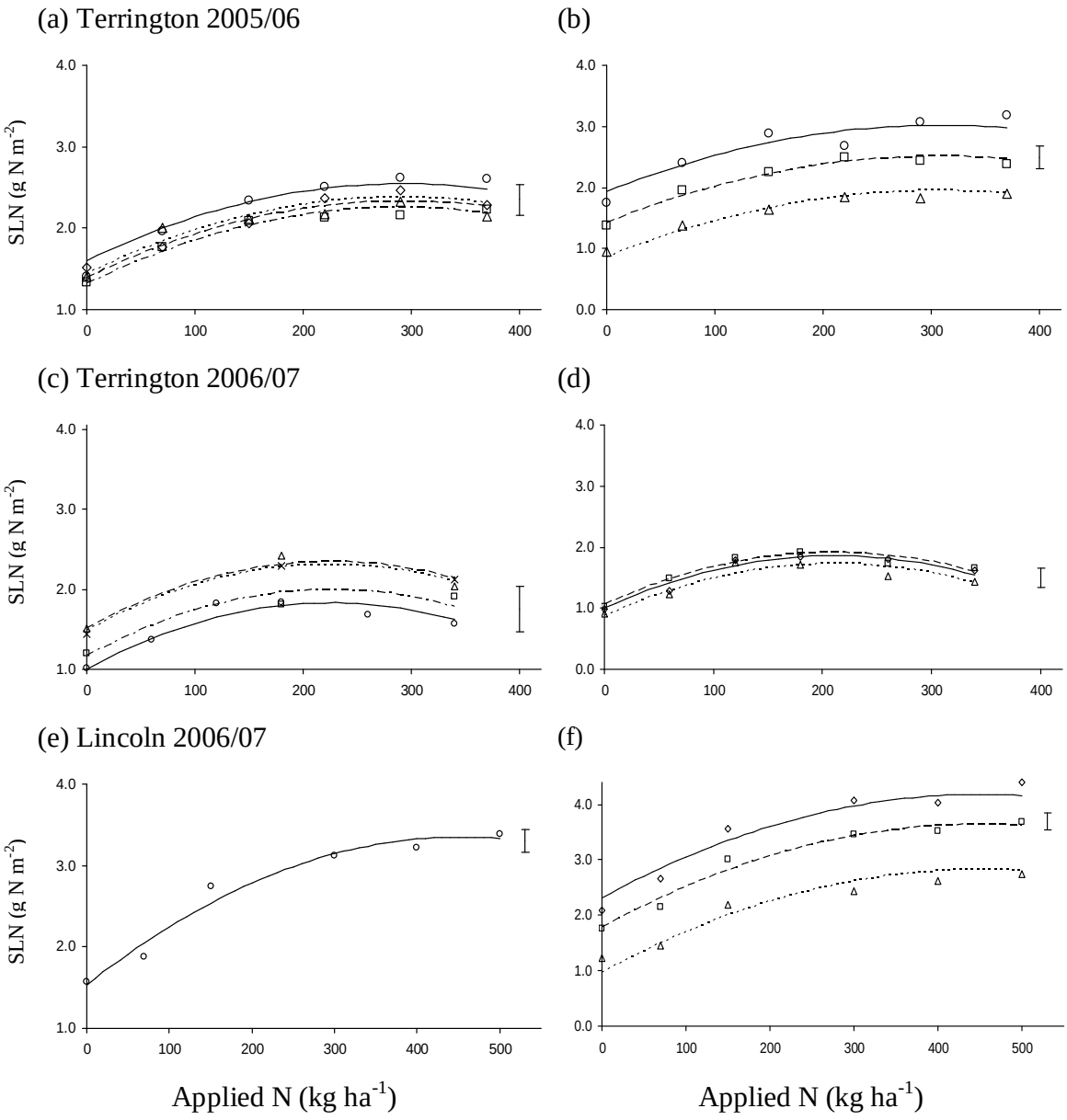


Figure 5.11 (a, c, e) Effect of applied N on the specific leaf N (SLN; all lamina; g N m^{-2}) at anthesis in TT06, TT07 and LC07. Observed values at six N treatments for one (LC07) or four (TT06 and TT07) varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------); with SED bars for N (LC07; df = 15) and N x V (df = 36 (TT06) and 18 (TT07)). Model parameters for curves are presented in Appendix IV.

Figure 5.12 (b, d, f) Effect of applied N on the specific leaf N (SLN; leaf lavers; g N m^{-2}) at anthesis in TT06, TT07 and LC07. Observed values for Istabraq at six N treatments for three leaf layers; flag leaf (◇), leaf two (□) and leaf 3 and remaining (△). Fitted lines for each leaf layer; flag leaf (———), leaf two (— – —), and leaf 3 and remaining (-------); with SED N bar (df = 24 (TT06), 24 (TT07) and 51 (LC07)). Model parameters for curves are presented in Appendix IV.

5.3.8 Canopy light interception

The total amount of photosynthetically active radiation (PAR) intercepted by the canopy depends on the amount of incident radiation (see 4.2.2), the crop green canopy area (see 5.3.5), and the canopy light extinction coefficient of PAR (K_{PAR}).

5.3.8.1 Light extinction coefficient

The canopy light extinction coefficient for Istabraq at anthesis was unaffected by N treatment in each experiment; averaging across N treatments, K_{PAR} was 0.51 in TT06, 0.45 in LC07, and 0.40 in TT07. In the Terrington experiments, K_{PAR} was affected by variety in both site-seasons ($P<0.01$), the interaction was not significant in either experiment. Overall in TT06 Savannah had the highest K_{PAR} (0.56) (i.e. intercepts most light per unit of GAI), then Istabraq (0.51), Claire (0.50), and Atlanta had the lowest (0.45); cf. in TT07 showed lower values with Savannah (0.49), Claire (0.48), Atlanta (0.47), and Istabraq (0.40). The varietal pattern was therefore different in the two years.

Analysis of data from TT06 for GS39 and anthesis showed no effect of growth stage on K_{PAR} ($P=0.708$). This confirms the finding of other studies which have shown no change in K_{PAR} between GS31 and GS75 (Thorne *et al*., 1988). The K_{PAR} at anthesis can therefore be taken as a reliable estimate of values during the period from the onset of stem elongation to anthesis (Shearman *et al*., 2005).

5.3.9 Canopy interception of PAR

The amount of PAR intercepted by Istabraq between GS31 and anthesis (IR) increased with N supply between the zero and optimum N treatments in all experiments ($P< 0.05$), but was not further increased at the N max-trt. Averaging across N treatments, the crop at TT06 had highest IR (399 MJ m^{-2}), then LC07 (333 MJ m^{-2}), and TT07 (265 MJ m^{-2}). The crop at LC07 had lowest IR at the N zero-trt due to a smaller canopy green area than at Terrington, and the crop at TT07 had lower IR at the optimum and maximum N treatments than other site-seasons due to the dull weather during the stem elongation

period reducing incident PAR. Overall IR was higher in TT06 than in TT07 as a consequence of the longer duration of the stem-elongation phase (53 days and 47 days, respectively), and the higher solar radiation availability during April, May and June in TT06 (435 MJ m^{-2}) than in TT07 (411 MJ m^{-2}).

In the Terrington experiments, IR differed amongst varieties in both experiments ($P<0.01$); there was no N treatment x variety interaction in either experiment. In both experiments Savannah and Atlanta tended to have greater IR than Claire and Istabraq (in the ranges 399 to 420 MJ m^{-2}, and 265 to 295 MJ m^{-2} in TT06 and TT07, respectively). The greater IR by Atlanta and Savannah in both seasons compared with Claire and Istabraq was more associated with the varietal differences in GAI than in K_{PAR}.

5.3.10 Biomass production

5.3.10.1 Above-ground dry mass production

At GS31 averaging across the zero and optimum N treatments, AGDM ranged from 3.4 (TT06) to 1.1 t ha^{-1} (LC07). At anthesis averaging across N treatments, the crop at TT06 again produced more biomass (15.2 t ha^{-1}), than at LC07 (10.6 t ha^{-1}) or TT07 (10.0 t ha^{-1}). The greatest range of AGDM between the zero and optimum N treatments at anthesis, however, was observed at LC07 at 6.5 t ha^{-1} compared with 4.6 and 3.1 t ha^{-1} at TT06 and TT07, respectively (Figure 5.13).

In the Terrington experiments, AGDM at GS31 was affected by variety in TT06 and TT07 ($P<0.05$), the interaction was not significant in either experiment (Figure 5.14). In both experiments, Atlanta had the highest AGDM and Claire had the lowest (in the ranges 3.1-4.0, and 2.5-3.4 t ha^{-1} in TT06 and TT07, respectively). At anthesis AGDM differed amongst varieties in TT06 ($P<0.001$) but not in TT07, the interaction was not significant in either experiment. Averaging across N treatments, in TT06 Atlanta again had higher AGDM (16.3 t ha^{-1}) than other varieties (in the range 14.8-15.2 t ha^{-1}); cf. TT07 with non-significant differences in the range 10.0 to 10.9 t ha^{-1}. Parallel regression

analysis performed for AGDM data at anthesis in TT06 fitted parallel curves to the data for the four varieties indicating similar responses to applied N.

5.3.10.2 Dry matter partitioning between crop components at anthesis

At anthesis, the proportion of DM in the crop components for Istabraq was affected by N treatment for the lamina (TT07 and LC07 $P<0.01$), leaf sheath (LC07 $P<0.001$), true stem (all experiments $P<0.05$), and ear (TT07 and LC07 $P<0.05$). Generally all three experiments had a similar biomass partitioning with overall 41-49% of the dry matter in the true stem, 17-19% in leaf lamina, 16-20% in the leaf sheath and 15-20% in the ear. In each of the Terrington experiments, the proportion of DM in all components was affected by variety (except the lamina in TT06) ($P<0.05$), mainly due to shorter stem length associated with reduced stem partitioning for Atlanta. The N treatment x variety interaction was significant for the proportion of DM in the true stem in TT06 ($P<0.001$) with Atlanta decreasing more with N supply than the other varieties.

5.3.10.3 Biomass production efficiency

The biomass production efficiency (BPE, AGDM/AGN) at anthesis for Istabraq decreased with N supply in all three experiments ($P<0.001$) (Figure 5.15). At the N zero-trt BPE was highest amongst the three site-seasons at LC07 (106.1) cf. TT06 and TT07 (101.3 and 83.8, respectively). BPE was lowest at LC07 at the optimum and maximum N treatments (46.6 and 44.4, respectively) cf. TT06 and TT07 (58.7 and 54.7, and 52.0 and 52.6, respectively). In the Terrington experiments, BPE was not affected by variety in TT06 and TT07 in the ranges 68.6 to 70.7 and 60.3 to 65.8 respectively, and the interaction was not significant in either experiment.

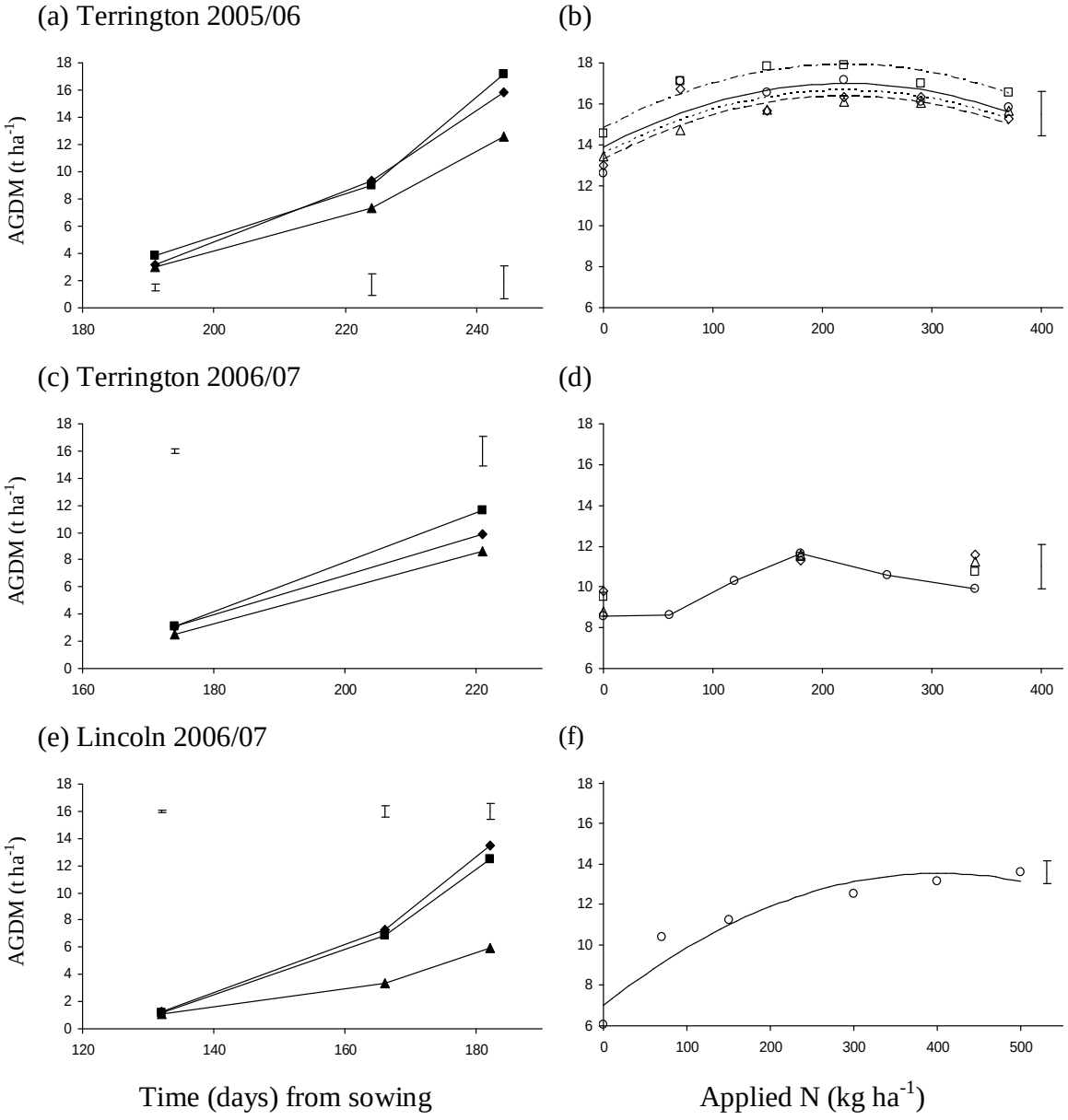
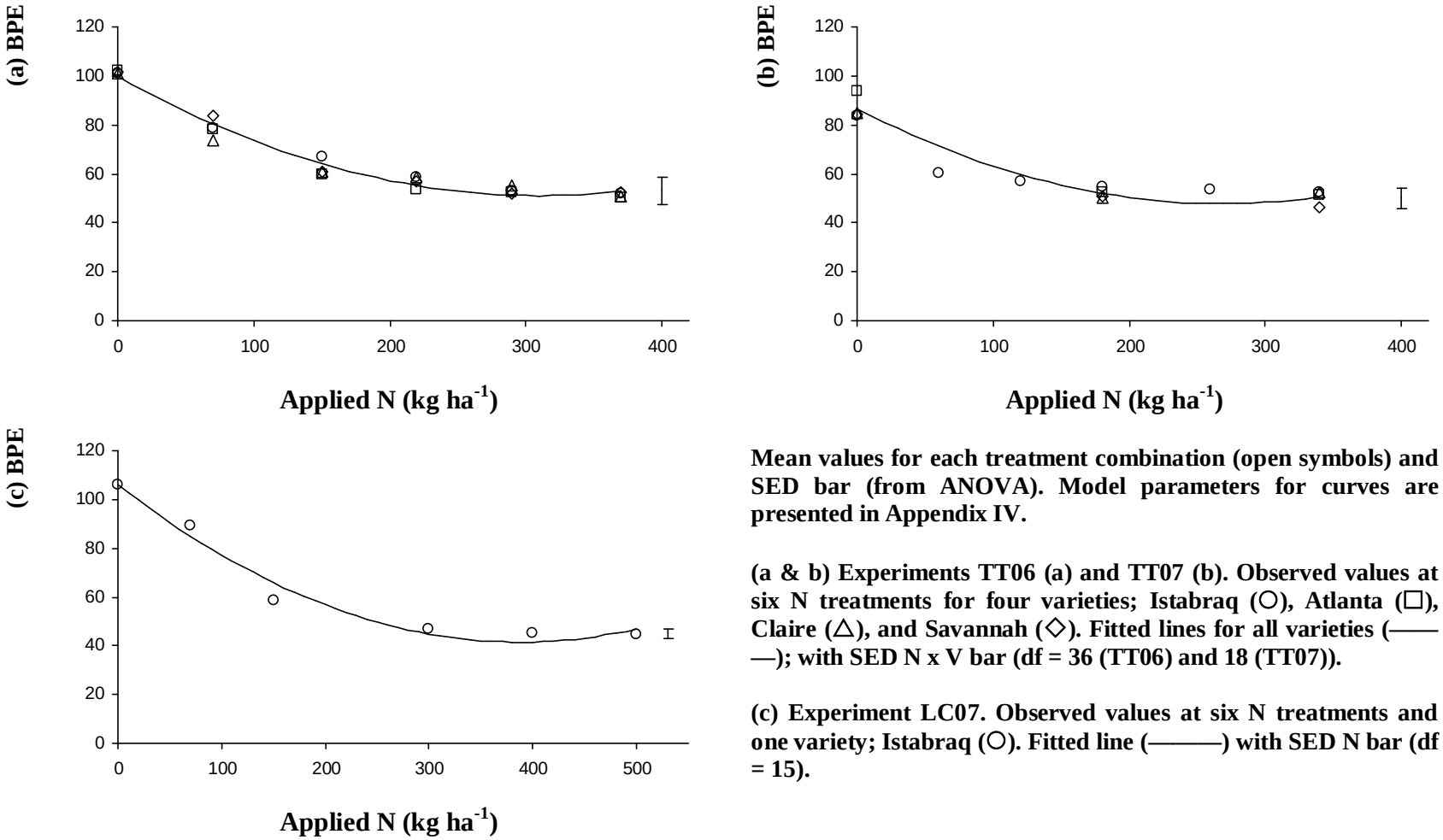


Figure 5.13 (a, c, e) Effect of applied N on the above-ground DM (AGDM; t ha^{-1}) accumulated between GS31 and anthesis in TT06, TT07 and LC07. Observed values for Istabraq at three growth stages; 31, 39, 61 and at three N treatments; zero (▲), optimum (■), and maximum (◆); with SED N bar at each growth stage (df = 2, 10 and 10 (TT06); 2 and 10 (TT07); 20, 25 and 25 (LC07), respectively).

Figure 5.14 (b, d, f) Effect of applied N and variety on the above-ground DM (AGDM; t ha^{-1}) at anthesis in TT06, TT07 and LC07. Observed values at six N treatments for one (LC07) or four (TT06 and TT07) varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------); with SED bars for N (LC07; df = 25) and N x V (df = 36 (TT06) and 18 (TT07)). Model parameters for curves are presented in Appendix IV.



Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values at six N treatments for four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for all varieties (———); with SED N x V bar (df = 36 (TT06) and 18 (TT07)).

(c) Experiment LC07. Observed values at six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

Figure 5.15 (a, b & c) Effect of applied N and variety on the biomass production efficiency (BPE) at anthesis in TT06, TT07 and LC07.

5.3.11 Radiation-use efficiency

Radiation-use Efficiency (RUE; g DM MJ^{-1}) was calculated as the slope of the linear relationship between intercepted AGDM and PAR at samplings between GS31 and anthesis. RUE for Istabraq during this period was affected by N treatment in TT06 ($P<0.05$), and was higher at the N opt-trt than the N zero-trt in TT07 and LC07. Thus RUE increased from the zero to optimum N treatment in TT06 from 2.73 to 3.10 g MJ^{-1}, in LC07 from 2.15 to 3.06 g MJ^{-1}, and in TT07 from 2.51 to 3.12 g MJ^{-1}.

In the Terrington experiments, RUE differed between varieties in TT06 ($P<0.05$) but not in TT07 ($P<0.11$); the interaction was not significant in either experiment. Averaging across N treatments, in TT06 Savannah had lower RUE (2.63 g MJ^{-1}) than Claire, Istabraq or Atlanta (range 2.95-2.97 g MJ^{-1}); cf. TT07 with non-significant differences (range 2.41 to 2.86). However, in both years there was tendency for Claire to show higher RUE than Savannah.

5.3.11.1 Relationship between RUE and SLN

A bi-linear model was fitted to the RUE and SLN data for Istabraq in all three site-seasons (Figure 5.16), with the slope of phase 2 fixed to zero (i.e. where RUE does not increase with increasing SLN) using the methods described in section 3.7.3. Data for the Terrington experiments was combined to increase the goodness of fit of the model. The breakpoint of these two lines is the minimum SLN required to maximise RUE (Table 5-3).

Table 5-3 Estimates of model parameters for the bi-linear model fitted to data for the regression of radiation-use efficiency (RUE; GS31 to anthesis) on specific leaf N content (SLN; all lamina at anthesis) for Istabraq.

Exp.	Slope 1	Slope 2	Breakpoint (SE)		% Variance accounted (SE)
			SLN	RUE	
Terrington	0.794 ± 0.282	0	2.13 (0.293)	3.10 (0.122)	31.5 (0.44)
Lincoln	2.310 ± 1.270	0	1.97 (0.182)	2.87 (0.129)	27.3 (0.54)

The estimates of the model parameters were associated with relatively high SE values due to the limited data available, and also due to the effect that the RUE was calculated for the period from GS31 to anthesis whilst the SLN was measured at anthesis. However, there is some consistency in breakpoint values across sites and seasons, and with previous studies (Foulkes *et al*., 2006; Hay and Porter, 2006). Model fitting to data for the relationship between RUE and the N content of the other crop components (i.e. leaf sheath, true stem and ear) did not optimise in all three experiments allowing the prediction of the breakpoint for these plant organs.

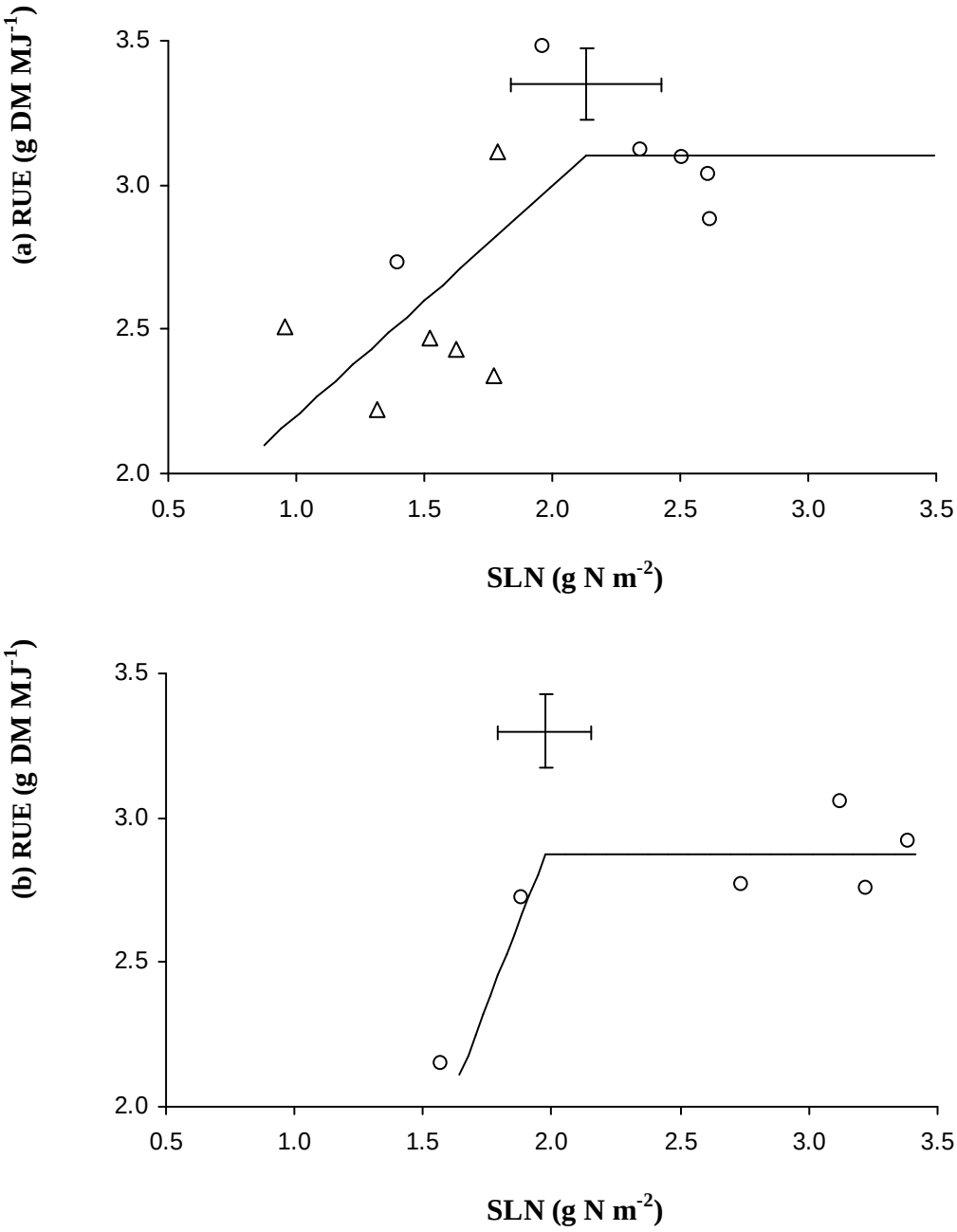


Figure 5.16 Relationship between radiation-use efficiency (RUE; GS31 to anthesis) and specific lamina N (SLN, all lamina at anthesis) for Istabraq at (a) Terrington (TT06 and TT07), and (b) Lincoln sites.

Observed values for each treatment (open symbols) and fitted 'bi-linear' model to data for Istabraq: (a) TT06 (○) and TT07 (△), and (b) LC07 (○). Values presented for six N treatments and one variety (Istabraq). Fitted lines to Istabraq (———). Bars represents SE at breakpoint.

5.3.12 Determining the critical crop N% for biomass production

To calculate a specific critical dilution curve for all the varieties used in this study would require the AGDM and N% to be measured over a range of N treatments for each variety for several sample stages leading up to and including anthesis. As there are insufficient data from these experiments to fit such curves, the data where available (Istabraq at GS31, GS39 and GS61, and all varieties at GS61 in relevant experiments) were compared to the critical N dilution curve for winter wheat reported by Justes *et al.* (1994) to validate its application in this study for estimating the critical crop N% and the crop N nutrition index, to then be applied across a wider range of treatment combinations.

5.3.12.1 Critical N concentration for biomass production

Justes *et al*. (1994) calculated the critical crop N concentration at a given point in time from the relationship between AGDM and the crop N% for a range of N treatments (Figure 5.17). The relationship between AGDM and N% was described as a bi-linear relationship composed of: (1) an oblique regression line corresponding to the increase in AGDM with the increase in N%, and (2) a vertical line corresponding to an increase of crop N% without any increase in AGDM. The critical N% ($N\%_{crit}$) is determined by the ordinate of the intersection point of the two lines and the critical dilution curve is obtained from plotting these points estimated at sequential samplings. The nitrogen nutrition index (NNI) is calculated as '$NNI = N_{actual} / N_{critial}$' (Equation 2-7).

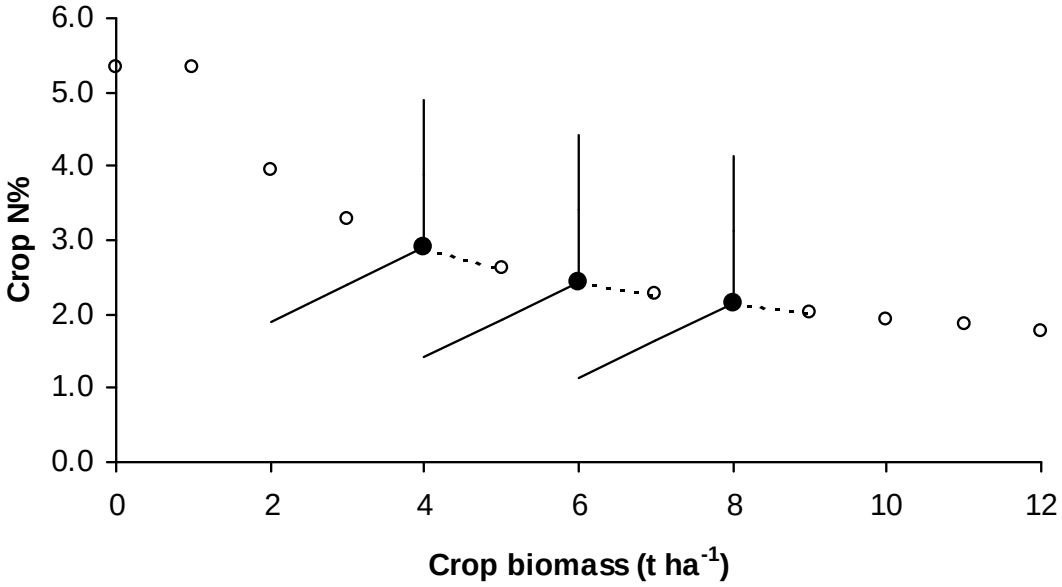


Figure 5.17 The critical N dilution curve for winter wheat. (Redrawn from Justes *et al*., 1994).

5.3.12.2 Determination of the critical N% intersection points from experiments in this study

From the experimental data in the present study an intersection point can be determined at each sequential sampling where the crop N% and AGDM are determined for all six N treatments for a given variety in an experiment. A bi-linear model was fitted to these data using the methods described in 3.7.3. The two lines intersect at the critical N%, where the NNI=1 (data presented in Table 5-4). These intersection points are plotted with SE bars, for each variety together with the critical dilution curve of Justes *et al*. (1994) on the same figure (Figure 5.18).

Intersection points were estimated for Istabraq at GS31 (TT06 and TT07), GS39 (TT06 and LC07) and anthesis (all three experiments). At GS31 the estimated critical N% was similar (TT06) or slightly lower (TT07) to that from the critical dilution curve of Justes *et al*. (1994). Data for LC07 at GS31 were not fitted to the model as the observed AGDM was 1.20 t ha^{-1} which is lower than the minimum of 1.55 t ha^{-1} for the dilution curve reported by Justes *et al*. (1994). At GS39 the critical N% for TT06 was again similar to

that predicted from the dilution curve of Justes *et al*. (1994), and the observed N% at the breakpoint for LC07 was slightly higher possibly due to the high rates of applied N or the different light environment at Lincoln compared with either the UK and/or France. At anthesis the N% at the breakpoint for TT06 and LC07 was slightly lower, and that for TT07 was slightly higher than predicted from the dilution curve (Justes *et al*., 1994).

In the Terrington experiments the critical N% was estimated for all varieties at anthesis, although in one case (Savannah in TT06) data could not be fitted successfully to the model (Table 5-4). In TT06, N% values for the three varieties were similar to that expected from the critical dilution curve. In TT07, breakpoints were based on fitted models using data from only 3 N treatments (as N% data was only available at 3 treatments) which lowered the % variance accounted for by the model. However, the observed N% for Istabraq and Claire was similar to that predicted from the critical dilution curve, whereas the observed N% for Atlanta and Savannah was slightly below that predicted.

Overall, there was sufficient similarity between the estimated critical N% from the curve fitting in the present study and that predicted from the critical dilution curve of Justes *et al*. (1994) that the critical dilution curve could be applied with confidence to present data to estimate the critical crop N% and the NNI from measured AGDM. The N theoretically required to maximize biomass production could then be compared to the actual crop N uptake across a range of treatment combinations.

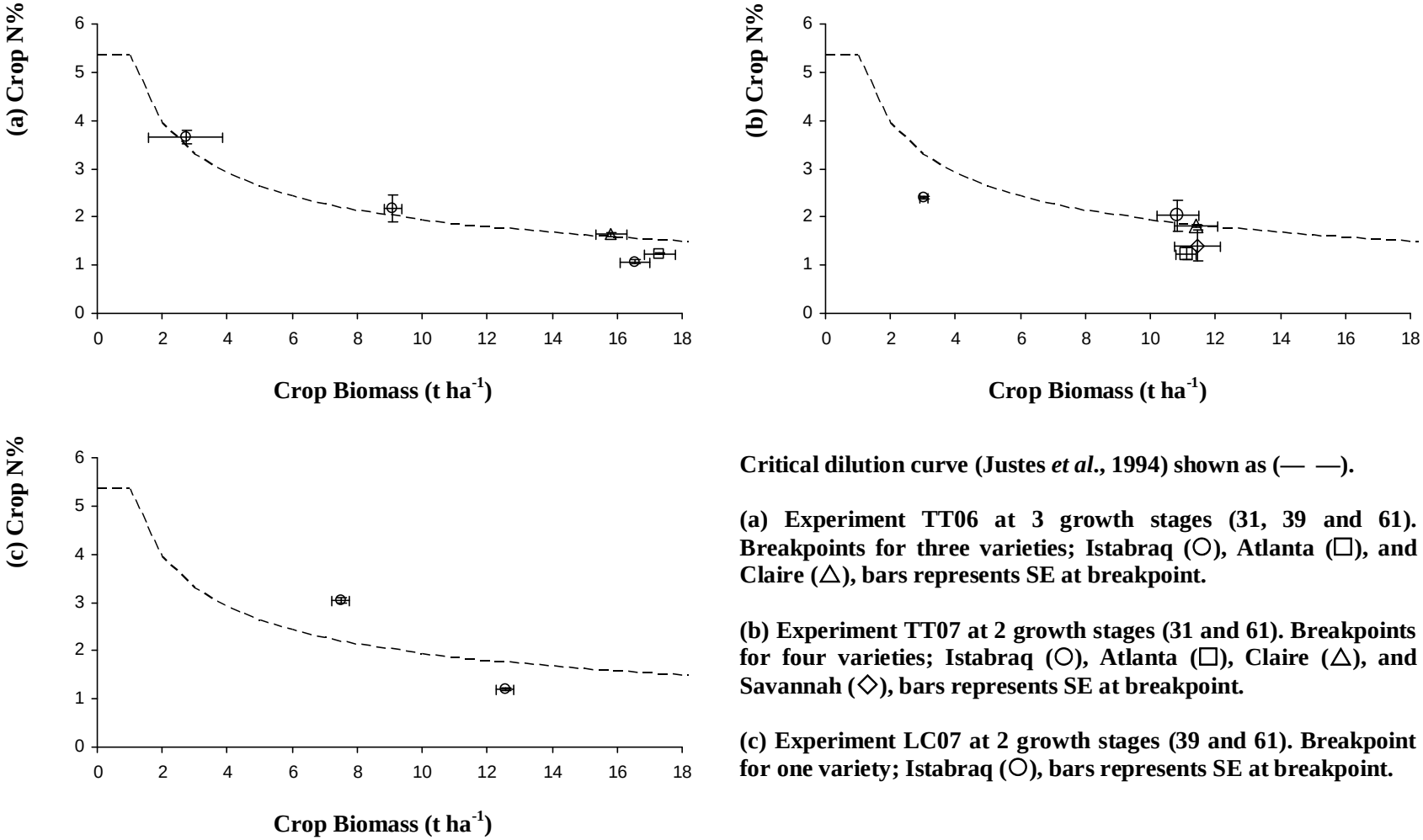
5.3.12.3 Critical N dilution curves and model fitting

Table 5-4 Critical N% for biomass production estimated from the bi-linear linear model for the relationship of crop N% on above-ground dry DM (AGDM) in TT06, TT07 and LC07. For varieties: Istabraq (Is), Atlanta (At), Claire (Cl) and Savannah (Sa).

Exp.	GS	Var.	Intersection point (SE)		% Variance accounted (SE)	Expected N% from Justes
			AGDM	N%		
TT06	31	Is	2.7 (1.15)	3.66 (0.15)	20.9 (0.47)	3.43
	39	Is	9.1 (0.27)	2.17 (0.27)	28.8 (0.94)	2.02
	61	Is	16.6 (0.47)	1.07 (0.04)	46.0 (1.82)	1.55
	61	At	17.3 (0.49)	1.23 (0.02)	22.0 (1.91)	1.52
	61	Cl	15.8 (0.47)	1.64 (0.05)	13.1 (1.67)	1.58
	61	Sa	-	-	-	-
TT07	31	Is	3.0 (0.13)	2.4 (0.04)	- (0.50)	3.28
	61	Is	10.8 (0.65)	2.03 (0.33)	8.1 (1.62)	1.87
	61	At	11.1 (0.31)	1.24 (0.12)	51.7 (0.77)	1.85
	61	Cl	11.4 (0.68)	1.81 (0.03)	26.9 (1.66)	1.82
	61	Sa	11.5 (0.70)	1.40 (0.315)	4.2 (1.76)	1.82
LC07	39	Is	7.5 (0.26)	3.03 (0.05)	77.7 (0.81)	2.20
	61	Is	12.6 (0.27)	1.20 (0.03)	85.9 (1.08)	1.75

5.3.13 N nutrition index

At a given crop AGDM, the crop N% at which NNI=1 can be estimated using the critical dilution curve (Equation 2-6) to give the 'critical N%'. The ratio between the 'actual N%' (determined in the experiment) and the 'critical N%' gives the NNI. The NNI for Istabraq at anthesis was affected by N treatment in all three experiments ($P<0.001$) (Figure 5.19). Averaging across N treatments, TT06 and LC07 had similar NNI both at 0.97, and TT07 had the lowest at 0.86. LC07 had the lowest NNI at the N zero-trt at 0.37 but the highest NNI at the optimum and maximum N treatments at 1.23 and 1.33, respectively; cf. TT06 at 0.57, 1.12 and 1.22, respectively; and TT07 at 0.58, 1.01 and 0.98, respectively. In the Terrington experiments, the NNI was affected by variety in TT06 ($P<0.01$) with a trend for varietal differences in TT07 ($P<0.07$); there was no interaction in either experiment. Averaging across N treatments, in TT06 Atlanta had the highest NNI (1.05), then Claire (0.98), and Istabraq and Savannah (both 0.97); cf. TT07 showed non-significant varietal pattern in the range 0.86 (Istabraq) to 0.96 (Savannah).



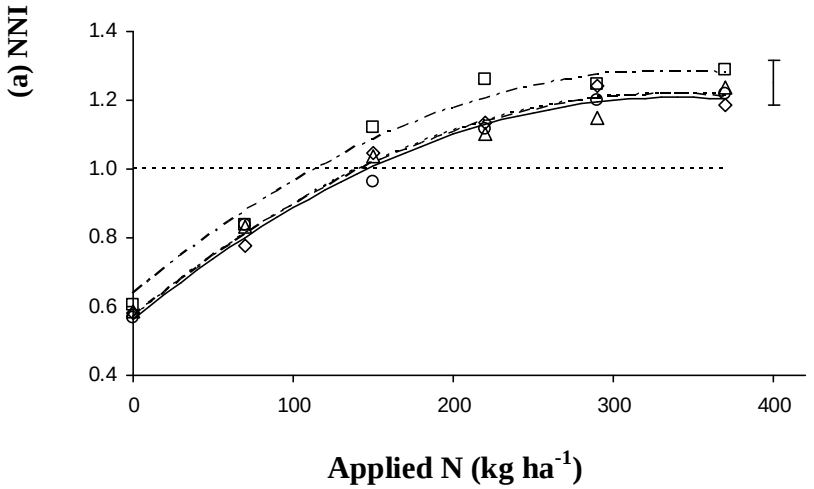
Critical dilution curve (Justes *et al.*, 1994) shown as (— —).

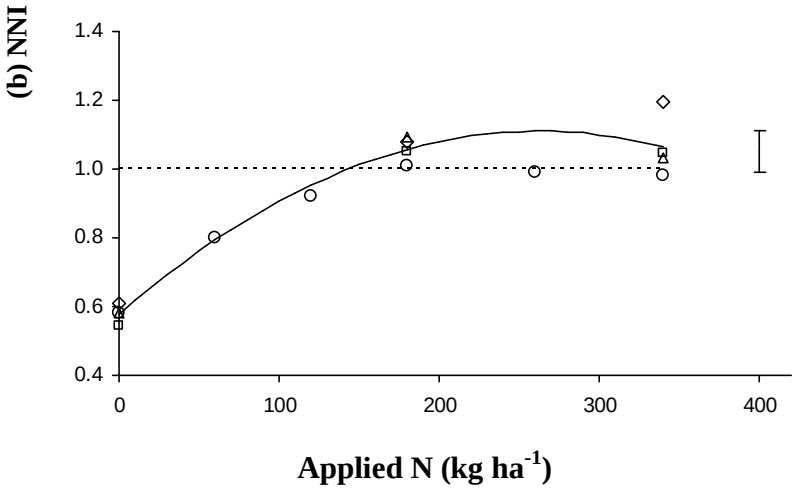
(a) Experiment TT06 at 3 growth stages (31, 39 and 61). Breakpoints for three varieties; Istabraq (○), Atlanta (□), and Claire (△), bars represents SE at breakpoint.

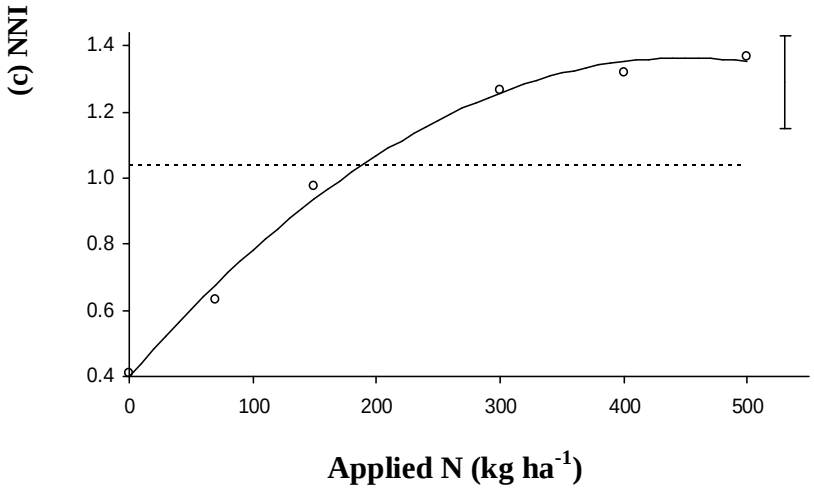
(b) Experiment TT07 at 2 growth stages (31 and 61). Breakpoints for four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇), bars represents SE at breakpoint.

(c) Experiment LC07 at 2 growth stages (39 and 61). Breakpoint for one variety; Istabraq (○), bars represents SE at breakpoint.

Figure 5.18 (a, b & c) Validating the critical dilution curve for winter wheat (Justes *et al.*, 1994) for application in this study.







Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV. Line of NNI = 1 shown as horizontal dashed line (— —) .

(a & b) Experiments TT06 (a) and TT07 (b). Observed values at six (TT06) or three (TT07) N treatments for four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for varieties; in TT06 - Istabraq (———), Atlanta (— – —), Claire (— —), and Savannah (-------) with SED N x V bar (df = 36); in TT07 – all varieties (———); with SED NxV bar (df = 18).

(c) Experiment LC07. Observed values for six N treatments and one variety; Istabraq (○). Fitted line for Istabraq (———); with SED for N (df = 15).

Figure 5.19 (a, b & c) Effect of applied N and variety on the N nutrition index (NNI) in TT06, TT07 and LC07.

5.3.13.1 Estimation of the amount of applied N and crop variables at NNI =1

By using the fitted curves for the NNI response to N supply (Figure 5.19), the amount of applied N where NNI=1 could be estimated for each variety in each experiment where data were available for all 6 N treatments (i.e. all varieties in TT06, and Istabraq only in TT07 and LC07) (Table 5-5). At this amount of applied N the values of GAI, LAI, AGDM and N% were estimated using the relevant regression curve of the variable on applied N. Finally, an estimated AGN at NNI=1 was calculated (i.e. from AGDM x N%). In TT07 the grand mean was used for AGDM as there was no effect of N supply.

Table 5-5 Estimated amount of applied N (kg ha^{-1}), green area index (GAI), leaf area index (LAI), above-ground DM (AGDM; t ha^{-1}) and N%, and calculated above-ground N (AGN; kg ha^{-1}) in the crop at anthesis at NNI=1 for each variety in TT06, TT07 and LC07.

Exp.	Variety	Applied N	GAI	LAI	AGDM	N%	AGN
TT06	Istabraq	147	6.35	4.15	16.64	1.59	264.2
	Atlanta	114	6.94	5.02	17.18	1.48	254.0
	Claire	143	6.80	4.46	15.98	1.58	251.7
	Savannah	141	6.60	4.45	16.23	1.57	254.7
TT07	Istabraq	143	5.75	3.91	10.52	1.83	192.3
LC07	Istabraq	189	4.37	3.02	11.62	1.80	208.9

5.3.13.2 Partitioning of AGN between crop components where crop NNI = 1

At the estimated amount of applied N where NNI=1, the amount of N in each canopy component was estimated using fitted curves for the response of the crop component N content to N supply for each variety in all three experiments at anthesis (Table 5-6). The 'sum' of the N content of the four crop components gives an approximated AGN (comparable with that calculated in Table 5-5) which will be used in calculations of the proportion of AGN in each canopy component at NNI=1 (Table 5-7).

Table 5-6 Estimated amount of N (kg ha^{-1}) in each crop component at NNI=1 at anthesis for each variety in TT06, TT07 and LC07.

Exp.	Variety	Lamina	Sheath	True stem	Ear	Sum
TT06	Istabraq	93.0	38.6	70.6	54.8	257.0
	Atlanta	92.5	34.9	64.2	72.2	263.8
	Claire	89.6	38.1	69.9	56.2	253.9
	Savannah	90.3	37.9	69.5	53.7	251.5
TT07	Istabraq	67.6	36.0	57.8	30.4	191.8
LC07	Istabraq	87.5	38.9	43.4	42.6	212.5

Table 5-7 Estimated proportion of above-ground N in each crop component at NNI=1 at anthesis for each variety in TT06, TT07 and LC07.

Exp.	Variety	Lamina	Sheath	True stem	Ear
TT06	Istabraq	0.36	0.15	0.27	0.21
	Atlanta	0.35	0.13	0.24	0.27
	Claire	0.35	0.15	0.28	0.22
	Savannah	0.36	0.15	0.28	0.21
TT07	Istabraq	0.35	0.19	0.30	0.16
LC07	Istabraq	0.41	0.18	0.20	0.20

5.3.13.3 Comparison of N uptake and partitioning in the crop at NNI=1 with optimum and maximum N treatments at anthesis

The estimated amount of N in the crop at NNI=1 can be compared with the observed amount of N in the crop at the optimum and maximum N treatments for each variety in all three experiments (Table 5-8). The difference in the AGN and the amount of N in each crop component estimates the quantity of N deficiency or excess for crop and plant organ, respectively. The change in the proportion of AGN in each component gives the

response to N supply, and whether the component responds proportionately or disproportionately to the increase or decrease in N content.

In comparison with the crop at NNI=1, the AGN at anthesis for Istabraq in all three experiments was higher at the optimum and maximum N treatments (except the N max-trt in TT07) by on average 41 and 52 kg N ha^{-1}, respectively. Averaging across the optimum and maximum N treatments, LC07 had more excess AGN (72 kg ha^{-1}), than TT06 (41 kg ha^{-1}), or TT07 (10 kg ha^{-1}). LC07 had most excess N in comparison with the crop at NNI=1 at both the optimum and maximum N treatments likely due to the higher N application rates compared with TT06 and TT07. At the maximum N treatment, the crop at TT07 had slightly lower AGN (-2 kg ha^{-1}) compared with the crop at NNI=1. In TT06 Atlanta had a larger amount of excess AGN (69 kg ha^{-1}) than other varieties in the range 38 to 41 kg ha^{-1} ($P<0.01$).

The amount of N in all crop components for Istabraq in all three experiments was in excess of that at NNI=1 at both the optimum and maximum N treatments (except N max-trt in TT07 all components). Averaging across experiments, N in the leaf lamina was most in excess by 15 and 19 kg ha^{-1}; then the true stem by 10 and 12 kg ha^{-1}, the leaf sheath by 9 and 10 kg ha^{-1}, and the ear by 2 and 4 kg ha^{-1}, respectively. In TT06 Atlanta had more excess leaf lamina N (24 kg ha^{-1}) than the other varieties (range 10 to 15 kg ha^{-1}) ($P<0.05$). Although there were no significant varietal differences for the other crop components, Atlanta tended to have more excess N in the other plant organs as well. There was little change in the proportion of AGN in each canopy component for Istabraq in all three experiments at both N treatments compared to NNI=1. Overall the lamina and sheath increased by 0.01, the true stem was unchanged, and the ear decreased by 0.02, and there were no statistically significant varietal differences in these overall changes in the proportion of AGN in each crop component of the two N treatments compared to NNI=1.

Table 5-8 Change in the amount of N and proportion of N between the estimated amount of N in the crop or crop components of NNI=1 and the observed amount of N at the optimum and maximum N treatments in each canopy component at anthesis for each variety in TT06, TT07 and LC07.

			Change in amount of N (kg ha^{-1})					Change in proportion of N			
Exp.	**N-trt**	**Variety**	**Lamina**	**Sheath**	**Tr. stem**	**Ear**	**AGN**	**Lamina**	**Sheath**	**Tr. stem**	**Ear**
TT06	Optimum	Istabraq	12.4	8.8	12.9	0.4	34.5	0.00	0.01	0.01	-0.03
		Atlanta	19.4	11.4	25.0	16.0	71.8	-0.02	0.00	0.02	-0.01
		Claire	8.8	0.2	7.5	6.1	22.4	0.00	-0.01	0.00	0.00
		Savannah	11.3	5.5	15.1	3.4	35.2	0.00	0.00	0.02	-0.01
TT06	Maximum	Istabraq	15.9	8.8	18.0	4.4	47.1	0.00	0.01	0.02	-0.02
		Atlanta	27.6	10.7	21.0	7.1	66.5	0.01	0.01	0.02	-0.04
		Claire	12.0	12.4	21.2	6.9	52.6	-0.02	0.01	0.02	-0.02
		Savannah	18.4	5.6	14.2	1.6	39.7	0.02	0.00	0.01	-0.03
TT07	Optimum	Istabraq	6.9	4.5	7.7	2.4	21.5	0.00	0.00	0.00	0.00
TT07	Maximum	Istabraq	2.4	-0.4	-3.8	-0.2	-2.0	0.01	0.00	-0.02	0.00
LC07	Optimum	Istabraq	26.7	14.4	10.2	3.5	54.8	0.02	0.02	0.00	-0.03
LC07	Maximum	Istabraq	40.0	20.9	21.3	7.3	89.5	0.01	0.01	0.01	-0.04
Average across 2 N-trts											
TT06		Istabraq	14.1	8.8	15.5	2.4	40.8	0.00	0.01	0.01	-0.02
TT06		Atlanta	23.5	11.1	23.0	11.6	69.1	0.00	0.01	0.02	-0.02
TT06		Claire	10.4	6.3	14.3	6.5	37.5	-0.01	0.00	0.01	-0.01
TT06		Savannah	14.8	5.5	14.7	2.5	37.5	0.01	0.00	0.02	-0.02
TT07		Istabraq	4.7	2.1	1.9	1.1	9.8	0.01	0.00	-0.01	0.00
LC07		Istabraq	33.4	17.7	15.8	5.4	72.2	0.01	0.02	0.00	-0.03
Average across varieties											
TT06		Optimum	12.9	6.5	15.1	6.5	41.0	0.00	0.00	0.01	-0.01
TT06		Maximum	18.5	9.4	18.6	5.0	51.5	0.00	0.01	0.02	-0.02

5.4 DISCUSSION

The first part of the discussion considers the response to N supply by Istabraq in the three site-seasons in relation to N uptake and partitioning, green area production, light interception, and radiation-use efficiency as drivers of biomass production, and varietal responses to N supply in the Terrington experiments. The second part considers the N nutrition status of the crop during the stem-extension period using the critical N content and N nutrition index models, and reviews the hypotheses addressed in this chapter.

5.4.1 Crop development and shoot production

Istabraq at Lincoln reached GS31 more quickly than at Terrington (132 and 174-191 days respectively), associated with higher over-winter temperatures. The stem-extension phase (GS31 to anthesis) was also shorter at Lincoln (47 days) than at Terrington (50-53 days). In the Terrington experiments, Atlanta reached anthesis 3-4 days earlier than the other varieties.

Plant establishment was broadly similar between the three site-seasons (range 172-204 m^{-2}), and differences in plant establishment could not explain site-season effects reported in this chapter. The crop at Lincoln produced fewest fertile shoots at GS31, probably due to the low SMN at Lincoln, and in the Terrington experiments Savannah consistently produced more potentially fertile shoots than Atlanta. Higher N supply increased tiller survival during the stem-extension phase in each experiment, particularly in LC07 where there was no significant tiller death at the optimum and maximum N treatments. Comparing between the Terrington experiments, dull and wet weather conditions in May 2007 were associated with reduced tiller survival resulting in fewer shoots at anthesis at all N treatments. Although high tiller death represented a loss of DM, the majority of N would be mobilised to the surviving tillers (Austin *et al.*, 1977; Thorne and Wood, 1987).

The site-season and N treatment effects on ear density at anthesis were considerably greater than varietal differences. Overall the crop at TT06 produced more ears per m^2 at

anthesis (514) than at LC07 (438), which in turn produced more than at TT07 (339). Ear density at the N opt-trt was sufficient in TT06 and LC07 to produce a high-yielding crop. However, in TT07 low ear density resulting from high tiller death for all varieties may have restricted yields below the attainable yield of the site-season. In TT06, Claire produced slightly more ears than other varieties (averaged across N treatments 569 m^{-2} cf. other varieties in the range 514-534 m^{-2}) associated with higher tiller survival. However, varietal differences in fertile shoot production tended to be either small or non-significant, likely due to the similar dates of release and some common parentage for the four varieties.

5.4.2 Crop N uptake

Crop N uptake at GS31 typically represents up to 25% of the N taken up by winter wheat crops by harvest (Sylvester-Bradley *et al.*, 2008). There were considerable differences between sites in early-season N uptake, with overall AGN at Lincoln around half that of Terrington at 31 and 73 kg N ha^{-1}, respectively, due to lower SMN at Lincoln (previously fallow grass) compared with Terrington (previously fertilised OSR). However, these differences were also partly a consequence of the N application regime and timing.

The majority of N is normally taken up between GS31 and flag-leaf emergence comprising around 40% of the AGN at harvest (Karlen and Sadler, 1990), and by anthesis AGN represents around 70-90% of the total crop N uptake at harvest (Austin *et al.*, 1977; Dalling, 1985; Oscarson *et al.*, 1995). Averaged across site-seasons at anthesis, AGN increased considerably from the N zero-trt (93 kg ha^{-1}) to the N opt-trt (256 kg ha^{-1}) but thereafter only marginally to the N max-trt (265 kg ha^{-1}). Site-season differences for AGN at anthesis overall followed a similar pattern to ear density: highest in TT06 (240 kg ha^{-1}) then LC07 (206 kg ha^{-1}) and TT07 (169 kg ha^{-1}). N uptake at LC07 was lower than other experiments in the unfertilised crop due to low SMN availability, whereas in the fertilised treatments AGN was lower at TT07 than in other experiments associated with lower fertiliser N recovery.

Above-ground N at the zero and sub-optimal N treatments was limited by N availability, whereas at the optimal and supra-optimal N treatments it was limited by the rate of N uptake and assimilation, which in turn depended on crop growth and environmental conditions. At the supra-optimal N treatments, the maximum rate of N uptake during the stem-extension period was 5.6 kg m^{-2} day^{-1} in LC07 cf. 3.9 and 2.2 kg m^{-2} day^{-1} in TT06 and TT07, respectively. Rates of N uptake in TT06 and LC07 are consistent with the findings of Sinclair and Amir (1992) of approximately 4-5 kg m^{-2} day^{-1} (equivalent to N uptake of around 200-250 kg ha^{-1} during the stem-extension phase). In TT07 a lower rate of N uptake possibly related to dull conditions in May 2007 reducing fertiliser recovery, and to lower plant establishment than in TT06.

Overall there were only small varietal differences in AGN at anthesis in TT06 (in the range 236-271 kg ha^{-1}) and no statistically significant differences in TT07. Although genetic variation in N uptake at anthesis was reported in the GREEN Grain data set in GGTT07 ($P<0.001$), the largest differences in winter wheat are usually observed between old and new cultivars (Foulkes *et al*., 1998; White *et al*., 1998) or between semi-dwarf and tall genotypes (Austin *et al*., 1977). However, the cultivars in this study are all modern, semi-dwarf, feed/biscuit wheats with similar release dates and anthesis dates, and this may partly explain why varietal differences in N uptake were relatively small compared to some varietal ranges reported in previous investigations.

5.4.3 Crop N partitioning

Averaging across experiments at anthesis, between the zero and optimum N treatments the proportion of AGN in the leaf lamina increased from 0.31 to 0.38, and in the ear decreased from 0.26 to 0.17, while the proportion in the leaf sheath and true stem changed little with N supply (0.16 to 0.18, and 0.26 to 0.27, respectively). With increasing N supply above the N opt-trt, the proportion of AGN in all of the crop components was unchanged. These results confirm previous findings that the plant organ containing most canopy N at anthesis is the leaf lamina (Grindlay, 1997) and that a significant amount of N is also contained in the true stem (Critchley, 2001; Wilhelm *et*

al., 2002). Since the N concentration was much higher in leaf lamina than the stem, variations in the leaf to stem ratio were the main cause of variation in the N content of plants of a given weight.

Overall the crop at Lincoln had relatively more AGN in the leaf lamina and less in the true stem compared to those at Terrington. More N in the leaf lamina at Lincoln was possibly due to the higher light intensity at this site increasing the requirement for lamina N to maximise photosynthetic efficiency. Less N in the true stem at Lincoln was associated with reduced height compared with the Terrington experiments. Comparing seasons at Terrington, averaging across N treatments the crop at TT06 had more N in the ear (21%) and less in the leaf sheath (15%); cf. TT07 (17% and 19%, respectively). In TT06 overall Atlanta partitioned more N to the ear (27%) and less to the leaf sheath (13%) and true stem (25%) compared to the other varieties (ranges 21-23%, 14-15%, and 28-29% respectively), while the leaf lamina was similar (range 34-35%); with a similar trend observed in TT07.

Although results indicated the proportion of AGN in the ear was apparently higher in Atlanta than the other varieties, this should be treated with caution as Atlanta reached anthesis 3-4 days earlier than the other varieties, and all varieties were sampled on the same calendar date. Thus, Atlanta was sampled at 'GS61+3d' and this may have favoured N accumulation in the ear. Atlanta was also the shortest cultivar in both experiments and this could partly account for relatively less N in the leaf sheath and true stem. Overall, there was little varietal difference in N partitioning between the four varieties, and the difference between Atlanta and the other three varieties may have been partly due to timing to anthesis in relation to sampling date rather than intrinsic physiological effects.

5.4.4 Green canopy area production

Green area index at GS31 was higher at Terrington than Lincoln reflecting the difference in SMN supply between sites. The majority of the canopy area was formed by GS39, reaching its maximum at around anthesis. Overall GAI nearly doubled between the zero

and optimum N treatments but remained unchanged up to the N max-trt. As expected in the N-limited treatments, N shortages were expressed through restricted canopy expansion (Grindlay, 1997) whilst at the optimal and supra-optimal N treatments the canopy reached its potential size. At the N opt-trt, GAI was lower at Lincoln than Terrington (5.1 and 6.6, respectively) associated with a shorter stem extension period, and at the sub-optimal N treatments with low SMN, whereas overall GAI was similar between seasons at Terrington (range 5.8-5.9). In TT06 Atlanta had higher GAI than other varieties associated with higher AGN. Overall, though genetic variation in canopy size was observed, it was small and was not consistent across seasons.

Canopy N requirement (amount of N per unit of green area) at anthesis averaged across experiments for Istabraq, increased from the zero (22.4 kg ha^{-1}) to the optimum N treatment (37.7 kg ha^{-1}) but thereafter increased only slightly to the N max-trt (38.8 kg ha^{-1}). Sylvester-Bradley *et al.* (1997) suggested that the CNR was relatively constant at around 30 kg ha^{-1} for UK-grown winter wheat. However, this excluded unfertilised and excessively fertilised crops. Other studies have also shown an effect of N supply on CNR (Stokes *et al.*, 1997; Foulkes *et al.*, 1998), and Grindlay *et al.* (1993) found that CNR of unfertilised wheat was reduced to 22-25 kg ha^{-1} while the CNR of well fertilised wheat was increased up to 51 kg ha^{-1}.

Crop N requirement was considerably higher in Lincoln than Terrington, and in turn higher in TT06 than TT07. Differences between sites and seasons were also reported by Sylvester-Bradley and Chambers (1992) who were able to explain only a minority of the variation between site-seasons. Some of these differences may partly be related to the light environment of the site-seasons, whereby light intensity was higher at Lincoln than Terrington, and in turn higher at TT06 than TT07. Higher light intensity may have increased the optimal N content of the green canopy required for light-saturated photosynthesis to occur. At the N max-trt, CNR in TT06 and LC07 was observed to reach 40.1 and 50.3 kg ha^{-1}, respectively, indicating luxury uptake and accumulation of excess N within the canopy (Grindlay *et al.*, 1993). In the Terrington experiments there were varietal differences in CNR, but these were not consistent across seasons. However,

Atlanta tended to have a lower CNR with relatively more canopy N allocated to the ear (which is not included in the CNR calculation) compared to the other varieties. In general, as there was little varietal difference in N uptake (excluding ear N) at anthesis in both seasons, the varietal pattern in CNR was similar to the inverse of that for GAI.

In all three site-seasons SLN increased with N supply between the zero and optimal N treatments but was unchanged at the N max-trt; averaged across site-seasons at 1.31, 2.50 and 2.54 g N m^{-2}, respectively. Overall SLN was higher at Lincoln (2.69 g N m^{-2}) than at Terrington, and higher in TT06 (2.17 g N m^{-2}) than TT07 (1.42 g N m^{-2}). However average values in all three site-seasons were close to the range 1 to 2 g N m^{-2} suggested for optimisation of photosynthetic function (Field and Mooney, 1986) thought to be controlled through limitation of leaf expansion in N-limited conditions (Grindlay, 1997). At the N opt-trt, SLN in TT06 (2.51 g N m^{-2}) and LC07 (3.13 g N m^{-2}) were higher than the reported optimal range for photosynthesis, suggesting reserve N accumulation in the leaf lamina, while the corresponding value in TT07 was lower at 1.79 g N m^{-2}. These results are broadly consistent with previous studies on UK-grown winter wheat at non-N limited supply which have reported ranges in SLN of 2.1-2.2 (Critchley, 2001), 2.3-3.6 (Foulkes *et al*., 1998), and 3.0-3.5 g N m^{-2} (Whaley, 2001). Varietal effects were observed in the Terrington experiments, but again the varietal pattern was not consistent across seasons. Varietal differences in SLN were mainly the consequence of differences in lamina area, and there was a similar inverse relationship between the varietal patterns of SLN and LAI in both seasons.

The vertical distribution of SLN between the leaf layers for Istabraq was not uniform, with the upper leaves (flag and leaf two) having higher SLN than the 'leaf 3 and below' at all N treatments in all experiments. Comparing the vertical distribution of SLN between experiments at the N opt-trt, the gradient in SLN with leaf layer was observed to be different; LC07 had a steeper gradient than TT06 which in turn had a steeper gradient than TT07. Previous studies have shown decreased SLN with increasing GAI deeper into the canopy for wheat (Grindlay *et al*., 1997), and the gradient follows the light availability profile within the canopy. However, the high SLN observed in the upper

leaves may be more than required to optimise the photosynthesis and the lamina may therefore be acting as a reservoir for reserve N accumulation. Additionally SLN in the shaded leaves at the bottom of the canopy may be greater than the optima predicted from photosynthesis/canopy light distribution models (Sinclair and Horie, 1989) possibly as the predicted optimum SLN levels are below that required for maintenance (Hirose and Werger, 1987) and/or that significant non-functional N remains in the lower leaves. Data for the vertical distribution of SLN between the leaf layers was available for Istabraq only so cultivar differences were not tested.

5.4.5 Canopy light interception

Whilst the canopy expanded with N supply in all experiments, the canopy architecture (as indicated by K_{PAR}) was not affected by N supply but differed between site-seasons; overall K_{PAR} for Istabraq was highest in TT06 at 0.51, then LC07 at 0.45, and lowest in TT07 at 0.40. The amount of light intercepted during the stem-extension phase increased with N supply to the N opt-trt paralleling the increase in GAI and little additional interception was observed at the supra-optimal N treatments. In the present study, a GAI of around 5-6 at non-limiting N treatments was optimum for almost complete interception of incident PAR (90-95%) in agreement with the findings of Sylvester-Bradley *et al.* (1997).

Varietal effects on K_{PAR} and intercepted radiation during the stem-extension period were observed in both seasons at Terrington. However, there were no N treatment x variety interactions in either experiment. The varietal pattern in radiation interception from GS31 to anthesis was consistent across both seasons; Savannah intercepted the most and Istabraq intercepted the least. Differences in interception were associated with differences in the light extinction coefficient; Savannah had the highest K_{PAR} and Istabraq had the lowest K_{PAR}. Varietal differences in K_{PAR} have been observed in previous studies on UK winter wheat (Shearman *et al.*, 2005), although the varietal pattern in K_{PAR} was different across seasons in the present study.

5.4.6 Biomass production, biomass production efficiency and radiation-use efficiency

Biomass production at GS31 was predominantly driven by SMN availability, consequently AGDM was higher at Terrington than Lincoln. This difference in AGDM continued to be evident at GS39 and anthesis, although the biomass was partitioned in a similar way between the crop components in all experiments. At anthesis, there was a strong trend for increasing AGDM with N supply; averaged across site-seasons AGDM overall was 9.0 and 13.7 t ha^{-1} at the zero and optimum N treatments, respectively. The plant organ accounting for most of the biomass at anthesis was the true stem in all the experiments. In the Terrington experiments there was a varietal effect in TT06 but not in TT07, and there were no N treatment x variety interactions in either experiment. Overall in TT06, Atlanta produced more biomass than other varieties as a consequence of both higher IR compared to Claire and Istabraq and higher RUE compared to Savannah.

Biomass production efficiency at anthesis for Istabraq decreased with increasing applied N between the zero and optimum N treatments. However, there was only a small decrease in BPE at the supra-optimal N treatments. Overall BPE was similar in all three site-seasons (in the range 64-71). However, Lincoln had a higher BPE than Terrington at the N zero-trt (106 and 93, respectively) likely due to higher N stress, whilst at the N opt-trt Lincoln had a lower BPE than Terrington experiments (47 and 57, respectively) likely due to low GAI at LC07 restricting canopy light interception and thereby biomass production. Comparing between seasons at Terrington, TT06 had a higher BPE at all N treatments than TT07, likely due to higher biomass production during the stem-extension phase in TT06. In both seasons varietal differences in BPE at anthesis were not observed, and there were no interactions in either experiment. Varietal differences in BPE were observed by Ortiz-Monasterio *et al*. (1997) associated with year of release (old cultivars out-performed newer cultivars). In the present study, as mentioned above, varieties had similar release dates.

The production of biomass from GS31 to anthesis showed a strong linear relationship with the cumulative radiation intercepted at all N treatments in all three site-seasons,

supporting observations of Gallagher and Biscoe (1978) and the 'solar engine' approach to modelling biomass production of Azam-Ali *et al*. (1994). Biomass production was therefore principally affected by N supply in the experiments as a result of its effects on light capture (Monteith, 1977). Comparing amongst site-seasons at anthesis, the range of the response to N was greater in LC07 at 7.5 t ha^{-1} due to low interception at the N zero-trt (through low GAI) and high interception at the N max-trt (through high incident solar radiation and high IR) than for the Terrington experiments (TT06 and TT07 at 3.3 and 1.3 t ha^{-1}, respectively).

Radiation-use efficiency has been shown to be generally constant during the growing season for a given environment and cultivar (Sinclair and Muchow, 1999). In present experiments, in all site-seasons there was a trend for lower RUE at the N zero-trt compared with the optimal and maximum N treatments. This was likely due to low lamina N content at the N zero-trt limiting photosynthetic processes, which was especially evident in LC07, as lamina N content is strongly linked with the concentration of photosynthetic enzymes (e.g. Rubisco) (Lawlor *et al*., 2001). At the N opt-trt RUE was similar in all three site-seasons (range 3.06-3.12), and at the high end of the range of published values for winter wheat at 2.6-3.0 g MJ^{-1} (Monteith, 1977; Kiniry *et al*., 1989; Scott *et al*., 1994; Shearman *et al*., 2005).

In the Terrington experiments a small varietal effect on RUE was observed in TT06 but not in TT07, and there were no interactions in either experiment. Previous studies show that genetic variation in RUE exists (Foulkes *et al*., 2001; Shearman *et al*., 2005) which may be associated with lower K_{PAR} (Sylvester-Bradley and Kindred, 2008) and/or higher flag-leaf specific weight (Shearman *et al*., 2005). In TT06 Savannah had a significantly lower RUE (2.63 g MJ^{-1}) than the other three varieties (range 2.95-2.97 g MJ^{-1}), possibly as a result of a high K_{PAR} which may have been associated with light saturation of the upper leaf layers and poor light distribution to the lower leaves, thereby reducing RUE. Sinclair and Muchow (1999) and Shearman *et al*. (2005) reported that RUE was affected by genotype in wheat. However, the small varietal differences observed in the present

experiments may relate to the varieties being genetically similar (i.e. as outlined above they were all feed/biscuit wheats from elite UK germplasm with similar release dates).

Despite the small number of data points, a relatively consistent relationship was found for the two-line relationship between RUE and SLN across all three site-seasons; combining data for TT06 and TT07 increased the goodness of fit of the model, indicating consistency across the two seasons at Terrington. Overall, the breakpoint (when no further increase in RUE with SLN) was found to be at a SLN in the range 1.97-2.13 g m^{-2} and RUE in the range 2.87-3.10 g MJ^{-1}. Below the breakpoint RUE was limited by leaf N content, whilst above the breakpoint further increases in SLN gave no further increases in RUE. These findings are in general agreement with published optima for SLN at 2 g N m^{-2} (Foulkes *et al*., 2005) and for RUE at 2.8 g MJ^{-1} (Hay and Porter, 2006). The relatively small differences in the breakpoint between sites suggests that a value of around 2 g N m^{-2} was sufficient to for light-saturated photosynthesis during grain filling even under the relatively high light intensity conditions of New Zealand.

5.4.7 Crop N requirement for growth

The critical N% dilution curve (Justes *et al*., 1994) and N nutrition index model (Lemaire *et al*., 1989) of crop N nutrition were used to consider the crop N requirements for growth. A close fit of the observed data to the critical N dilution curve justified the use of the N nutrition index in this analysis. At the N zero-trt, Lincoln had a lower NNI (0.37) compared with Terrington (0.58); whilst at the optimum and maximum N treatments Lincoln had a higher NNI (1.23 and 1.33, respectively) compared to Terrington (1.07 and 1.10, respectively). Previous studies have not shown significant differences in NNI between wheat varieties (Justes *et al*., 1994), with the majority of field grown crops falling on about the same line (Greenwood *et al*., 1991). In the present experiments, there was an effect of variety in TT06 but not in TT07, and there were no interactions in either experiment. At the N opt-trt in TT06 Atlanta had higher NNI (1.26) than the other varieties (range 1.10-1.13), associated with high N uptake by Atlanta raising crop N%.

At all three site-seasons less fertiliser N was required to maximise biomass growth at anthesis (i.e. at NNI=1) than was required to maximise grain yield at harvest (i.e. at the N opt-trt). The amount of excess fertiliser N applied at the optimum N treatment ranged from 37-111 kg N ha^{-1}. At Terrington, in TT06 as a consequence of the higher N uptake efficiency of Atlanta, the estimated amount of applied N where NNI=1 was lower for Atlanta (114 hg ha^{-1}) compared to the other varieties (range 141-147 kg ha^{-1}). This suggests that Atlanta required considerably less applied N to maximise growth at anthesis than the other varieties, and that there was a greater amount of excess fertiliser N for anthesis growth at the optimum and maximum N treatments (106 and 256 kg ha^{-1}; cf. range 73-79 and 223-229 kg ha^{-1}, respectively). Higher N uptake efficiency at anthesis for Atlanta may be due to larger root system (length, density and/or distribution) or shoot-root DM partitioning than the other varieties.

5.4.8 Identifying crop excess N accumulation

In comparison with the estimated AGN of the crop at NNI=1 a considerable amount of additional N uptake was observed at the optimum and maximum N treatments in all three site-seasons (except N max-trt in TT07). Despite the considerable difference in applied N between the optimum and maximum N treatments, there was only a small increase in AGN. The highest crop excess N accumulation was observed in LC07 possibly due to higher N rates at the supra-optimum N treatments, while the lowest accumulation was observed in TT07 due to low fertiliser N recovery likely as a result of dull weather conditions during May 2007.

Comparing the estimated N content of each canopy component with that at NNI=1 indicated that the leaf lamina N increased the most in proportion to the other crop components at the optimum and maximum N treatments. Calculation of the SLN in the crop at NNI=1 for TT06, TT07 and LC07 gave values of 2.24, 1.73, and 2.90 g m^{-2}, respectively, generally lower than observed at the optimum and maximum N treatments. This indicated that N was accumulating in the leaf lamina. Examination of the other crop components showed that overall the N content of the true stem and leaf sheath both

increased by similar amounts with increasing N supply above NNI=1, but less so than the leaf lamina N. This increase was consistent with an increase in both shoot density and shoot height with N supply. However, the amount of N per unit of true stem length per m^{-2} (specific stem N; SSN) increased between NNI=1 and the optimum and maximum N treatments, as did the leaf sheath N% but to a lesser extent than that observed for the leaf lamina. Present results therefore indicated that excess N accumulation was occurring in both the true stem and leaf sheath, but generally less so than in the leaf lamina. There was little evidence of accumulation of excess N in the ear in all three site-seasons with a slight decrease in the proportion of AGN in the ear with increasing N supply.

Overall there was little difference in the partitioning of excess N accumulation between the crop components at the optimum and maximum N treatments; with only a slight increase in the proportion of N in the leaf lamina and leaf sheath, possibly indicating excess N accumulation in the photosynthetic tissues (Lawlor *et al*., 1987a; Evans, 1989). In TT06 there was a small increase in the proportion of N in the true stem and therefore as a potential site of excess N accumulation (Blum, 1998), although this was not observed in the other experiments. A small varietal difference was observed in TT06 in the additional N uptake above NNI=1 at the optimum and maximum N treatments as a consequence of Atlanta taking up more N than the other varieties, however this occurred similarly across all crop components.

5.4.9 Conclusions

Use of the critical N concentration and the NNI models allowed an estimation of the amount of applied N required to maximise growth, and the estimation of key growth parameters. In all experiments the estimated amount of applied N at NNI=1 was found to be considerably less than the amount of applied N at the N opt-trt, and additional N uptake (‘luxury uptake’) occurred at anthesis at the optimum and supra-optimum N treatments which caused excess N accumulation in the crop canopy so confirming the hypothesis (2) ‘that N accumulates in the plant organs of wheat canopies at anthesis, which is in excess of that required for structural and photosynthetic uses’. In all

experiments a large amount of additional N was applied at the N max-trt compared to that required at the NNI=1. The AGN was found to increase with N supply to the optimum and maximum N treatments, although limitations of N uptake and assimilation likely occurred at the supra-optimal N treatments. Consequently excess N accumulation was observed to increase with N supply, supporting hypothesis (3) 'that excess N accumulation responds disproportionately to the availability of N, and occurs particularly at high (i.e. supra-optimum) N availabilities'.

Comparison of N content of the crop components in the crop at NNI=1 with that of the crop at the optimum and maximum N treatments indicated that the N content of all crop components increased. Overall the majority of this additional N uptake was partitioned to the leaf lamina, but a considerable proportion was also partitioned to the true stem and leaf sheath; the true stem therefore had a significant role in crop N accumulation. In the Terrington experiments there was a small amount of genetic variation in N partitioning between crop components and their responses to N supply, as proposed in hypothesis (4) 'that there is genetic variation in the partitioning of N and the amount of excess N accumulated in the plant organs of wheat canopies and their responses to N supply'. However, despite being chosen for contrasting whole stem N content at anthesis, the limited genetic variation observed was likely due to the similarity of the study varieties in parentage and release date, and possibly therefore all modern UK varieties are similar. This also explained why all varieties were similar in the lamina SLN required for photosynthetic function and that only limited differences in RUE was found between varieties in one of the Terrington experiments, thereby overall not supporting hypothesis (5) 'that RUE increases linearly with increasing SLN to the maximum RUE, and there are genetic differences in lamina SLN required for photosynthetic function and hence differences in RUE between varieties'.

6 POST-ANTHESIS GROWTH PHASE

6.1 INTRODUCTION

This chapter describes the physiological processes of crop growth in the period from anthesis to harvest, and examines the sequence of yield-forming processes in this phase in relation to N supply and variety treatments.

Data are presented for crop development and growth between anthesis and harvest for each variety in each site-season. Effects on N uptake and partitioning, canopy senescence, dry matter production, N remobilisation, and grain N content at harvest are quantified. The crop N content at anthesis and harvest is quantified according to the 'functional' and 'non-functional' N pools (Lemaire and Gastal, 1997), and the amount of photosynthetic N, structural N and reserve N in the crop components determined using several assumptions based on observations from this study. N remobilisation data is used to identify the amount and location of *storage* N (remobilised RN) and/or *accumulation* N (non-remobilised RN) at anthesis, determining efficiency of canopy N use.

The chapter concludes with a discussion of the findings in relation to testing the study hypotheses: (6) 'that crop components differ in their accumulation of reserve N, and the true stem has a more significant role in the accumulation of RN at anthesis than other crop components'; (7) 'that *storage* N has an important physiological role in wheat crops, especially true stem *storage* N at low N availabilities'; (8) 'that *accumulation* N creates inefficiencies in crop N use by reducing NRE and increasing straw N content, especially at high N availabilities'; (9) 'that *storage* N provides a buffer against premature redistribution of photosynthetic N and hence canopy senescence'; and (10) 'that there are genetic differences in N remobilisation efficiencies of the plant organs (leaf laminae, leaf sheath and true stem) and their responses to N linked to patterns of senescence' (thereby quantitatively related to UTE and which could provide selection traits for improved UTE).

6.2 METHODOLOGY

6.2.1 Treatment combinations and statistical analysis

The effect of N supply for Istabraq is analysed in all three experiments at GS75 and harvest at six N treatments. Unless otherwise specified, results for the effect of N supply and variety are presented for all four varieties in the Terrington experiments at harvest at six (TT06) or three (TT07; zero, optimum and maximum) N treatments, and at three specified N treatments (TT06 and TT07) for results requiring N% data. ANOVAs for the effect of N supply for Istabraq are at six N treatments in all three experiments, and for all four varieties at six (TT06) or three (TT07) N treatments, or three (TT06 and TT07) N treatments for results requiring N% data. SEDs from ANOVAs are shown as error bars in figures, and model parameters for curves are found in Appendix IV.

6.2.2 Calculation of N remobilisation components

6.2.2.1 Post-anthesis N remobilisation

N remobilisation (NR; kg ha^{-1}) is the amount of N in the crop or crop component at anthesis which is not recovered in the straw or straw component at harvest (Cox *et al*., 1986). NR is calculated by the 'apparent remobilisation' method;

NR = N content at anthesis – N content in straw component at harvest **Equation 6-1**

6.2.2.2 Post-anthesis N uptake

Post-anthesis N uptake (PANU; kg ha^{-1}) is the amount of N in the crop at harvest which is not present in the crop at anthesis and is assumed to be the result of post-anthesis N uptake;

PANU = AGN at harvest – AGN at anthesis **Equation 6-2**

Estimation of NR and PANU using the 'apparent remobilisation' method can be subject to large experimental errors due to the necessity to combine data obtained at two different sampling dates, and since it does not take into account the loss of N through volatilization or mobilisation from the roots. These calculations therefore assume that loss of N from the system are negligible. Alternatively the use of ^{15}N stable isotope labeling allows the estimation in a less biased and more precise manner (Kichey *et al.*, 2007). However this was not practical in this field study.

6.2.2.3 Post-anthesis N remobilisation efficiency

N remobilisation efficiency (NRE; %) is the fraction of N in the crop or crop component at anthesis which is not recovered in the straw or straw component at harvest (Cox *et al.*, 1986);

NRE = (NR / N content at anthesis) * 100 **Equation 6-3**

6.2.2.4 N contribution to the grain

N contribution (NC; %) is the percentage of grain N at harvest contributed by the remobilisation of N from the crop or crop component between anthesis and harvest;

NC = (NR of crop or organ / grain N content) * 100 **Equation 6-4**

6.2.3 Grain N concentration and N per grain N response curves

The grain N% and N per grain (NPG) response to applied N were fitted to a 'Normal Type curves with Depletion' (NTD) using GENSTAT (see Murray and Nunn, 1987);

y = d+c*exp(-exp(-a*(N-b)) **Equation 6-5**

Where y is grain N% or NPG, N is the amount of applied N, and a, b, c and d are parameters determined by curve fitting.

6.3 RESULTS

6.3.1 Crop development

In each site-season Istabraq was sampled at GS75 ('mid-grain filling'); representing 28 (TT06), 26 (TT07) and 22 (LC07) days after anthesis (DAA). The date of complete canopy senescence (CCS) is shown for N treatment x variety combinations in Table 6-1. Istabraq at the N opt-trt reached CCS at 60 (TT06), 51 (TT07) and 75 (LC07) DAA. The date of sampling at harvest was the same for all varieties and N treatments in the Terrington experiments; TT06 (305-DAS) and TT07 (281 DAS). In LC07, the sub-optimal N treatments were harvested first; 70N (245 DAS), 0N and 150N (250 DAS); and then the 300N, 400N and 500N treatments (257 DAS).

6.3.2 Shoot production

The fertile shoot density for Istabraq at GS75 increased with N supply in LC07 ($P<0.001$), and there was a trend for increasing shoot density with N supply in both Terrington experiments. Fertile shoot density at harvest was described in section 4.4.2.1. and increased with N supply in all three experiments ($P<0.01$). There were differences amongst varieties in the Terrington experiments ($P<0.05$) at harvest, and the interaction was significant in TT07 ($P<0.05$). During the period from anthesis to harvest the fertile shoot density for Istabraq increased overall from 355 to 404 m^{-2} in TT07 ($P<0.01$), but there was no change in TT06 and LC07.

6.3.3 Crop N uptake

6.3.3.1 Above-ground N uptake

Averaging across N treatments, at GS75 TT06 and LC07 had higher AGN (both 240 kg ha^{-1}), than TT07 (218 kg ha^{-1}). Istabraq at the N zero-trt at Terrington had around double the AGN than it acquired at Lincoln; 149 kg ha^{-1} and 72 kg ha^{-1}, respectively. However, at the N opt-trt LC07 had higher AGN (294 kg ha^{-1}) than TT06 (270 kg ha^{-1}) or TT07 (243 kg ha^{-1}).

AGN at harvest was described in section 4.4.5. AGN at harvest increased with N supply in all experiments ($P<0.001$). There were no differences amongst varieties in either of the Terrington experiments; the interaction was not significant in either experiment. Averaging across N treatments, AGN for Istabraq was slightly higher at TT06 (284 kg ha^{-1}) than LC07 (265 kg ha^{-1}), and lowest in TT07 (224 kg ha^{-1}). Averaging across N treatments, AGN in TT06 was in the range 273 to 284 kg ha^{-1}, and in TT07 was in the range 224 to 254 kg ha^{-1}.

6.3.3.2 Apparent fertiliser recovery at harvest

Apparent fertiliser recovery for Istabraq decreased with N supply in all three site-seasons ($P<0.01$). Averaging across N treatments, TT06 had the highest AFR (0.80), then LC07 (0.68) and TT07 (0.59). The low mean AFR in TT07 may in part be associated with low fertiliser N uptake during dull and wet conditions during the first half of the grain-filling phase. In the Terrington experiments, AFR was not affected by variety, and there was no interaction in either experiment. In TT06 variety differences were in the range 0.69 to 0.80, and in TT07 in the range 0.54 to 0.65.

6.3.4 N partitioning between crop components

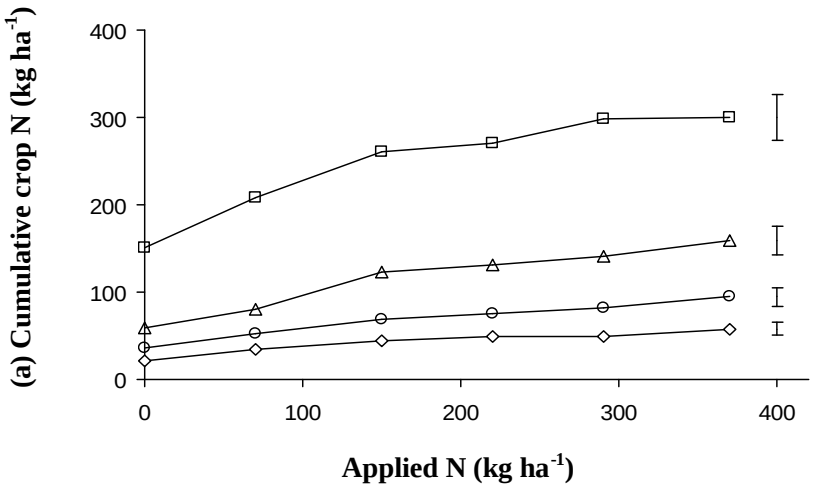
6.3.4.1 Effects at GS75

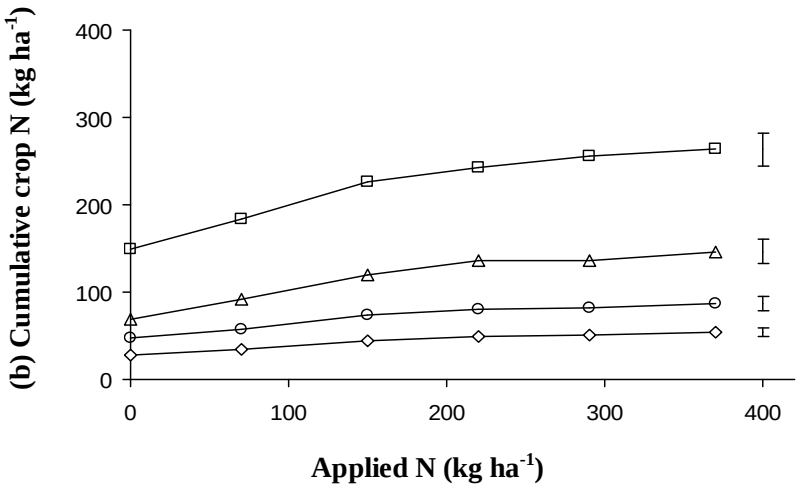
The amount of N in all crop components for Istabraq increased with N supply in all three experiments ($P<0.01$) (Figure 6.1). The proportion of AGN in each crop component was affected by N treatment in TT06 (ear $P<0.01$), TT07 (lamina and ear $P<0.05$), and LC07 (all components $P<0.05$). As N supply increased, all three experiments showed a trend for a higher proportion of crop N in the lamina, sheath, and true stem, while the proportion of N in the ear decreased.

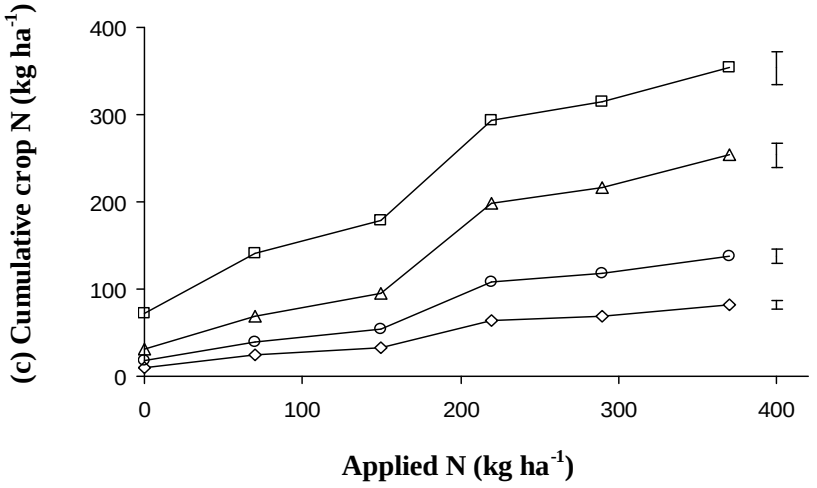
6.3.4.2 Effects at harvest

Overall for Istabraq the amount of N in all crop components in all three experiments increased with N supply ($P<0.01$) (Figure 6.2). Averaged across N treatments in TT06, TT07 and LC07 the amount of N in the straw was 87, 78 and 73 kg ha^{-1}, respectively; and in the grain was 196, 146, and 192 kg ha^{-1}, respectively. In TT06 most of the N retained in the straw was in the true stem (36 kg ha^{-1}), then leaf lamina (24 kg ha^{-1}), leaf sheath (17 kg ha^{-1}) and chaff (11 kg ha^{-1}). A broadly similar pattern was observed in TT07 (29, 17, 15, 17 kg ha^{-1}, respectively) and in LC07 (24, 18, 13, 17 kg ha^{-1}, respectively), although with relatively more N remaining in the chaff than the leaf sheath in these two site-seasons.

N partitioning for Istabraq was affected by N treatment in LC07 (all components $P<0.01$), TT07 (true stem $P<0.05$), but not in TT06. As N supply increased in LC07, the proportion of N in the lamina, sheath and true stem and chaff increased, but in the grain decreased. Turning to consider variety effects (Figure 6.3), in TT06 there was a variety effect on the proportion of AGN in the lamina, true stem, chaff and grain ($P<0.01$), and the interaction was significant for the lamina and grain ($P<0.01$). Overall Savannah increased lamina N and decreased grain N relatively more with N supply than other varieties. In TT07 there were no significant variety effects, and no consistent varietal patterns across site-seasons.



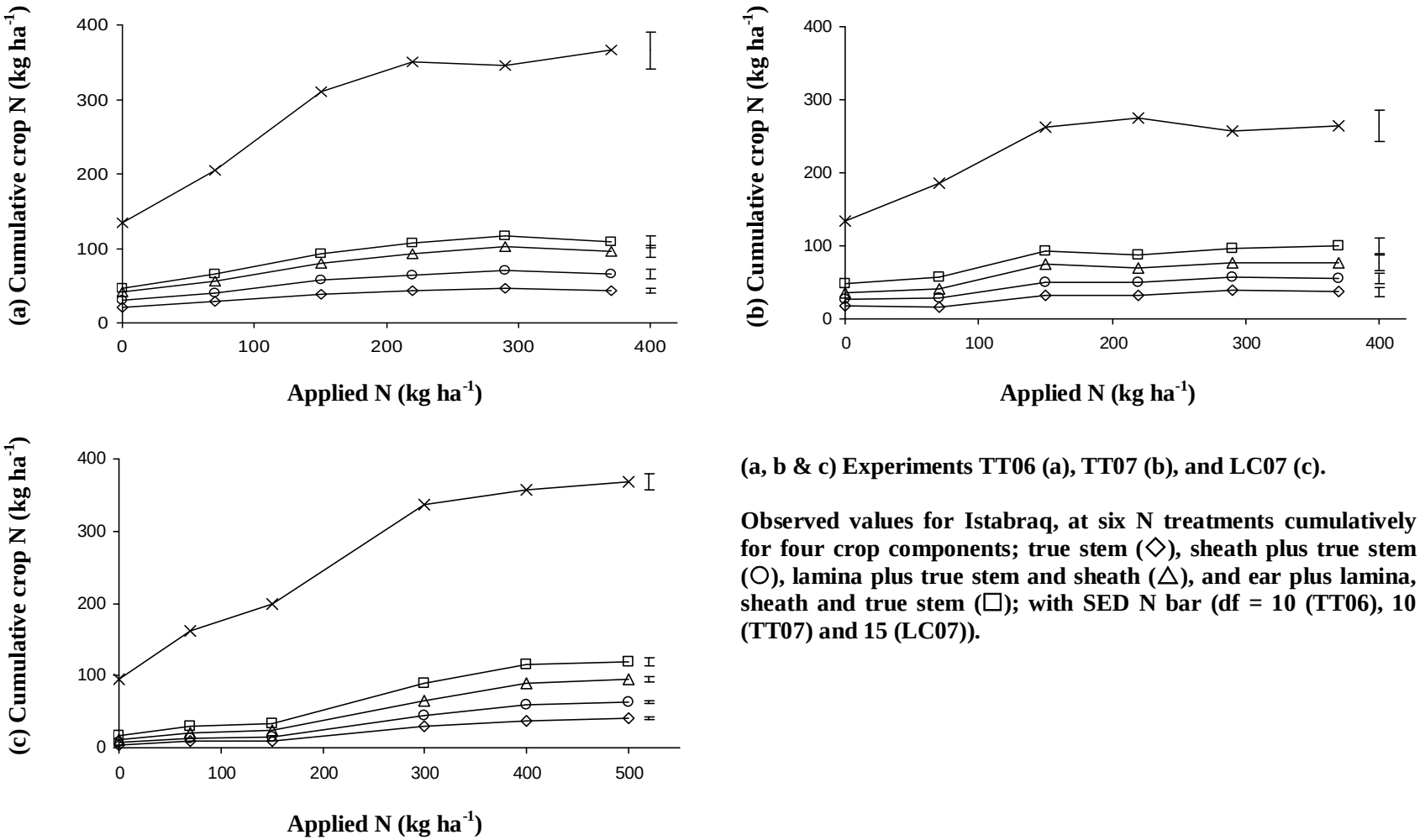




(a, b & c) Experiments TT06 (a), TT07 (b), and LC07 (c).

Observed values for Istabraq at six N treatments cumulatively for four crop components; true stem (◇), sheath plus true stem (○), lamina plus true stem and sheath (△), and ear plus lamina, sheath and true stem (□); with SED N bar (df = 10 (TT06), 10 (TT07) and 15 (LC07)).

Figure 6.1 (a, b & c) Effect of applied N on the crop N cumulatively for four crop components at GS75 for Istabraq in TT06, TT07 and LC07.



(a, b & c) Experiments TT06 (a), TT07 (b), and LC07 (c).

Observed values for Istabraq, at six N treatments cumulatively for four crop components; true stem (◇), sheath plus true stem (○), lamina plus true stem and sheath (△), and ear plus lamina, sheath and true stem (□); with SED N bar (df = 10 (TT06), 10 (TT07) and 15 (LC07)).

Figure 6.2 (a, b & c) Effect of applied N on the crop N cumulatively for four crop components at harvest for Istabraq in TT06, TT07 and LC07.

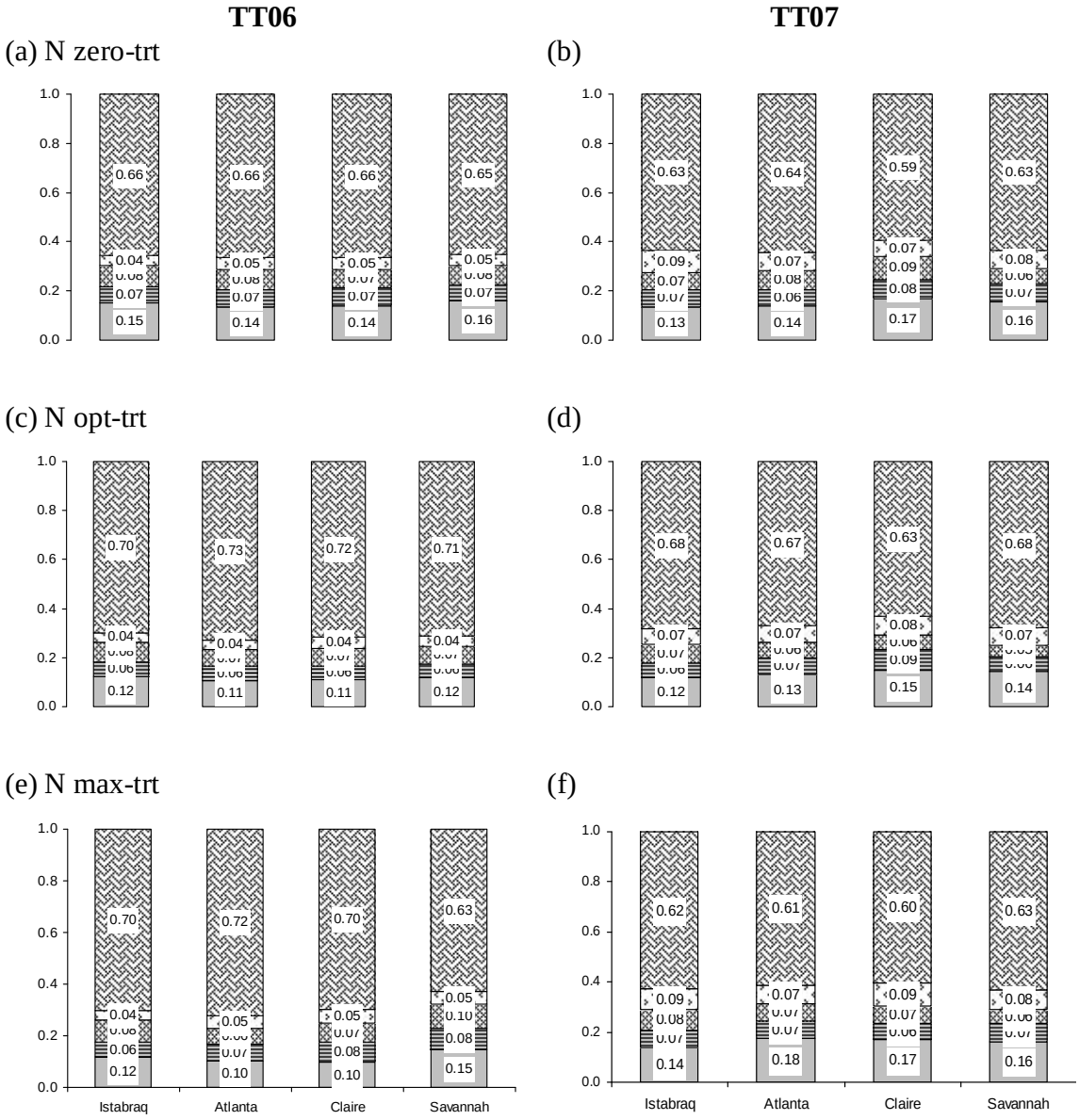


Figure 6.3 (TT06; a, c, e) (TT07; b, d, f) - Effect of applied N on the N partitioning at harvest for the Terrington experiments. Observed values for four varieties, at three N treatments (zero, optimum and maximum), for five crop components (true stem, sheath, lamina, chaff and grain: in ascending order with true stem nearest x axis). Values represent proportion of above-ground N in component.

6.3.5 Canopy Green Area

6.3.5.1 Green area index

Green area index for Istabraq was affected by N treatment at GS75 in all three experiments ($P<0.01$) (Figure 6.5); averaging across N treatments GAI was highest in TT06 (4.5), then LC07 (3.8), and TT07 (3.2). The pattern of GAI with N treatment at GS75 broadly reflected that at anthesis in all three experiments; GAI for the unfertilised Istabraq crop was similar in TT06 and TT07 (1.9 and 2.2, respectively) but lower in LC07 (1.3). Whereas, at the N opt-trt GAI was higher in TT06 (5.7) than TT07 (3.3) and LC07 (4.6). The reduction in GAI between anthesis and GS75 was greater in the unfertilised than in the fertilised treatments (averaged across experiments at 1.7, 1.5 and 1.1 for the zero, optimum and maximum N treatments, respectively), and differed between site-seasons at 1.5 (TT06), 2.6 (TT07) and 0.4 (LC07). Data at GS75 were collected for Istabraq only and as a result variety differences could not be tested.

6.3.5.2 Canopy interception of PAR

The amount of PAR intercepted by Istabraq between anthesis and GS75 was affected by N treatment all three site-seasons ($P<0.01$), averaging across N treatments TT06 had the highest IR (246 MJ m^{-2}), then TT07 (216 MJ m^{-2}), and LC07 (175 MJ m^{-2}). Interception was lower at the N zero-trt than the optimum or maximum N treatments which were similar in all three experiments. However LC07 had the longest period from GS75 to CCS which increased total IR during the grain-filling phase for this crop.

6.3.5.3 Canopy senescence

6.3.5.4 Global leaf lamina senescence

The percentage of total lamina area remaining green between GS75 and harvest (stages labelled ‘M’ and ‘H’ in Figure 6.5, respectively) was scored visually in each plot every 3-4 days. Complete canopy senescence (CCS) was taken as the date when all green lamina area had senesced and there was <10% stem green area remaining. In LC07, the optimal

and supra-optimal N treatments were harvested before CCS (but when less than 5% green lamina area remained). The date of CCS for Istabraq was affected by N treatment in all three experiments ($P<0.01$) (Table 6-1); the N-limited treatments reached CCS earliest but there was little difference between the optimum and maximum N treatments. In the Terrington experiments, date of CCS was affected by variety ($P<0.05$) in both experiments, but there was no N treatment x variety interaction in either experiment. In both experiments, there was a trend for Claire to reach CCS slightly earlier than the other varieties.

Table 6-1 Dates of complete canopy senescence (DAS, days after sowing) for N treatment x variety combinations in TT06, TT07 and LC07.

Exp.	Variety	N zero-trt	N opt-trt	N max-trt
TT06	Istabraq	20 July (282)	02 August (295)	02 August (295)
	Atlanta	20 July (282)	31 July (293)	02 August (295)
	Claire	20 July (282)	31 July (293)	31 July (293)
	Savannah	20 July (282)	31 July (293)	31 July (293)
TT07	Istabraq	02 August (276)	07 August (281)	07 August (281)
	Atlanta	30 July (273)	07 August (281)	07 August (281)
	Claire	02 August (276)	02 August (276)	02 August (276)
	Savannah	30 July (273)	07 August (281)	07 August (281)
LC07	Istabraq	14 February (251)	20 February (257)*	20 February (257)*

* incomplete canopy senescence at time of harvest (green lamina area <5%).

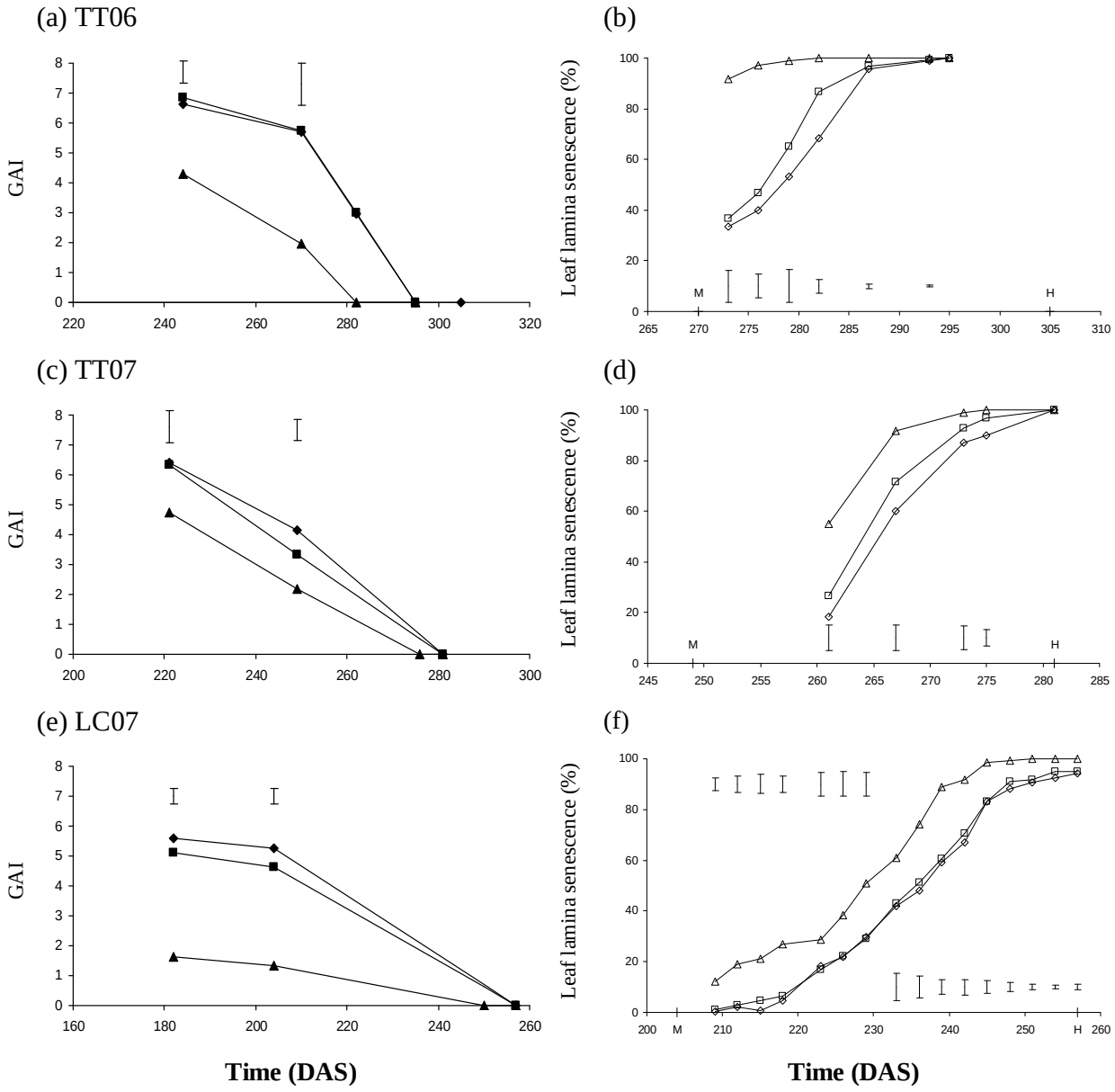


Figure 6.4 (a, c, e) Effect of applied N on the green area index (GAI) between anthesis and harvest in TT06, TT07 and LC07. Observed values for one variety (Istabraq) at three growth stages; 31, 39, 61 and at three N treatments; zero (▲), optimum (■), and maximum (); with SED N bar at each growth stage (df = 10 (TT06); 10 (TT07); 25 (LC07)).

Figure 6.5 (b, d, f) Effect of applied N on leaf lamina senescence score (percentage of total lamina area remaining green) in TT06, TT07 and LC07. Observed values for one variety (Istabraq) at three N treatments; zero (△), optimum (□), and maximum (◇); with SED N bar at each sample date (df = 10 (TT06), 10 (TT07) and 25 (LC07)).

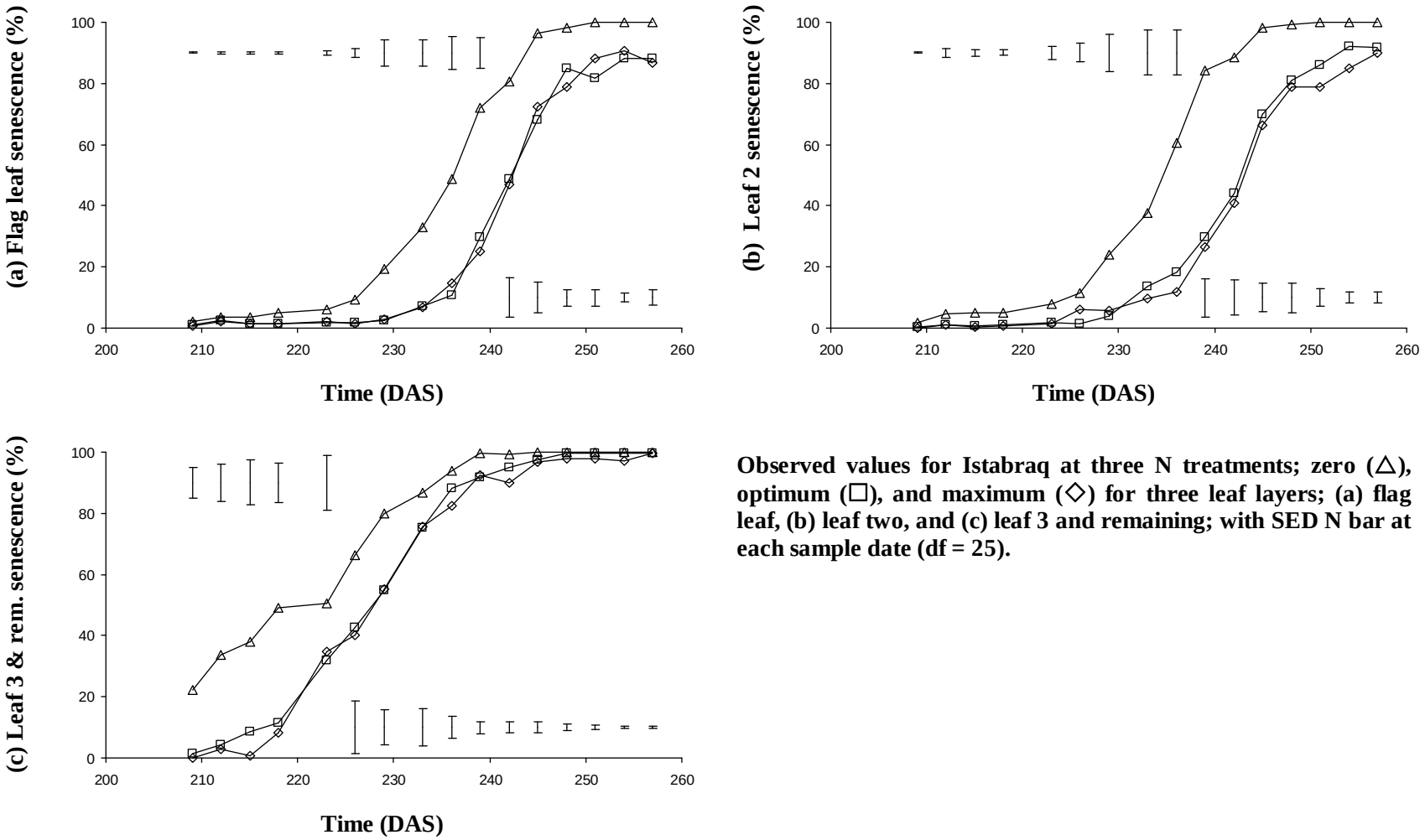
6.3.5.5 Leaf lamina senescence for individual leaf layers

The senescence of the leaf lamina for Istabraq in LC07 was assessed separately for the 'flag leaf', 'leaf two' (penultimate leaf), and 'leaf three and below' (Figure 6.6). Senescence data were converted to a score of 1 to 10 based on % canopy senescence, and plotted against degree days (^{o}Cd, base temperature of 0^{o}C) using SIGMAPLOT version 11 (Systat Software Inc., USA; 2008) fitting a five parameter function (Génard *et al*., 1999) to estimate the start date, end date, rate and duration of the main phase of rapid senescence (Table 6-2). Although CCS was not reached for the flag leaf and leaf two at the optimal and supra-optimal N treatments prior to harvest, the individual leaf layers had reached a score of 10 (i.e. >95% senescence).

Averaging across leaf layers, the start of the rapid phase of senescence in the N zero-trt (224 DAS) occurred earlier than it did in the optimum and maximum N treatments (232 DAS); a similar pattern was observed for the end date of senescence (243 and 248 DAS, respectively). Averaging across N treatments, leaf 3 and below started to senesce earlier (215 DAS) than leaf 2 and flag leaf (at around 235 DAS), and were also the first to completely senescence (240 DAS; cf. 250 DAS). Overall, the duration of senescence was longest for the N zero-trt (19 days), then N opt-trt (17 days) and N max-trt (16 days); and leaf 3 and below took longer to senesce (22 days) than leaf 2 and the flag leaf which were similar (15 days).

Table 6-2 Estimated start date, end date, rate (with SE) and duration of the main phase of rapid senescence as thermal degree-days after anthesis (^oCd; base temperature of 0^oC) and calendar days after sowing (DAS) for individual leaf layers at three N treatments in TT06, TT07 and LC07.

Leaf position	N-trt	Start date		End date		Rate		Duration	
		^oCd	DAS	^oCd	DAS	$1/d^oC$	SE	^oCd	Days
Flag	Zero	694	231 (25-Jan)	943	246 (09-Feb)	0.041	0.0039	249	15
	Opt	796	237 (31-Jan)	1035	253 (16-Feb)	0.045	0.0023	239	16
	Max	806	238 (01-Feb)	1029	252 (15-Feb)	0.048	0.0030	222	14
Leaf 2	Zero	681	230 (24-Jan)	903	244 (07-Feb)	0.045	0.0037	221	14
	Opt	794	237 (31-Jan)	1048	254 (17-Feb)	0.042	0.0033	254	17
	Max	810	238 (01-Feb)	1040	253 (16-Feb)	0.045	0.0044	230	15
Leaf 3 & below	Zero	364	210 (04-Jan)	827	239 (02-Feb)	0.023	-	463	29
	Opt	542	221 (15-Jan)	846	240 (03-Feb)	0.035	-	304	19
	Max	542	221 (15-Jan)	846	240 (03-Feb)	0.035	-	304	19



Observed values for Istabraq at three N treatments; zero (△), optimum (□), and maximum (◇) for three leaf layers; (a) flag leaf, (b) leaf two, and (c) leaf 3 and remaining; with SED N bar at each sample date (df = 25).

Figure 6.6 (a, b & c) Effect of applied N on the leaf lamina senescence for individual leaf layers of Istabraq in LC07.

6.3.6 Biomass production

6.3.6.1 Above-ground biomass production

Above-ground biomass for Istabraq at GS75 was affected by N treatment in TT06 and LC07 ($P<0.01$), but not in TT07 although there was a trend for increasing AGDM with N supply. Averaging across N treatments, AGDM was higher at TT06 (19.3 t ha^{-1}) than at TT07 (15.9 t ha^{-1}) or LC07 (15.0 t ha^{-1}). AGDM at harvest was described in section 4.4.4. In summary, AGDM for Istabraq increased with N supply in all three experiments ($P<0.001$). In the Terrington experiments AGDM was not affected by variety, in the ranges 19.7-20.7 t ha^{-1} in TT06 and 15.7-16.7 t ha^{-1} in TT07; the interaction was not significant in either experiment.

Biomass production between anthesis and harvest for Istabraq was affected by N treatment in all three site-seasons ($P<0.01$). Overall AGDM increased the most in the post-anthesis period in LC07 (10.4 t ha^{-1}), then TT07 (6.2 t ha^{-1}), and TT06 (5.1 t ha^{-1}). In the Terrington experiments, the post-anthesis biomass production was affected by variety in TT06 ($P<0.05$) but not in TT07; the interaction was not significant in either experiment. Averaging across N treatments, in TT06 Savannah produced the most AGDM (5.8 t ha^{-1}) and Atlanta produced the least (4.4 t ha^{-1}); cf. TT07 where non-significant variety differences were observed in the range 5.1 to 6.2 t ha^{-1}.

6.3.6.2 Radiation-use Efficiency

Radiation-use efficiency for Istabraq in the period from anthesis to GS75 was not affected by N treatment in any of the three experiments. Averaging across N treatments, RUE was higher in TT07 (2.61 g MJ^{-1}), and LC07 (2.48 g MJ^{-1}) than in TT06 (1.67 g MJ^{-1}); the low RUE in TT06 may have been associated with dry soil conditions limiting AGDM production during this period. Comparison of RUE at anthesis and at GS75 showed no difference in TT07 and LC07, but a significant decrease in TT06 ($P<0.001$).

6.3.7 N remobilisation components

N remobilisation ('unloading') from each crop component to the grain during the post-anthesis phase is described in Figure 6.7, 6.8 and 6.9. Negative values (on the left side of the y-axis) are the amount of N remaining in each crop component at harvest, and positive values (on the right side of the y-axis) are the amount of N remobilised to the grain from each component as well as the post-anthesis N uptake (PANU). The contribution of each crop component to the grain N (NC; %), and the efficiency of N remobilisation (NRE; %) are calculated.

6.3.7.1 Post-anthesis N remobilisation

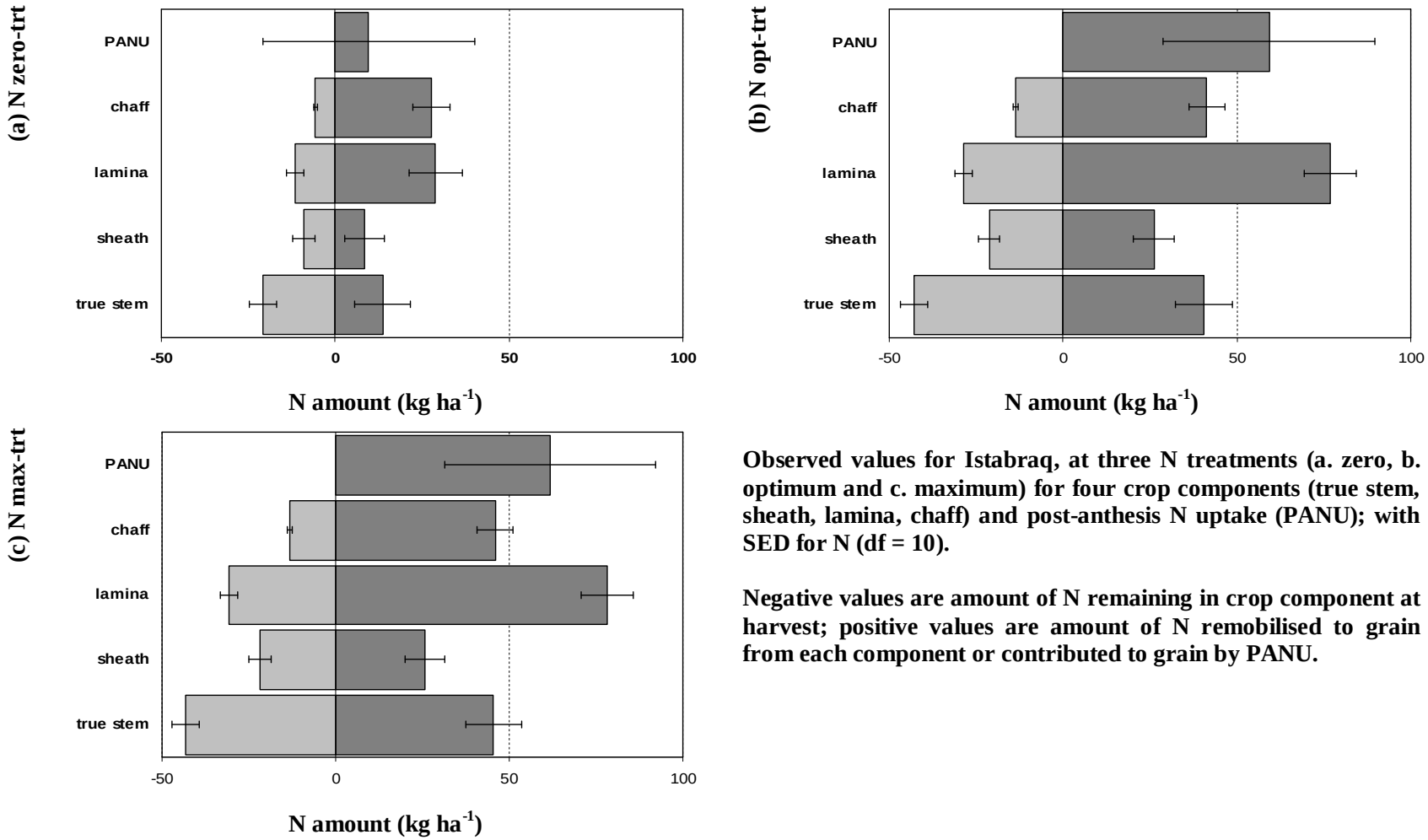
The total amount of N remobilisation from all crop components for Istabraq increased with N supply in all site-seasons, averaging across N treatments in TT06, TT07 and LC07 at 153, 90, and 132 kg ha^{-1}, respectively. The total amount of NR at the N zero-trt was higher in the Terrington experiments than LC07 (range 54-79 and 35 kg ha^{-1}, respectively), but TT06 and LC07 were similar at the optimum and maximum N treatments (185 and 179 kg ha^{-1}; and, 196 and 183 kg ha^{-1}, respectively) while TT07 was lower (126 and 90 kg ha^{-1}, respectively).

The amount of NR from each crop component for Istabraq increased with N supply in TT06 (lamina $P<0.001$; true stem and chaff $P<0.05$) and LC07 (all components $P<0.001$), but the effect of N supply was not significant in TT07. In TT06, NR was overall larger for the leaf lamina (61 kg N ha^{-1}) than the chaff, true stem and leaf sheath (38, 33 and 20 kg ha^{-1}, respectively). In LC07 NR for the leaf lamina was again larger than the leaf sheath, chaff, and true stem (at 67, 27, 20 and 18 kg ha^{-1}, respectively). Whereas, in TT07 NR for the leaf lamina was greater than the true stem, leaf sheath and chaff (at 42, 21, 17 and 10 kg ha^{-1}, respectively). There was an interaction between crop component NR and N treatment in LC07 ($P<0.001$) but not in TT06 or TT07. In LC07 NR from the true stem and chaff increased to the N opt-trt and thereafter decreased slightly at the supra-optimal N treatments, whereas NR from leaf lamina and ear continued to increase above the N opt-trt.

Turning to consider the variety effects, in TT06 Atlanta had higher NR than other varieties for the leaf lamina (74 kg ha^{-1}) and chaff (59 kg ha^{-1}) with the other varieties in the range 61-62 kg ha^{-1} and 35-40 kg ha^{-1}, respectively; and there was an N treatment x variety interaction for chaff NR ($P< 0.05$). In TT07 there were no effects of variety or interactions. However, Atlanta again remobilised the most N from the chaff, at 16 kg ha^{-1} and range 10-13 kg ha^{-1}, respectively. The small interaction for the chaff NR in TT06 was a consequence of the different response by Atlanta which increased NR more at the supra-optimum N treatment compared to the other varieties.

6.3.7.2 Post-anthesis N uptake

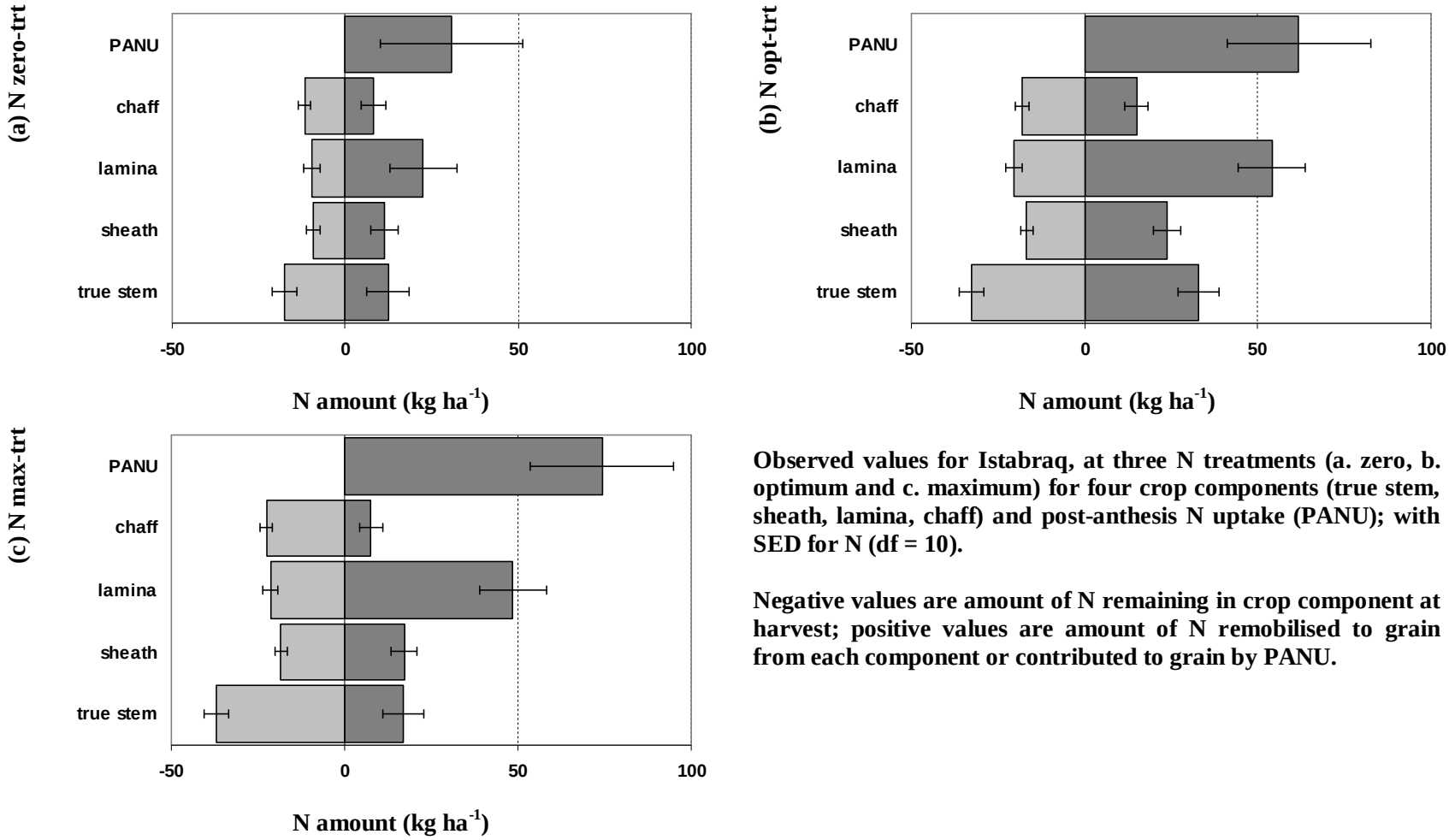
Post-anthesis N uptake for Istabraq was affected by N supply in LC07 ($P<0.01$), and in TT06 and TT07 there was a trend for increased PANU with N supply. Averaging across N treatments, PANU was similar in all three experiments; LC07 (60 kg ha^{-1}), TT07 (56 kg ha^{-1}) and TT06 (44 kg ha^{-1}). PANU was higher at the N zero-trt in LC07 than TT06 and TT07 (45, 10 and 31 kg ha^{-1}, respectively), but similar at the optimum and maximum N treatments (70, 59 and 62 kg ha^{-1}, respectively; and 67, 62 and 74 kg ha^{-1}, respectively). In the Terrington experiments, PANU differed amongst varieties in TT06 ($P<0.05$) but not in TT07, and there was no interaction in either experiment. Averaging across N treatments, in TT06 Savannah, Istabraq and Claire had similar PANU (range 48-34 kg ha^{-1}), whereas for Atlanta PANU was lower at 9 kg ha^{-1}; cf. TT07 with all varieties in the range 56-71 kg ha^{-1}.



Observed values for Istabraq, at three N treatments (a. zero, b. optimum and c. maximum) for four crop components (true stem, sheath, lamina, chaff) and post-anthesis N uptake (PANU); with SED for N (df = 10).

Negative values are amount of N remaining in crop component at harvest; positive values are amount of N remobilised to grain from each component or contributed to grain by PANU.

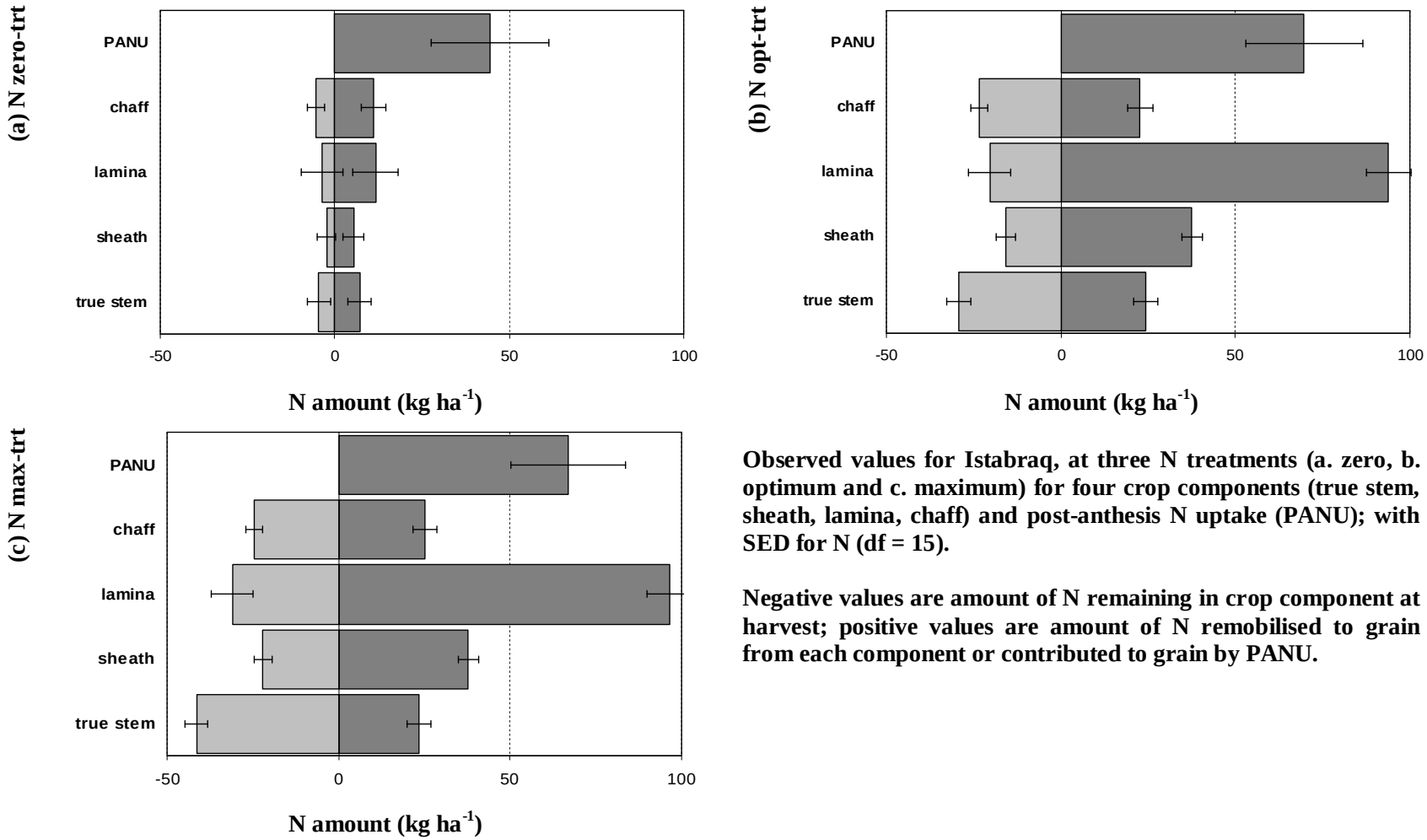
Figure 6.7 (a, b & c) TT06; Amount of N remaining in components of straw at harvest (negative values) and amount of N remobilised to the grain or contributed to grain by post-anthesis N uptake (positive values) for Istabraq at three N treatments.



Observed values for Istabraq, at three N treatments (a. zero, b. optimum and c. maximum) for four crop components (true stem, sheath, lamina, chaff) and post-anthesis N uptake (PANU); with SED for N (df = 10).

Negative values are amount of N remaining in crop component at harvest; positive values are amount of N remobilised to grain from each component or contributed to grain by PANU.

Figure 6.8 (a, b & c) TT07; Amount of N remaining in components of straw at harvest (negative values) and amount of N remobilised to the grain or contributed to grain by post-anthesis N uptake (positive values) for Istabraq at three N treatments.



Observed values for Istabraq, at three N treatments (a. zero, b. optimum and c. maximum) for four crop components (true stem, sheath, lamina, chaff) and post-anthesis N uptake (PANU); with SED for N (df = 15).

Negative values are amount of N remaining in crop component at harvest; positive values are amount of N remobilised to grain from each component or contributed to grain by PANU.

Figure 6.9 (a, b & c) LC07; Amount of N remaining in components of straw at harvest (negative values) and amount of N remobilised to the grain or contributed to grain by post-anthesis N uptake (positive values) for Istabraq at three N treatments.

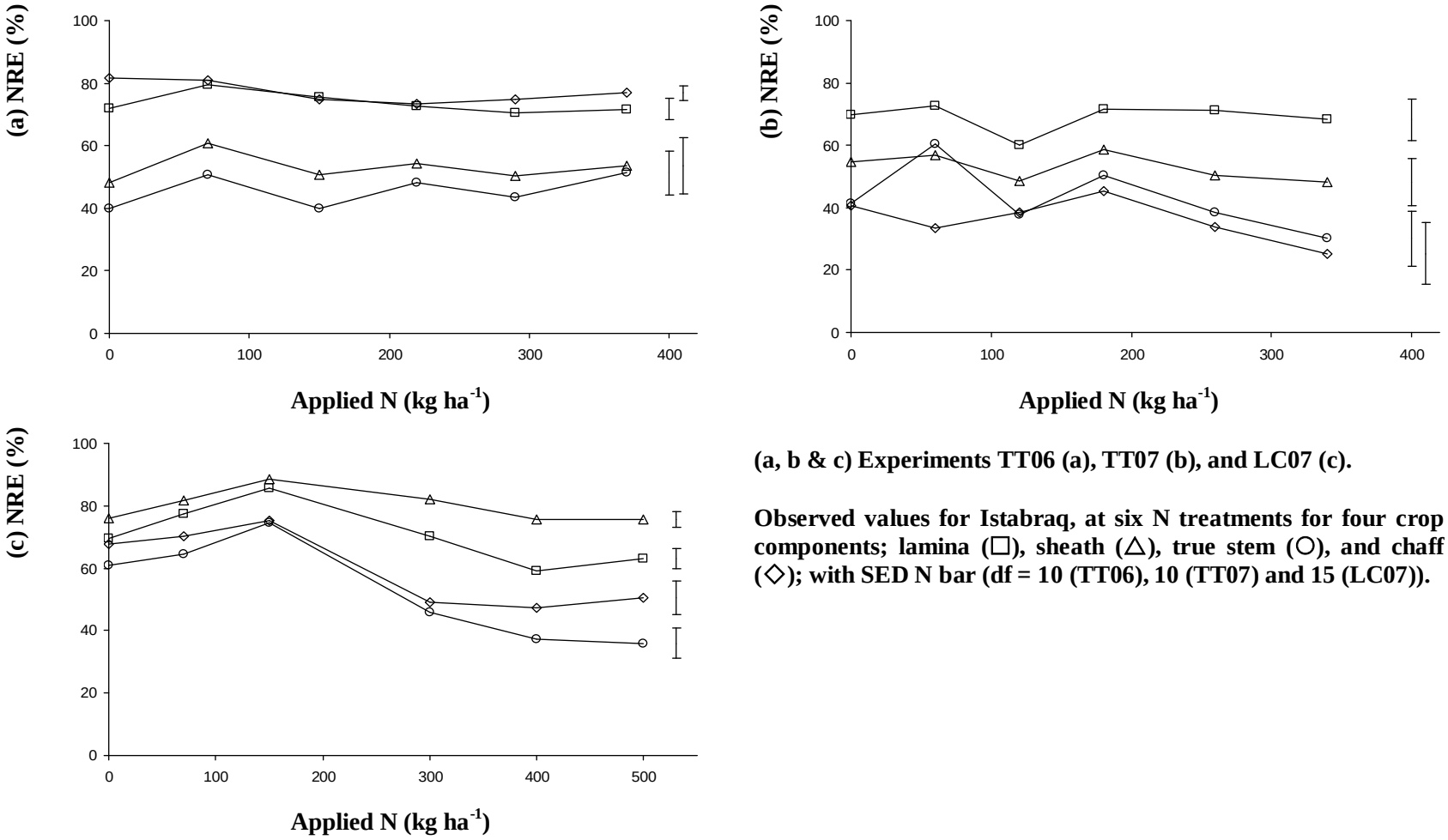
6.3.7.3 N contribution to the grain

The percentage of grain N contributed (NC) from the straw components for Istabraq decreased with N supply in TT06, was inconsistent in TT07, and increased in LC07. Averaging across N treatments NC from all crop components for TT06, TT07 and LC07 was 80, 62 and 63%, respectively. NC for Istabraq was affected by N treatment in TT06 (chaff $P<0.05$), LC07 (all components $P<0.01$), but not in TT07. The main effects with increasing N supply across site-seasons were that lamina NC increased in TT07 and LC07, but decreased in TT06; sheath NC increased in LC07; true stem NC increased in TT06; chaff NC decreased in all experiments; and PANU increased in TT06, was inconsistent in TT07, and decreased in LC07. In each experiment, the leaf lamina contributed the largest percentage of grain N. PANU also contributed a large percentage of the grain N, although this varied considerably between site-seasons. In the Terrington experiments, NC was affected by variety in TT06 (chaff $P<0.001$), there were no interactions in either experiment. Overall, in TT06 Atlanta had higher chaff NC (31%) than other varieties (in the range 21-23%); in TT07 Atlanta again had a slightly higher NC but not significantly (11%, and range 7-9%, respectively).

6.3.7.4 Post-anthesis N remobilisation efficiency

N remobilisation efficiency for all crop components for Istabraq was affected by N treatment in LC07 ($P<0.001$), but not in TT06 or TT07; averaging across N treatments, NRE was similar in TT06 and LC07 (both at 62%) but lower in TT07 (50%). NRE for individual crop components for Istabraq was affected by N treatment in TT06 (chaff $P<0.05$) and LC07 (all components $P<0.001$), but not in TT07 (Figure 6.10). Overall, in the three experiments NRE was higher for the lamina (70-78%) compared to the chaff (37-77%), leaf sheath (52-70%) and true stem (41-61%). As N supply increased to the optimum N treatment, in TT06 chaff NRE decreased (82 to 73%), whereas in LC07 leaf lamina NRE increased (76 to 82%), leaf sheath was unchanged but decreased at the N max-trt (70 to 63%), true stem decreased (61 to 46%), and chaff decreased (68 to 49%).

In the Terrington experiments there was a trend for a difference in crop NRE amongst varieties ($P<0.10$); there was no interaction in either experiment. Averaging across N treatments, NRE was in the range 57-65% in TT06, and 49-51% in TT07. NRE for individual crop components was affected by variety in TT06 (lamina $P<0.05$, and chaff $P<0.001$) and in TT07 there was again a trend for differences for the lamina and chaff ($P<0.07$); there were no interactions in either experiment. The varietal effects for lamina and chaff in TT06 resulted from Atlanta having a higher NRE (78% and 81%, respectively) than other varieties (ranges 72-76%, and 72-77%, respectively). In TT07, Atlanta also showed a trend for higher NRE for the chaff (49%) than other varieties (range 37-40%).



(a, b & c) Experiments TT06 (a), TT07 (b), and LC07 (c).

Observed values for Istabraq, at six N treatments for four crop components; lamina (□), sheath (△), true stem (○), and chaff (◇); with SED N bar (df = 10 (TT06), 10 (TT07) and 15 (LC07)).

Figure 6.10 (a, b & c) Effect of applied N on N remobilisation efficiency (NRE) for individual crop components during the post-anthesis phase for Istabraq in TT06, TT07 and LC07.

6.3.7.5 Rate of N mobilisation

For Istabraq in each site-season the rate of N mobilisation per calendar day from the leaf lamina, leaf sheath, and true stem and of PANU (kg N ha^{-1} day^{-1}) to the ear (chaff and grain) during the post-anthesis phase was estimated for the first half of the phase (anthesis to GS75) and the second half of the phase (GS75 to complete canopy senescence) (Table 6-3). Averaging across components (leaf lamina, sheath and true stem) the overall rate of N mobilisation in the first half of the phase was highest in TT06, then TT07, and LC07 (at 2.90, 0.85 and 0.40 kg ha^{-1} d^{-1}, respectively); and in the second half of the phase was highest in LC07, then TT06, and TT07 (at 1.97, 1.87 and 1.84 kg ha^{-1} d^{-1}, respectively). The rate of PANU was highest in TT07 and LC07 in the first half of the phase (1.78 and 1.50 kg ha^{-1} d^{-1}, respectively) compared with TT06 which showed no PANU in the first half of grain filling; and in the second half of the phase was highest in TT06 (1.59 kg ha^{-1} d^{-1}), cf. TT07 and LC07 at (0.15 and 0.54 kg ha^{-1} d^{-1}, respectively).

Rate of N mobilisation to GS75 for individual crop components was affected by N treatment in LC07 (all components $P<0.05$), but not in TT06 or TT07; although in all three experiments there was a trend for an increased rate of N mobilisation from the leaf lamina, leaf sheath, true stem and PANU to the ear with increased N supply. The rate of accumulation of ear N increased with N supply in TT07 and LC07 ($P<0.05$) and there was a trend for an increase in TT06. In TT06 rate of N mobilisation for leaf lamina was greater than for the true stem, leaf sheath and PANU; and similar effects were observed in TT07. In LC07 the crop showed a slightly different pattern with net accumulation in the true stem (possibly temporary storage). Rate of N mobilisation from GS75 to harvest was affected by N treatment in LC07 (all components $P<0.001$). However, there was again a general trend for an increased rate of N mobilisation to the ear from the leaf lamina, leaf sheath, true stem and PANU with N supply. The rate of accumulation of ear N per calendar day was affected by N supply in all three experiments ($P<0.05$). In TT06 rate of N mobilisation for leaf lamina was again greater than that for the leaf sheath and true stem, with PANU still occurring during this phase; LC07 and TT07 showed a similar pattern.

Table 6-3 Rate of N mobilisation (kg N ha^{-1} day^{-1}) for Istabraq at three N treatments in the crop components during the post-anthesis phase for each variety in TT06, TT07 and LC07. Positive value indicates net gain, and negative value indicates net loss. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

		Anthesis to GS75					GS75 to harvest				
Exp.	**N-trt**	**lamina**	**sheath**	**tr. stem**	**ear**	**PANU**	**lamina**	**sheath**	**tr. stem**	**ear**	**PANU**
TT06	Zero	-0.66	-0.16	-0.48	2.26	-0.96	-1.13	-0.37	-0.07	0.36	1.20
	Opt	-1.97	-0.78	-1.33	3.25	0.83	-1.05	-0.24	-0.23	4.86	-3.34
	Max	-1.73	-0.41	-1.17	3.16	0.15	-1.32	-0.60	-0.60	5.15	-2.63
	SED N	0.417	0.274	0.374	0.522	1.163	0.478	0.193	0.3263	1.269	1.526
	df (P)	10 ns	10 ns	10 ns	10 ns	10 ns	10 ns	10 ns	10 ns	10 *	10 ns
TT07	Zero	-0.36	-0.07	-0.05	2.13	-1.65	0.49	0.36	0.43	-0.71	0.57
	Opt	-0.72	-0.29	-0.60	2.68	-1.07	1.07	0.49	0.50	-3.05	-0.99
	Max	-0.38	-0.08	-0.00	3.08	-2.62	1.18	0.47	0.53	-2.21	-0.03
	SED N	0.394	0.184	0.347	0.250	1.033	0.264	0.161	0.323	0.632	1.202
	df (P)	10 ns	10 ns	10 ns	10 *	10 ns	10 ns	10 ns	10 ns	10 *	10 ns
LC07	Zero	-0.12	-0.01	-0.06	1.12	-0.93	-0.19	-0.11	-0.12	0.94	-0.51
	Opt	-1.15	-0.42	0.50	2.29	-1.22	-1.30	-0.53	-0.67	3.31	-0.81
	Max	-0.53	-0.17	0.75	2.29	-2.34	-1.60	-0.64	-0.75	3.29	-0.29
	SED N	0.375	0.184	0.239	0.329	0.951	0.145	0.060	0.103	0.170	0.377
	df (P)	15 *	15 *	15 *	15 *	15 ns	15 ***	15 ***	15 ***	15 ***	15 ns
Average across experiments											
	Zero	-0.38	-0.08	-0.20	1.84	-1.18	-0.28	-0.04	0.08	0.20	0.42
	Opt	-1.28	-0.50	-0.48	2.74	-0.49	-0.43	-0.09	-0.13	1.71	-1.71
	Max	-0.88	-0.22	-0.14	2.84	-1.60	-0.58	-0.26	-0.27	2.08	-0.98

6.3.8 N Harvest Index

N harvest index (NHI) for Istabraq was affected by N treatment in LC07 ($P<0.001$), but not in TT06 or TT07 (Figure 6.11). Averaging across N treatments, NHI was highest in LC07 (0.75), then TT06 (0.69), and TT07 (0.65). In LC07 NHI decreased with increased N supply from the N zero-trt to the N max-trt from 0.83 to 0.68, respectively, in TT06 it increased from 0.66 to 0.70, respectively, and in TT07 NHI it was broadly consistent at ca. 0.65. Regression analysis fitted a line to the data in LC07, but not in TT06 or TT07. In the Terrington experiments, NHI differed amongst varieties in TT06 ($P<0.05$), and the interaction was significant in TT06 ($P<0.05$). In TT06 Atlanta had the highest NHI (0.70), then Istabraq and Claire (both 0.69), and Savannah (0.67); cf. in TT07 varieties were in the range 0.61-0.65. The interaction in TT06 was the result of Savannah decreasing sharply at the supra-optimal N treatments while the other varieties decreased only slightly.

6.3.9 Grain N concentration and N per grain

Grain N% for Istabraq was affected by N treatment in all three site-seasons ($P<0.001$); averaging across N treatments, grain N% was higher in TT06 (1.93%) than in LC07 (1.79%) and TT07 (1.73%). Grain N% increased with N supply from the zero to the maximum N treatment; in TT06 from 1.38 to 2.24%, in LC07 from 1.44 to 2.04%, and in TT07 from 1.33 to 1.81% (Figure 6.12). The decrease in grain N% from the N opt-trt to the N max-trt in TT07 was likely due to lodging/leaning of this crop reducing N mobilisation to the grain. Regression analysis fitted curves to Istabraq in all three site-seasons. In the Terrington experiments grain N% differed amongst varieties in both experiments ($P<0.05$); there was no interaction in either experiment. In TT06 Claire (1.95%) and Istabraq (1.93%) had higher grain N% than Atlanta (1.84%) and Savannah (1.80%); whereas in TT07 there was a different varietal pattern with Atlanta (1.92%), Claire (1.86%), Savannah (1.85%), and Istabraq (1.73%). Overall varieties differed within a relatively narrow range in both years. The N content per grain (NPG) followed a similar pattern to grain N% in all three site-seasons, NPG data are referred to in chapter 7 (Figure 6.13).

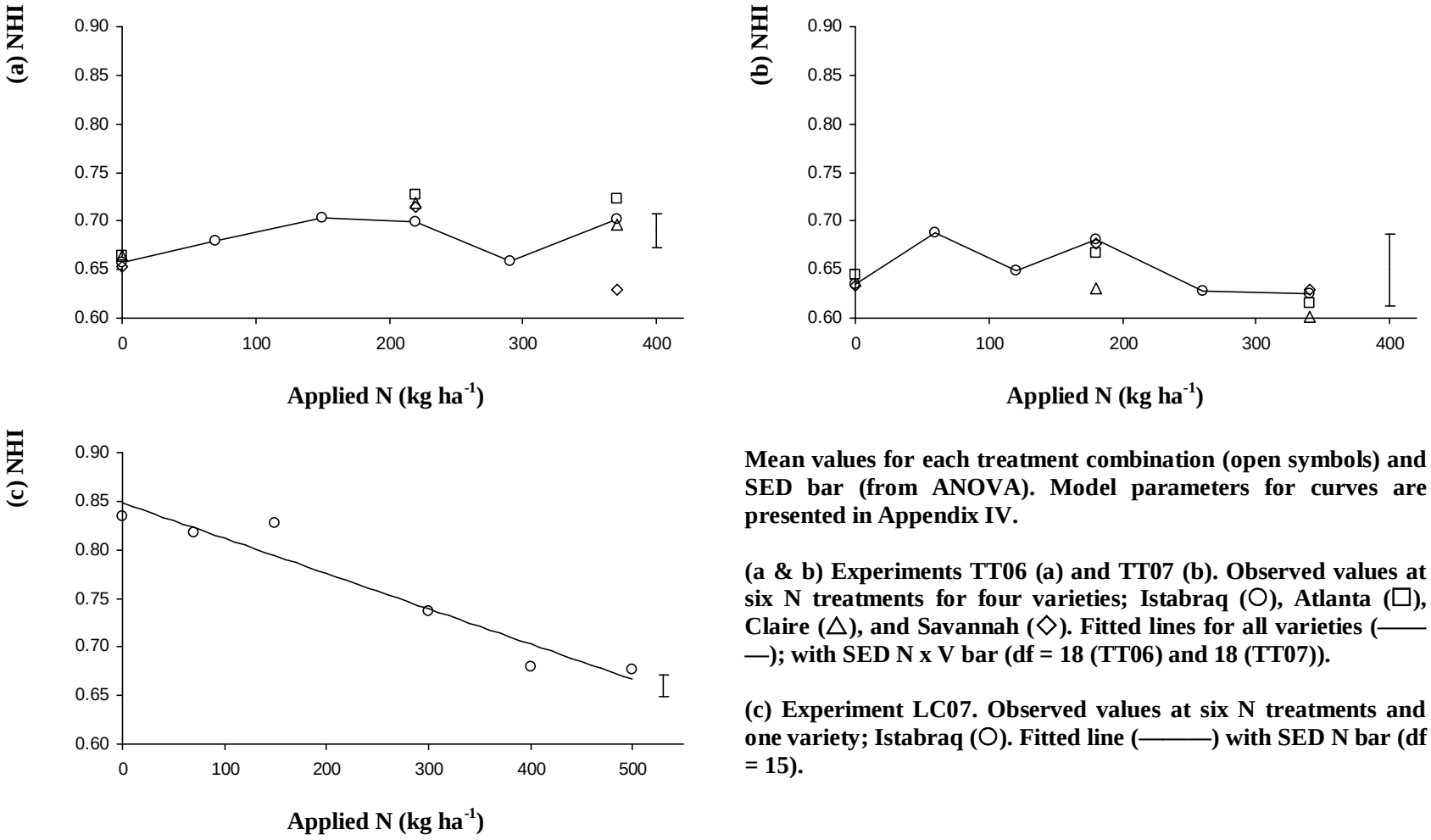
Normal Type curves with Depletion (as described in 6.2.3), were fitted to the grain N% and NPG response to N supply for Istabraq in TT06, TT07 and LC07 (model parameters shown in Table 6-4 and Table 6-5, respectively).

Table 6-4 Model parameters for fitted Normal Type curves with Depletion for the relationship between grain N concentration (%) and fertiliser N applied for Istabraq.

Exp.	A (SE)	B	C (SE)	D (SE)
TT06	0.000075 (5.6E-05)	11.0122	-0.7682 (0.0987)	2.1388 (0.0633)
TT07	0.000107 (1.8E-04)	-14.3142	-0.6211 (0.2170)	1.9408 (0.0630)
LC07	0.000018 (5.7E-06)	23.2491	-0.6054 (0.0378)	2.0392 (0.0329)

Table 6-5 Model parameters for fitted Normal Type curves with Depletion for the relationship between N per grain (mg) and fertiliser N applied for Istabraq.

Exp.	A (SE)	B	C (SE)	D (SE)
TT06	0.000096 (5.3E-05)	13.3358	-0.2979 (0.0268)	0.9261 (0.0162)
TT07	0.000099 (1.9E-04)	-7.3517	-0.1764 (0.0623)	0.7256 (0.0269)
LC07	0.000027 (1.6E-05)	29.7781	-0.2202 (0.0160)	0.8402 (0.0131)

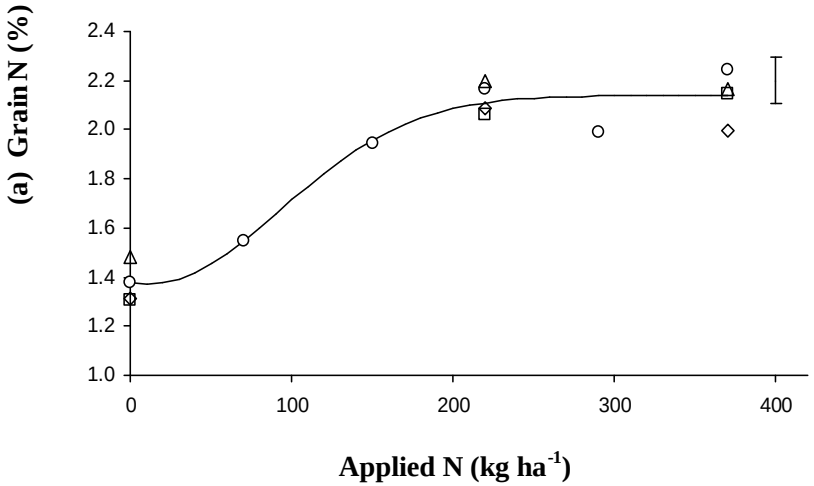


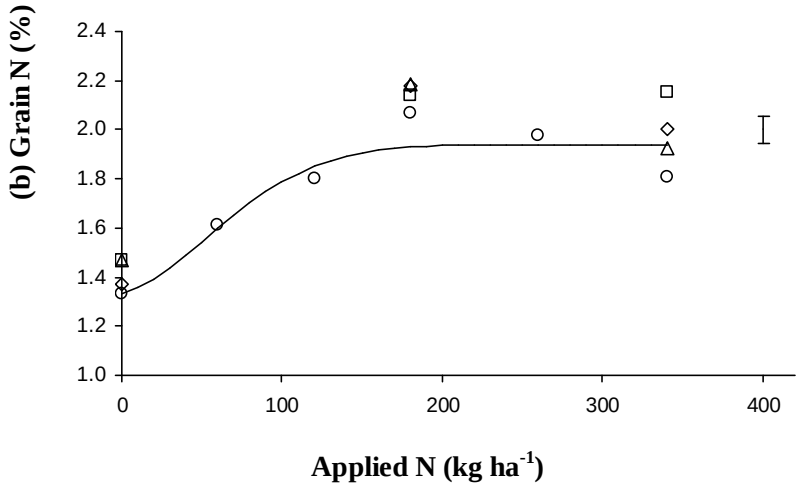
Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Appendix IV.

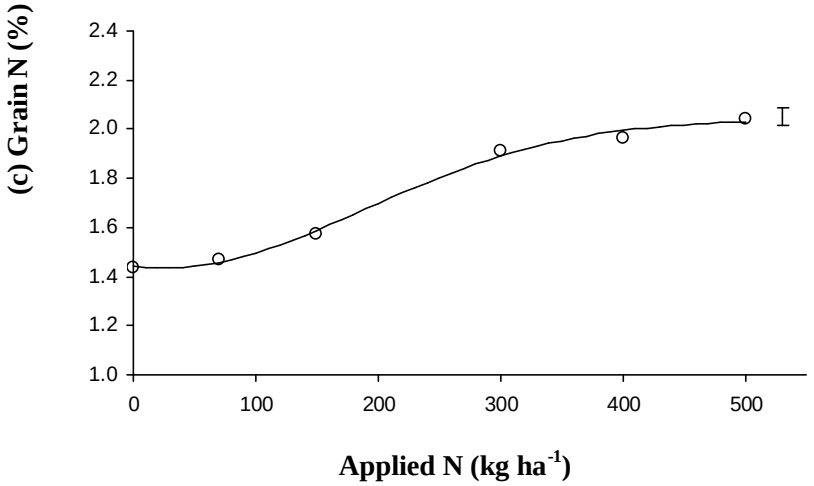
(a & b) Experiments TT06 (a) and TT07 (b). Observed values at six N treatments for four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for all varieties (———); with SED N x V bar (df = 18 (TT06) and 18 (TT07)).

(c) Experiment LC07. Observed values at six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

Figure 6.11 (a, b & c) Effect of applied N and variety on N harvest index (NHI) in TT06, TT07 and LC07.





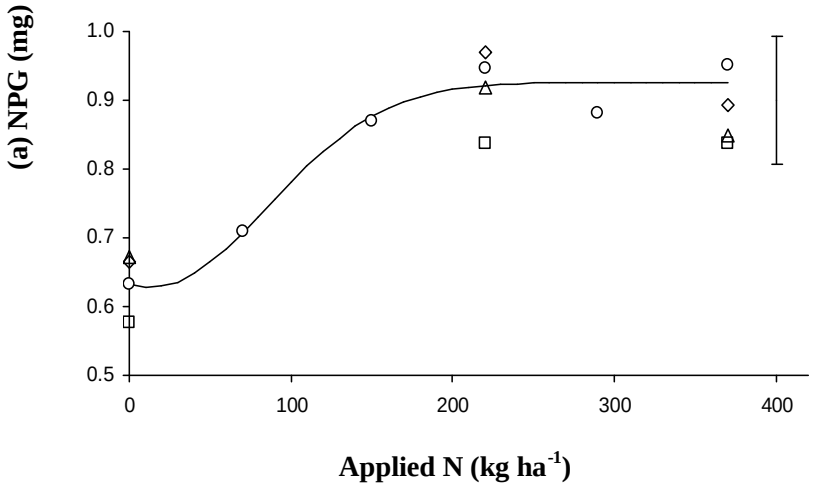


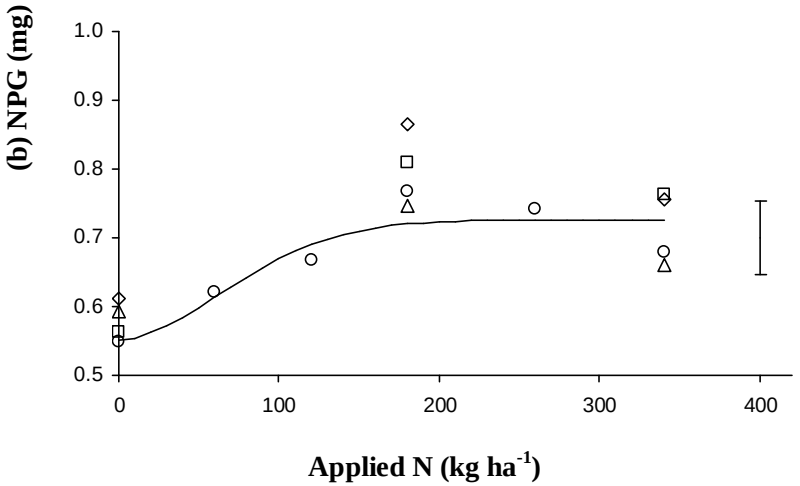
Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Table 6-4.

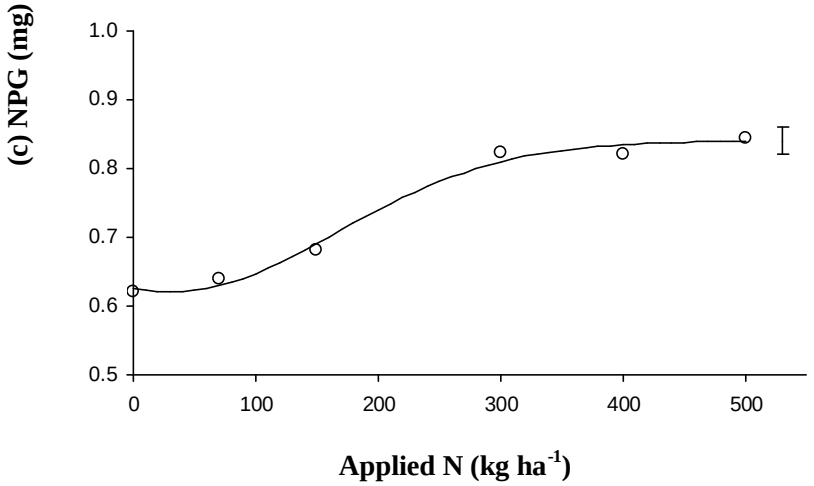
(a & b) Experiments TT06 (a) and TT07 (b). Observed values at six N treatments for four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for all varieties (———); with SED N x V bar (df = 18 (TT06) and 18 (TT07)).

(c) Experiment LC07. Observed values at six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

Figure 6.12 (a, b & c) Effect of applied N and variety on grain N concentration (%) at harvest in TT06, TT07 and LC07.







Mean values for each treatment combination (open symbols) and SED bar (from ANOVA). Model parameters for curves are presented in Table 6-5.

(a & b) Experiments TT06 (a) and TT07 (b). Observed values at six N treatments for four varieties; Istabraq (○), Atlanta (□), Claire (△), and Savannah (◇). Fitted lines for all varieties (———); with SED N x V bar (df = 18 (TT06) and 18 (TT07)).

(c) Experiment LC07. Observed values at six N treatments and one variety; Istabraq (○). Fitted line (———) with SED N bar (df = 15).

Figure 6.13 (a, b & c) Effect of applied N and variety on N content per grain (NPG) at harvest in TT06, TT07 and LC07.

6.3.10 Crop N pools

6.3.10.1 Estimation of the amount of N in crop N pools.

Above-ground N at anthesis and during the post-anthesis phase was partitioned into three conceptual pools; composed of structural N (SN), photosynthetic N (PN), and the reserve N (RN) pool. The amount of N in each pool was calculated from the study data, using several assumptions based on observations from this study:

- Structural N:
    - SN is determined first.
    - SN content of each crop component for each treatment combination is calculated by 'AGDM at harvest x minimum N% observed at harvest'.
    - SN can not be greater than AGN (if so SN is reduced to AGN).
    - SN remains constant from anthesis to harvest.
- Photosynthetic N:
    - PN is determined after SN.
    - Functional N content (i.e. FN = SN + PN) of photosynthetic tissue (i.e. projected green area) is set at the breakpoint for the regression of SLN on RUE (i.e. breakpoint value for TT06 and TT07 at 2.13 g N m^{-2}, and for LC07 at 1.97 g N m^{-2}) at GS61 and GS75 (see note below *).
    - For the lamina and sheath (where the green area is taken as the total component area), PN = FN – SN.
    - For the true stem and ear (where the green area is a fraction of the total component projected area) the PN of the photosynthetic tissue (i.e. green true stem or ear) is calculated as PN = FN (green true stem or green ear x breakpoint value for leaf lamina‡) x (lamina PN/lamina FN)
    - SN + PN can not be greater than AGN (if so PN is reduced; i.e. to below the 'optimum' for RUE).
- Reserve N:
    - RN is determined after SN and PN.
    - RN is calculated by 'RN = AGN – (SN + PN)'
    - RN is equal to or greater than zero.

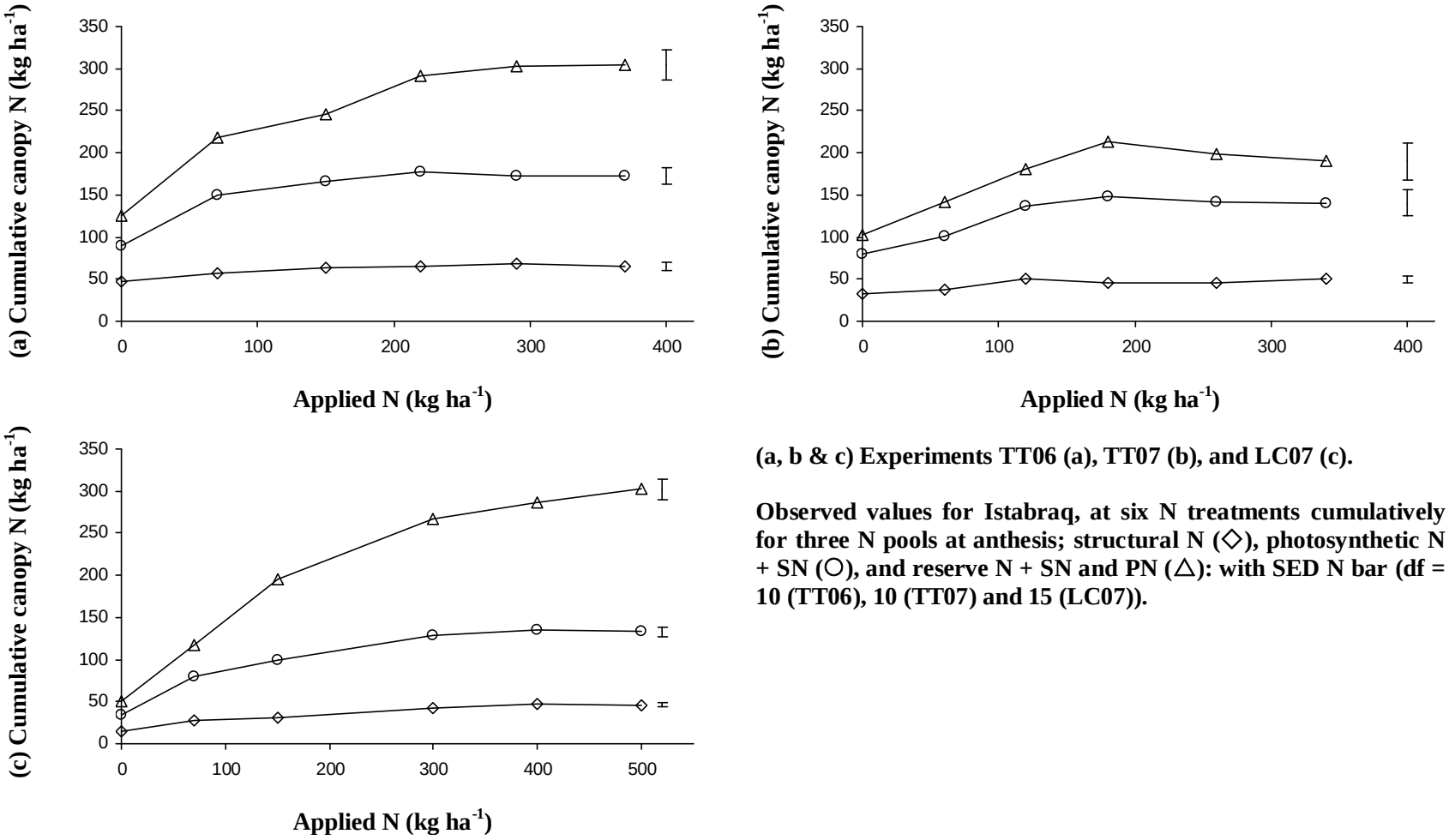
* Comparison of RUE at anthesis and GS75 showed no change in TT07 and LC07, and a marginal decrease in TT06; there were no variety x GS interactions in either experiment. Based on the conservative nature of RUE in the early phase of grain filling, it is assumed that the optimal functional N requirement of the photosynthetic tissue is the same at GS75 as that estimated at GS61.

‡ The breakpoint for the regression of N content of the green area and RUE for the leaf sheath, true stem and ear could not be assessed, so the leaf lamina breakpoint was applied to these organs.

6.3.10.2 Estimated amount of N in the structural, photosynthetic and reserve N pools at anthesis

Nitrogen in each of the three pools increased with N supply in each experiment; averaging across N treatments, SN was highest in TT06 (59 kg ha^{-1}), then TT07 (43 kg ha^{-1}), and LC07 (34 kg ha^{-1}); cf. PN at 88, 80 and 64 kg ha^{-1}, respectively, and RN at 94, 46 and 108 kg ha^{-1}, respectively (Figure 6.14). Averaging across experiments, as the N supply increased up to the optimum N treatment, the proportion of AGN as SN decreased (from 0.32 to 0.20), as PN was unchanged (at 0.39), and as RN increased (from 0.28 to 0.41). At the N max-trt the proportion of AGN in the RN pool increased slightly (to 0.42), as that in the PN pool decreased (to 0.37). Overall, TT07 had the lowest proportion of AGN in the RN (0.26), cf. TT06 (0.37) and LC07 (0.47).

In the Terrington experiments there was an effect of variety on PN in TT06 ($P<0.001$) and on RN in both experiments ($P<0.05$), there were no interactions in either experiment (Figure 6-15). Averaging across N treatments, PN in TT06 was in the range 88 to 107 kg ha^{-1} (Istabraq - Atlanta). In TT06 overall Atlanta had higher RN (108 kg ha^{-1}) than the other varieties in the range 83-94 kg ha^{-1}; cf. in TT07 which showed a different varietal pattern with Savannah (64 kg ha^{-1}), Atlanta (61 kg ha^{-1}), Claire (56 kg ha^{-1}) and Istabraq (46 kg ha^{-1}). Thus, across seasons there was a trend for Atlanta to have higher RN than Claire and Istabraq. Atlanta tended to have a higher proportion of N in the RN pool (due to the higher amount of N in the ear), and a lower proportion of N in the SN (due to the shorter stem length) compared to other varieties.



(a, b & c) Experiments TT06 (a), TT07 (b), and LC07 (c).

Observed values for Istabraq, at six N treatments cumulatively for three N pools at anthesis; structural N (◇), photosynthetic N + SN (○), and reserve N + SN and PN (△): with SED N bar (df = 10 (TT06), 10 (TT07) and 15 (LC07)).

Figure 6.14 (a, b & c) Effect of applied N on the three crop N pools at anthesis for Istabraq in TT06, TT07 and LC07.

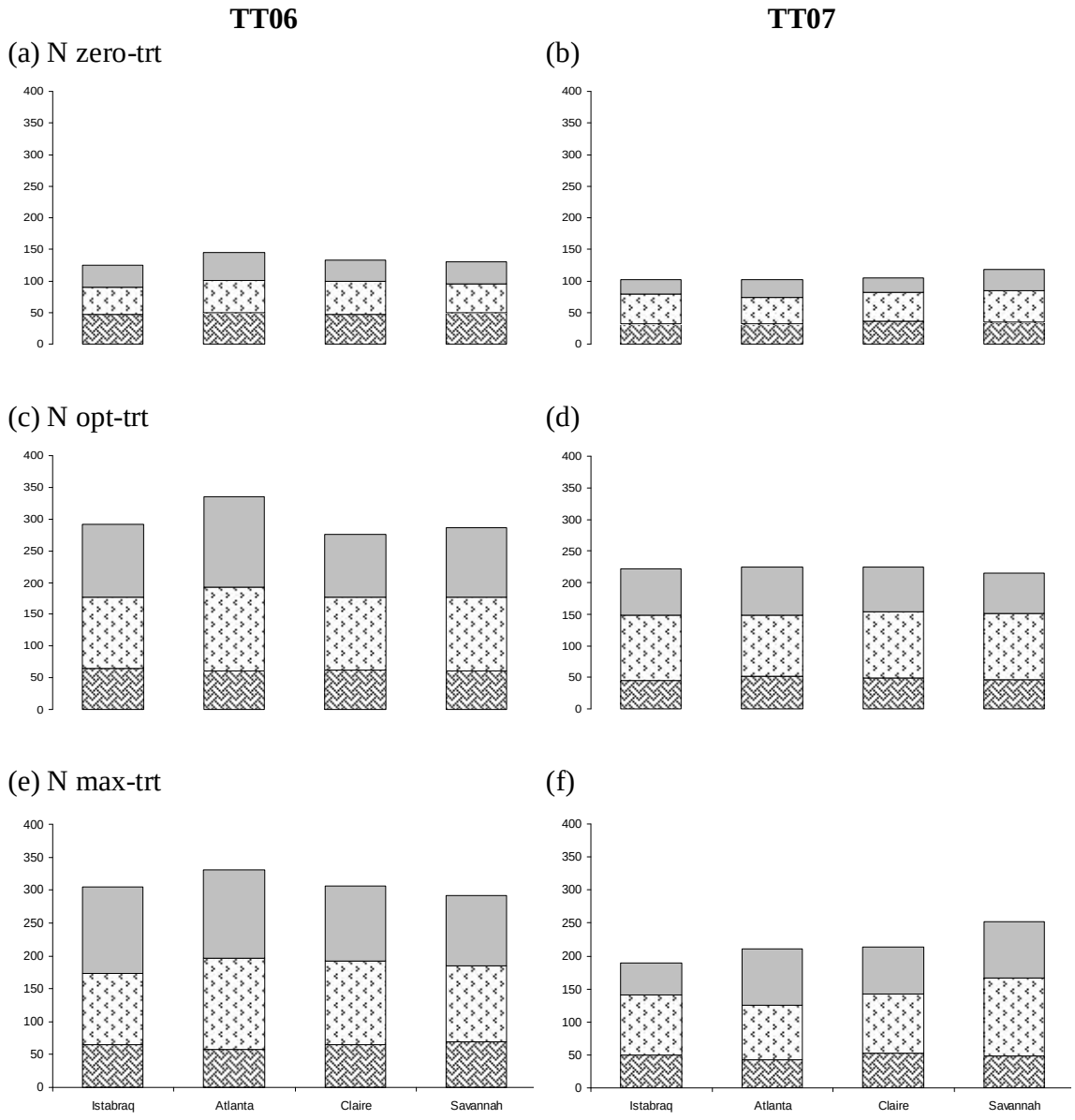


Figure 6-15 (TT06; a, c, e) (TT07; b, d, f) - Effect of applied N on crop N pools (kg ha^{-1}) at anthesis for the Terrington experiments. Observed values for four varieties, at three N treatments (zero, optimum and maximum N) for structural N, photosynthetic N, and reserve N (in ascending order with SN nearest x-axis).

6.3.10.3 Differences in N allocation to crop N pools amongst crop components

Averaged across experiments, at the N opt-trt the true stem (21 kg ha^{-1}) contained the largest amount of SN, then leaf lamina (12 kg ha^{-1}), ear (10 kg ha^{-1}) and leaf sheath (9 kg ha^{-1}). The largest amount of PN was in the leaf lamina, then leaf sheath, ear and true stem (at 67, 24, 7 and 2 kg ha^{-1}, respectively); and most RN was in the true stem, then ear, leaf lamina and leaf sheath (at 45, 28, 19 and 14 kg ha^{-1}, respectively). The pattern in N allocation to crop N pools between crop components was consistent across N treatments (at zero and maximum N treatments) and site-seasons (Table 6-6).

Table 6-6 Estimated amount of structural N (SN), photosynthetic N (PN) and reserve N (RN) (kg ha^{-1}) in the crop components (leaf lamina, leaf sheath, true stem and ear) for Istabraq at anthesis for three N treatments (zero, optimum and maximum) in TT06, TT07 and LC07. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

		SN				PN				RN			
Exp.	**N-trt**	**lamina**	**sheath**	**true st**	**ear**	**lamina**	**sheath**	**true st**	**Ear**	**lamina**	**sheath**	**true st**	**ear**
TT06	Zero	11	9	20	6	29	8	1	5	0	0	12	23
	Opt	17	13	27	8	73	28	3	8	16	6	54	38
	Max	17	13	27	8	71	25	3	8	20	10	59	43
	SED N	1.2	1.0	2.0	0.3	6.9	4.0	0.4	0.9	4.0	3.5	7.7	5.0
	df (P)	10 **	10 **	10 *	10 ***	10 *	10 **	10 *	10 *	10 **	10 *	10 ***	10 *
TT07	Zero	6	6	15	5	26	14	2	5	0	0	13	10
	Opt	9	8	21	8	66	28	3	7	0	5	42	19
	Max	9	9	24	8	61	21	3	6	0	6	28	16
	SED N	1.1	0.7	2.3	0.3	9.0	4.7	0.6	0.7	n/a	3.7	7.0	2.0
	df (P)	10 *	10 *	10 *	10 ***	10 **	10 ns	10 ns	10 ns	n/a	10 ns	10 *	10 *
LC07	Zero	4	2	4	5	13	5	0	3	0	1	7	9
	Opt	10	6	14	13	61	16	1	6	40	31	39	27
	Max	12	7	15	12	62	16	2	7	53	37	48	31
	SED N	0.9	0.5	1.2	0.5	5.2	1.5	0.3	0.5	3.9	1.9	3.9	2.4
	df (P)	15 ***	15 ***	15 ***	15 ***	15 ***	15 ***	15 **	15 ***	15 ***	15 ***	15 ***	15 ***
Average across experiments													
	Zero	7	6	13	5	22	9	1	4	0	0	11	14
	Opt	12	9	21	10	67	24	2	7	19	14	45	28
	Max	13	10	22	9	65	21	3	7	24	18	45	30

6.3.10.4 N remobilisation from the crop N pools during the post-anthesis phase

Comparison of the anthesis and harvest data sets allows the amount of N remobilised (NR) from each pool for each crop component to be calculated. For each component, it is assumed:

- If N at harvest < N at anthesis, then the difference is the amount of N remobilised (NR) to the grain:
    - SN is not remobilised and remains in the straw at harvest.
    - RN is remobilised first in preference to PN (see note below †).
    - PN is remobilised when the amount of N remobilised > RN.
    - RN remobilised at harvest is termed '*storage* N'
    - RN not remobilised at harvest is termed '*accumulation* N'

Therefore;

- NR to grain = *storage* N + remobilised PN
- Straw AGN = SN + non-remobilised PN + *accumulation* N + PANU

† Observations from this study support the assumption that RN is remobilised first in the photosynthetic tissues in preference to the PN between anthesis and GS75 (Figure 6-16). Comparison of the estimated amount of PN (based on canopy green area) at the two growth stages in all three experiments indicate that the PN is broadly maintained during this phase.

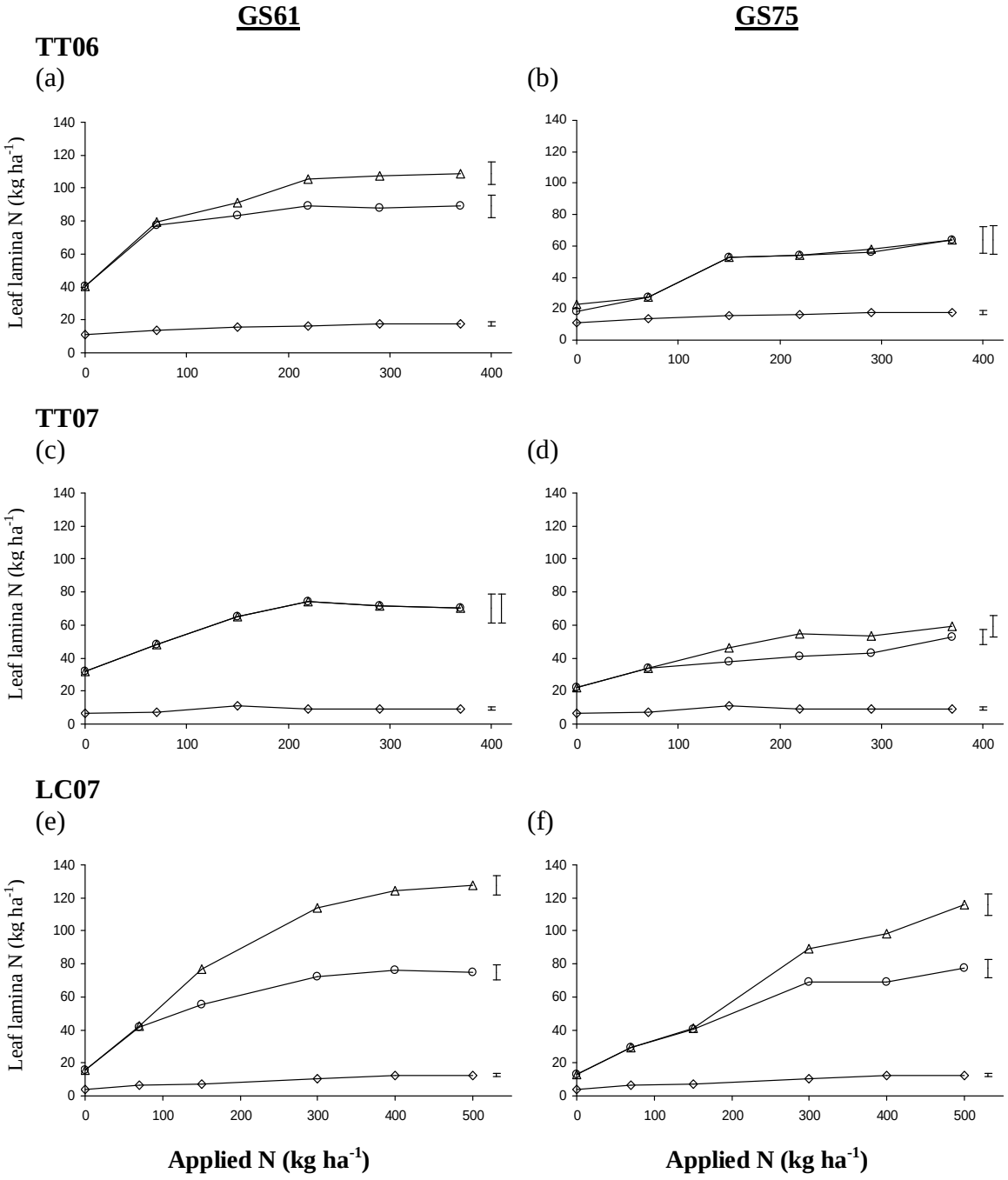


Figure 6-16 (TT06; a, b) (TT07; c, d) (LC07; e, f) – Observed values for the N amount in leaf lamina (kg N ha^{-1}) of Istabraq at anthesis and GS75 at six N treatments cumulatively for structural N (◇), photosynthetic N plus SN (○), and reserve N plus SN and PN (△); with SED N bar (df = 10 (TT06), 10 (TT07) and 15 (LC07)).

6.3.10.5 Estimated amount of N remobilised from PN and RN pools during the post- anthesis phase

The N remobilised and non-remobilised from the RN pool will henceforth be referred to as '*storage* N' and '*accumulation* N', respectively (Table 6-7). Averaging across N treatments, the amount of *storage* N was higher in LC07 (92 kg ha^{-1}) than TT06 (85 kg ha^{-1}) and TT07 (34 kg ha^{-1}); cf. *accumulation* N at 17, 9 and 12 kg ha^{-1}, respectively. As N supply increased up to the N opt-trt, averaging across experiments the percentage of RN remobilised decreased from 96 to 85%, and continued to decrease to 77% at the N max-trt. A similar pattern of remobilisation was observed for the PN pool, where the percentage of PN remobilised was 89, 71 and 64%, respectively.

Varieties differed in the amount of *accumulation* N in both Terrington experiments ($P<0.05$) and *storage* N in TT06 ($P<0.01$); there was an interaction in TT06 for *accumulation* N ($P<0.001$). *Accumulation* N in TT06 was in the range 5 to 12 kg ha^{-1} (Claire to Savannah), and in TT07 in the range 12 to 22 kg ha^{-1} (Istabraq to Claire). Overall there was a trend for Savannah to have higher *accumulation* N than other varieties. *Storage* N in TT06 was overall in the range 71 to 101 kg ha^{-1} (Savannah to Atlanta). The percentage of RN remobilised was affected by variety in TT06 ($P<0.001$) but not in TT07, and there was an interaction in TT06 ($P<0.001$). In TT06 Atlanta remobilised the highest percentage of RN (95%), then Claire (94%), Istabraq (92%) and Savannah (88%); cf. in TT07 varieties were in the range 60 to 76% (Claire to Istabraq). Overall, across the two experiments there was a trend for Atlanta to remobilise the highest percentage of RN. The basis of the interaction in TT06 was at the N max-trt, the percentage of RN remobilised for Claire continued to decrease, for Savannah increased, and for Istabraq and Atlanta was unchanged.

Table 6-7 Estimated amount of reserve N and photosynthetic N (kg ha^{-1}) remobilised (R), non-remobilised (non-R), and percentage remobilised (%R) during the post-anthesis phase for Istabraq in TT06, TT07 and LC07. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

		Reserve N			Photosynthetic N		
Exp.	**N-trt**	**Non-R.**	**R.**	**% R.**	**Non-R.**	**R.**	**% R.**
TT06	Zero	0	35	100	1	42	98
	Opt	13	101	89	29	84	74
	Max	14	118	89	30	78	72
	SED N	2.5	15.1		3.9	9.5	
	df (P)	10 ***	10 ***		10 ***	10 **	
TT07	Zero	3	20	87	12	34	74
	Opt	13	53	80	30	73	71
	Max	19	30	61	30	60	67
	SED N	4.5	8.6		3.4	13.6	
	df (P)	10 *	10 ns		10 ***	10 ns	
LC07	Zero	0	16	100	1	19	95
	Opt	18	121	87	28	58	67
	Max	32	138	81	41	45	52
	SED N	2.9	8.8		1.3	7.1	
	df (P)	15 ***	15 ***		15 ***	15 ***	
Average across experiments							
	Zero	1	24	96	5	32	89
	Opt	14	92	85	29	72	71
	Max	22	95	77	34	61	64

6.3.10.6 Comparison of N remobilisation from PN and RN pools between crop components

Overall for Istabraq the amount of *storage* N and *accumulation* N in the crop components increased with N supply in all three experiments (Table 6-8). Averaged across experiments, at the N opt-trt the true stem had the largest amount of *storage* N (33 kg ha^{-1}), then the ear (25 kg ha^{-1}), leaf lamina (19 kg ha^{-1}) and leaf sheath (14 kg ha^{-1}). At the N zero-trt *storage* N was observed mainly in the true stem and ear. However, at the optimum and maximum N treatments *storage* N was observed in all crop components,

especially the leaf lamina in LC07. The largest amount of *accumulation* N occurred in the true stem, with smaller amounts in the ear and leaf sheath, and there was no *accumulation* N in the leaf lamina. At the N zero-trt, no *accumulation* N was observed in all crop components in all three experiments (except 2 kg ha^{-1} in both the true stem and ear in TT07). As the N supply increased to the optimum and maximum N treatments the amount of *accumulation* N was observed to increase in the true stem and ear.

In the Terrington experiments there was an effect of variety in TT06 on *storage* N (lamina and ear $P<0.001$), but not in TT07; and on *accumulation* N in both experiments (true stem $P<0.05$); there was an interaction in TT06 for *storage* N (lamina $P<0.001$) and *accumulation* N (true stem $P<0.05$). Averaging across N treatments, in TT06 Istabraq had the highest leaf lamina *storage* N (12 kg ha^{-1}) and Claire the lowest (2 kg ha^{-1}); the basis of the interaction was at the N max-trt leaf lamina *storage* N continued to increase for Istabraq whilst for Claire it decreased. Atlanta had higher ear *storage* N (54 kg ha^{-1}) than other varieties in the range 32-35 kg ha^{-1}. In TT06 Savannah had higher true stem *accumulation* N (12 kg ha^{-1}) than Claire (5 kg ha^{-1}), and in TT07 Claire was higher (18 kg ha^{-1}) than Istabraq (7 kg ha^{-1}); the basis of the interaction in TT06 was that stem *accumulation* N for Claire decreased at the supra-optimum N treatment, while for other varieties it continued to increase.

Table 6-8 Estimated amount of photosynthetic N (PN) and reserve N (RN) (kg ha^{-1}) remobilised and non-remobilised in the leaf lamina (leaf), leaf sheath (sh.), true stem (ts.) and ear for Istabraq for three N treatments (zero, optimum and maximum) in TT06, TT07 and LC07. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

		***Accumulation* N**				***Storage* N**				**PN Non-remobilised**				**PN Remobilised**			
Exp.	**N-trt**	**leaf**	**sh.**	**ts.**	**ear**	**leaf**	**sh.**	**ts.**	**ear**	**leaf**	**sh.**	**ts.**	**ear**	**leaf**	**sh.**	**ts.**	**ear**
TT06	Zero	0	0	0	0	0	0	12	23	0	0	0	0	29	8	1	5
	Opt	0	0	13	0	16	6	41	38	12	9	3	5	61	20	0	3
	Max	0	0	14	0	20	10	46	43	13	9	3	5	58	16	0	3
	SED N	na	na	2.5	na	4.0	3.5	8.2	5.0	1.7	2.3	0.7	0.5	7.6	4.1	0.3	0.9
	df (P)	na	na	10 ***	na	10 **	10 *	10 *	10 *	10 ***	10 **	10 **	10 ***	10 *	10 ns	10 ns	10 *
TT07	Zero	0	0	2	2	0	0	12	8	3	3	1	5	23	11	1	0
	Opt	0	0	9	4	0	5	33	15	11	11	3	7	54	19	0	0
	Max	0	0	11	9	0	6	17	8	12	12	3	6	49	12	0	0
	SED N	na	na	4.2	1.8	na	3.7	6.0	3.4	2.0	1.7	0.8	0.7	9.7	4.6	0.3	na
	df (P)	na	na	10 *	10 *	na	10 ns	10 ns	10 ns	10 **	10 *	10 **	10 ns	10 ns	10 ns	10 *	na
LC07	Zero	0	0	0	0	0	1	7	9	0	0	0	0	12	4	0	2
	Opt	0	0	14	4	42	32	24	23	10	10	1	6	52	6	0	0
	Max	0	1	25	6	53	36	23	25	19	15	2	7	44	2	0	0
	SED N	na	0.3	1.6	1.7	3.9	1.9	3.5	3.3	0.8	0.6	0.4	0.5	5.5	1.6	0.3	0.3
	df (P)	na	15 ns	15 ***	15 **	15 ***	15 ***	15 ***	15 ***	15 ***	15 ***	15 **	15 ***	15 ***	15 ***	15 **	15 ***
Average across experiments																	
	Zero	0	0	1	1	0	0	10	13	1	1	0	2	21	8	1	2
	Opt	0	0	12	3	19	14	33	25	11	10	2	6	56	15	0	1
	Max	0	0	17	5	24	17	29	25	15	12	3	6	50	10	0	1

6.4 DISCUSSION

The first part of this discussion considers the response to N supply by Istabraq in all three site-seasons in relation to N uptake and partitioning, green area senescence, biomass production, and N remobilisation to the grain, and varietal responses to N supply in the Terrington experiments. The second part analyses the contribution of N from each of the crop N pools to the grain, and the quantification and location of '*storage*' N and '*accumulation*' N within the canopy, and reviews the specific hypotheses addressed in this chapter.

6.4.1 Crop development and shoot survival

The duration of the first half of the post-anthesis period up to GS75 was shorter at Lincoln than at Terrington, but the period from GS75 to physiological maturity was considerably longer due to the cooler weather conditions. Overall the post-anthesis period was shorter for TT06 and TT07 than for LC07 (by 15 and 24 days, respectively), although the total length of the growing season at Lincoln was shorter than at TT06 and TT07 due to the higher mean air temperature (by 24 and 38 days, respectively). There were only small non-significant changes in fertile shoot density for Istabraq during the post-anthesis phase likely due to within plot variation apparent between samplings, and therefore differences in post-anthesis resource capture are not used to explain site-season effects for fertile shoot density at harvest.

6.4.2 Crop N uptake

Above-ground N at harvest for Istabraq at the N opt-trt in the range 275-351 kg N ha^{-1} was broadly similar to that found in recent studies of UK winter wheat (Sylvester-Bradley *et al*., 1997; Foulkes *et al*., 1998). Although varietal differences in total N uptake at harvest have been observed in previous field studies of wheat (Halloran and Lee, 1979; Ortiz-Monasterio *et al*; 1997; Le Gouis *et al*., 2000), there were no variety differences observed in either of the Terrington experiments in AGN, and no consistent varietal trends across seasons. This lack of varietal difference in AGN was associated with similar

UPE amongst the varieties possibly resulting from similar breeding and parentage of the varieties tested (see Table 3-3) resulting in only small differences in traits such as date of anthesis potentially affecting rooting characteristics and rate and capacity of N uptake.

Several studies on UK-grown wheat have shown that N uptake during the post-anthesis phase typically represents around 10-30% of the AGN at harvest (Dalling, 1985; Widdowson *et al*., 1987; Oscarson *et al*., 1995; Scott *et al*., 1998). Austin *et al*. (1977) reported the mean for 47 winter wheat lines at 17%. However, Loffer *et al*. (1985) found it to be as low at 8%. In this study, PANU for Istabraq at the N opt-trt varied across experiments in the range 59-70 kg ha^{-1} (17-23% of AGN) and was within the reported range of previous studies (De Ruiter and Brooking, 1996; Andersson, 2005). PANU is affected by N availability (Masoni *et al*., 2006) and in all three site-seasons there was a trend for increased PANU with N supply. Previous investigations suggest that PANU is strongly influenced by the variation between site-seasons (Cox *et al*., 1985b; Webb *et al*., 1997) as a consequence of variation in soil type, N availability and soil moisture content (Austin *et al*., 1977; Cox *et al*., 1985b; Bly and Woodward, 2003).

In TT06 PANU was lower in Atlanta (9 kg ha^{-1}) than other varieties (range 34-48 kg ha^{-1}) but in the following season differences were non-significant in the range 56-71 kg ha^{-1}. Although several studies have reported significant varietal differences (Cataldo *et al*., 1975; Peterson *et al*., 1975), a lack of consistent varietal differences in the present study in AGN at harvest, PANU and AFR was possibly the consequence of the narrow breeding background of these four contemporary feed wheat varieties.

6.4.3 Crop N partitioning

At the optimally fertilised treatment N partitioning for Istabraq at harvest differed only slightly across site-seasons; the majority of the AGN was in the grain (68-74%) with most of the remainder in the true stem (8-12%), rather than the leaf lamina (6-8%), leaf sheath (4-6%) or chaff (4-7%). A small amount of N would be expected to be in the roots (10-20%; Andersson, 2005) but was not accounted for in this study. Overall the

proportion of AGN in the respective crop components for Istabraq was relatively similar in all three experiments.

For winter wheat the distribution of N between the grain and the straw is typically 70:30 (i.e. NHI of 0.70; McNeal *et al*., 1966; Austin and Jones, 1975). Overall TT06, TT07 and LC07 had a broadly similar NHI at 0.69, 0.65 and 0.75, respectively. The grain is a strong sink for N (Martre *et al*., 2003) and possesses the ability to accumulate most of the N absorbed by the plant (Vaidyanathan *et al*., 1987). NHI typically appears to be stable over a range of N treatments (Bloom *et al*., 1988), but has been observed to decrease at high N supply (Blacklow and Incoll, 1981) due to the diminishing increases in yield and grain N%, yet continued uptake of N in the components of the straw. NHI was observed to decrease with N supply in LC07, but there was no effect of N supply in TT06 and TT07.

N harvest index appears to be a generally conservative trait (Austin, 1980; Foulkes *et al*., 1998) with variation across environments in the range 0.70 to 0.80 (Austin *et al*., 1977; White *et al*., 1998; Andersson, 2005), although higher NHI has been reported (Van Sanford and MacKown, 1987; NHI of 0.83 for mean of 9 soft red winter wheat cultivars). There was an effect of variety in TT06, with a N treatment x variety interaction as Savannah showed a sharp decrease at the N max-trt compared to the other varieties. However the varietal pattern of NHI was not consistent across seasons, and other field investigations have revealed only small differences in NHI amongst UK winter wheat varieties in the ranges 0.76-0.79 (Foulkes *et al*., 1998) and 0.73-0.75 (White *et al*., 1998).

For the straw components the largest response to N supply was observed at LC07 ($P<0.001$; all components) with all straw components responding positively to N supply as was reported in previous studies (Cox *et al*., 1985a). The proportion of AGN in the true stem showed a relatively greater increase with N supply compared to the leaf lamina and leaf sheath. This may have been a consequence of the increased stem length with N supply, and an indication that the true stem was accumulating N at high N availability. Although Istabraq in TT07 showed a small increase in true stem N partitioning with increased N supply, overall in the Terrington experiments there was no effect of N supply

on the proportion of AGN in the crop components at harvest. Comparing sites-seasons LC07 had the least AGN in the true stem (8%) likely due to the shorter stem length; cf. TT06 and TT07 (both 13%).

Variety differences in N partitioning at harvest in the Terrington experiments were small and inconsistent. Overall there was a trend for Atlanta to partition less N to the true stem likely due to the shorter stem length compared to the other varieties. However although varietal differences and responses to N were inconsistent, further study may demonstrate larger varietal differences in a wider UK germplasm or amongst more exotic germplasm (e.g. synthetic wheats or diploid ancestor) as an avenue to breed for increasing NHI through a reduction in true stem N content at harvest. It is likely that significant varietal differences in N partitioning between straw components at harvest could be linked to distinct physiological traits; e.g. for the true stem length as indicated by the results of the present results or alternatively stem wall thickness (Sylvester-Bradley, personal communication).

6.4.4 Green canopy area, radiation interception and radiation-use efficiency

There was a small decrease in green canopy area in the first half of grain filling, notably in the N limited treatments. However canopy light interception probably remained close to the maximum in well fertilised crops, assuming no change in K during this period (Shearman *et al.*, 2005) and that canopy died from the base upwards (Hay and Walker, 1989). Post-anthesis green area loss was most rapid in TT07, although this was found not to have reduced LI much below other site-seasons. Overall total IR in the first half of grain fill was higher at Terrington than Lincoln, likely due to the dull and cloudy weather conditions and shorter duration of this period in LC07.

In the second half of grain fill the N-limited treatments continued to senesce more rapidly than the well fertilised treatments; overall the N zero-trt reached CCS 8 days earlier than the N opt-trt, with little difference between the optimum and maximum N treatments. N

deficiency accelerates leaf senescence due to faster N remobilisation from the leaf lamina to the grain (Morris and Paulsen, 1985; Sinclair and Amir, 1992), and green area loss is reported to accelerate in crops with low SLN at anthesis (Evans, 1983; Borrell and Hammer, 2000). At the optimum and maximum N treatments, the higher SLN is associated with maintenance of the green leaf area, referred to as 'stay-green' (Jenner and Rathjen, 1975). Lower SLN in TT07 could explain the faster senescence than in LC07 (overall SLN at anthesis of 1.42 and 2.69 g N m^{-2}, respectively). However the rate of senescence is also affected by soil moisture and temperature (Parameswaran *et al*., 1984); warmer and drier conditions in TT06 may have accelerated canopy senescence, whereas cool and cloudy conditions in LC07 may have prolonged green canopy area. Despite evidence of genetic control of plant senescence in wheat (Richards, 2000; Christopher *et al*., 2008) and in other crops such as sorghum (Borrell and Hammer, 2000), there was only a small effect of variety in TT06 and no effect in TT07; Claire apparently reached CCS earlier than the other varieties.

Assessment of leaf lamina senescence on individual leaf layers in LC07 showed that the lower leaves senesced earliest and most slowly, whilst the upper two leaves senesced at similar rates and over similar durations. There was an effect of N treatment on the start date and rate of senescence especially in the lower leaves, the N zero-trt started to senesce earliest and senesced showing a lower rate of senescence compared to the fertilised treatments. The upper two leaves senesced at a similar rate in all N treatments likely due to lower rate of grain N demand at the N zero-trt, but senescence again started earliest in the N zero-trt. Thus, where the start of senescence was delayed with increasing N supply, the rate was faster (i.e. there was an inverse relationship between start date and rate). However, overall the later start of senescence related to improved green area duration (i.e. 'stay-green' effect), was not completely counteracted by the faster rate of senescence leading to improved light interception during the post-anthesis period.

Although the lower leaves contribute to canopy light interception, the upper two leaves are particularly important as about half of the photo-assimilate moved to the grain originates from the flag and penultimate leaf (Rawson *et al*., 1983; Gooding *et al*., 2000).

The rate of reduction in gross photosynthesis of flag and penultimate leaves is closely related to the proportion of N already exported by them (Gregory *et al.*, 1981) and the increase in SLN at anthesis at the optimum and maximum N treatments was probably associated with the greater green area persistence and increased net photosynthesis.

Biomass production during the post-anthesis phase continued to be driven by N supply. During this phase crops at LC07 produced the most biomass and consequently had the highest AGDM at harvest, likely due higher incident solar radiation and duration of grain fill than TT06 and TT07, and also to the continued soil moisture availability facilitating canopy photosynthesis. There was no statistically significant effect of N treatment on RUE at GS75 for Istabraq in the experiments, although there was a trend for lower RUE at N zero-trt. RUE was overall higher at Lincoln than Terrington, but significantly lower in TT06 than TT07. Comparison of RUE during the stem-elongation phase with the first half of the post-anthesis phase showed no difference in TT07 and LC07, but a decrease in TT06 (from 2.96 to 1.67 g MJ^{-1}) again possibly due to restriction in biomass production due to water stress rather than reduced SLN (in agreement with Bingham *et al.*, 2007). For the calculation of the PN requirement of the green area, it was therefore assumed that the optimum SLN content for RUE remained the same at GS75 as at anthesis. In the second half of the post-anthesis phase N remobilisation from the leaf lamina is reported to accelerate leaf senescence causing a rapid reduction in RUE (Bingham *et al.*, 2007).

6.4.5 Crop N remobilisation components

Total grain N accumulation is equivalent to PANU plus the net remobilisation of N accumulated in the vegetative tissues prior to anthesis. High N remobilisation is important in the efficient utilisation of canopy N, although low N remobilisation may be more efficient for grain C accumulation per unit canopy N. PANU contribution to the grain is typically low (Austin *et al.*, 1977) and varies depending upon the soil N content and its availability (Dhugga and Waines, 1989). N remobilisation contributes at least 50% and potentially up to 100% of the grain N (Pearman *et al.*, 1977; Spiertz and Ellen, 1978; Pakakosta and Gagianas, 1991; Austin *et al.*, 1997) but typically is around 75%

depending on N supply (Masoni *et al.*, 2006). Overall N remobilisation contribution for Istabraq in TT06, TT07 and LC07 was within this range at 80%, 62% and 63%, respectively (153, 90 and 132 kg ha^{-1}, respectively).

N contribution to the grain by NR decreased with N supply between the zero and optimum N treatment in TT06 (from 88 to 76%) as found by Blacklow and Incoll (1981; from 84% to 65%) and by Masoni *et al*. (2006). However NR in TT07 was relatively unchanged (from 65% to 67%) and LC07 increased (from 45% to 72%). The leaf lamina was the most important source of N for the grain at all N treatments as found by Critchley (2001), overall in TT06, TT07 and LC07 representing 32%, 28% and 31% of the grain N contribution, respectively. In LC07 the amount of NR was lower at N zero-trt probably due to low leaf area (i.e. lower total leaf lamina N content) at anthesis, and was higher at the optimum and maximum N treatments possibly due to higher SLN (i.e. high leaf lamina N content) at anthesis. However, this major relocation of N from the leaf lamina exerts a strong negative effect on the capacity of the crop to assimilate carbon, mainly though the loss of green area in the second half of the grain-filling phase. Observed data in the present study supported the hypothesis of Sinclair and De Wit (1975) linking N remobilisation from photosynthetic enzymes with lamina senescence and reduction in photosynthetic capacity.

Comparing between experiments, the amount of N remobilised at the N opt-trt in the true stem NR in Terrington was ca. 50% greater than that of LC07 (37 and 24 kg ha^{-1}, respectively) associated with more true stem N at anthesis and longer stem length. This suggests that the true stem may act as an opportunistic location for N accumulation given its physical capacity and integration with the solute transport systems. Overall variation in the N content of the crop components at anthesis seemed to explain the majority of the variation observed in the amount of N remobilisation (rather than differences in NRE). Although under genetic control, relatively little genetic variation in the amount of N remobilisation was observed in the Terrington experiments, reflecting in turn, the limited varietal variation in true stem N loading at anthesis. As the amount of N remobilised also depends on the environmental conditions (Halloran, 1981, Simmons, 1987, Van Sanford

and MacKown 1987) repeatable genotypic differences may be difficult to obtain (May *et al*., 1991).

Overall, N remobilisation efficiency for all canopy components for Istabraq was similar in TT06 and LC07, but slightly lower in TT07 due to lower crop N content at anthesis. NRE decreased with N supply; consistent with the findings of Dalling (1985), Spiertz and Ellen (1987), Campbell *et al*. (1995), Delogu *et al*. (1998) and Barbottin *et al*. (2005). In each experiment NRE initially increased with N supply as grain N demand increased relative to AGN at anthesis. The subsequent decrease with increasing N supply observed in LC07 was due to both a reduction in grain N sink size relative to AGN and an increase in PANU at high N supply. For all three experiments at the N opt-trt NRE was highest for the leaf lamina, consistent with this organ containing the highest amount of potentially mobile photosynthetic and metabolic N at anthesis (Lawlor *et al*., 2001). The leaf sheath is similar in function to the leaf lamina, and NRE for this component was higher in LC07 (70%) than TT06 or TT07 (54% and 59%, respectively). The true stem had the lowest NRE in all experiments, at 48% (TT06), 50% (TT07) and 46% (LC07). In comparison with the other canopy components the low NRE values for the true stem imply that a higher proportion of the N content is immobile (i.e. as structural N).

Varietal differences in NRE were reported in previous investigations in the range 51-91% (Van Sanford and MacKown, 1987) and 61-81% (Papakosta and Gagianas, 1991). In TT06 small genetic effects on leaf lamina and chaff NRE were related to differences between Atlanta and the other varieties. Overall, chaff NRE was highest for Atlanta (82%, and range 72-77%) related to the higher ear N content at anthesis (possibly due to an earlier calendar date of anthesis), with a similar trend in TT07 (49%, and range 37-40%). Leaf lamina NRE in TT06 was also highest for Atlanta (79%, and range 72-76%) associated with high lamina N content at anthesis (through high LAI). Van Sanford and MacKown (1987) also found leaf lamina NRE of fertilised soft red winter wheat was subject to genetic variability. Low lamina NRE may be associated with increased canopy photosynthesis in the grain-filling phase (and therefore increase UTE). However varietal differences in TT06 were not sufficiently large to test this in this study.

6.4.6 Rate of N mobilisation, and relationship with canopy senescence

Overall ear N accumulation occurred more quickly in the second half of grain fill than in the first, particularly at the N zero-trt. Grain N accumulation has been observed to occur at a generally linear rate in fertilised winter wheat in Australia (Richards, 2000) related to decreases in N content of the vegetative tissues and PANU. In the present study, overall in the first half of the phase remobilisation was most rapid for the leaf lamina and true stem, and in the second half for the leaf lamina and leaf sheath. PANU was found to be an important source of grain N at all N treatments; for the N zero-trt in the first half of the phase, and for the optimum and maximum N treatments in both halves of the phase.

The amount of N mobilisation from the true stem was overall higher in the first half of the phase, indicating that the true stem acts as an initial N source for grain filling thereby buffering grain N demand on the PN pool. The rate of N mobilisation from leaf lamina was considerable in both halves of the phase, whilst the majority of leaf sheath senescence occurred in the second half of the phase. Present results strongly imply that in the first half of the phase most of the N remobilised was from the RN pool, and was probably used before the PN pool since photosynthetic activity was broadly maintained through the first half of grain fill. This is consistent with the findings of Peoples and Dalling (1988) who observed that N remobilisation from photosynthetic proteins was strongly linked to canopy senescence. Consequently N remobilised in the first half of the phase was apparently mainly RN, whilst in the second half N was from both the RN and PN pools.

The analysis presented in chapter 5 indicated that both the leaf lamina and true stem contained significant RN at the optimum and maximum N treatments to facilitate grain N supply without significant loss of photosynthetic tissues or reduction in photosynthetic capacity. Consequently the green canopy area persisted during grain filling despite the apparent mobilisation of N from the tissues. The maintenance of leaf lamina area after

anthesis is a major determinant of grain yield (Richards, 2000). Present findings support the original hypothesis that the canopy RN has important physiological function in maintaining photosynthetic capacity by buffering green tissue senescence, particularly in the first half of the grain-filling phase. However testing for varietal differences in the temporal pattern of canopy N dynamics and senescence was not possible as data at GS75 was available for Istabraq only in the three site-seasons.

6.4.7 Grain N%

Grain N concentration response to N supply for Istabraq followed a typical Normal Type curve with Depletion pattern in all three seasons, as observed in previous investigations (Austin, 1980; Murray and Nunn, 1987; Vaidyanathan *et al*., 1987). As the amount of applied N increased towards the N opt-trt, the grain dry matter yield initially increased more than grain N uptake and consequently slower increase in grain N content was observed at the sub-optimal N treatments. Above the N opt-trt, grain N content increased linearly but grain yield increase diminished, and overall the grain N% increased slightly.

Grain N% is a complex trait that results from an interaction of several component traits: N uptake and assimilation prior to anthesis, PANU and N remobilisation during the post-anthesis phase, grain yield and HI; and environmental conditions during the grain-filling period. Small site-season effects were observed. Overall, grain N% for Istabraq was highest in TT06 (1.93%), cf. LC07 (1.80%) and TT07 (1.73%) possibly due to the warmer post-anthesis period (July 2006) reducing the duration of the grain filling period and consequently decreasing carbohydrate supply to the grain. Increases in grain N% with shorter grain filling period were observed by Martre *et al*. (2003), where C accumulation decreased more than N accumulation. LC07 had a relatively cool post-anthesis period which extended the duration of grain filling; this increased the amount of IR and consequently grain assimilate supply resulting in high yields with lower grain N% (i.e. dilution effect). TT07 had the lowest grain N% likely due to dull and wet weather reducing N uptake prior to anthesis and post-anthesis N remobilisation.

Although determined by genetic factors (Cox *et al.*, 1985b; Beninati and Busch, 1992), grain N% is relatively conservative parameter at 1.5 to 2.5% N (Foulkes *et al.*, 1998) and varies more across end-use groups (e.g. feed vs. bread-making) than between varieties within a group (Foulkes *et al.*, 1998). There was an effect of variety in both Terrington experiments, although the varietal pattern was not consistent across seasons and overall grain N% was relatively similar amongst the varieties. Turning to consider the relationship between grain yield and grain N% amongst varieties, overall in TT06 Atlanta had the highest yield but the lowest grain N%, consistent with the negative yield-grain N% relationship often observed in wheat (Kramer, 1979; Cox *et al.*, 1985a; Triboi *et al.*, 2006). The negative relationship was again observed amongst varieties in TT07. Improvements in grain yield and HI, whether genetic or agronomic, are therefore often linked to reduced grain protein content. Present results indicate that low grain protein varieties may be useful in improving UTE and possibly may be obtained via reduced N remobilisation helping to maintain canopy greenness and photosynthetic capacity for grain filling.

Overall Savannah in TT06 and Istabraq in TT07 showed negative departures from the general negative relationship between yield and grain N% (i.e. lower grain N% in relation to grain yield than expected). However, no consistent varietal effects (either positive or negative) were identified across the two seasons perhaps due to the relative similarity between the study varieties. Although further experimentation may elucidate consistent varietal patterns between these varieties, inclusion of varieties from wider UK or worldwide germplasm is likely required to identify the underlying traits associated with such negative or positive departures from the yield-grain N% relationship. However, genotypes with consistently lower grain N% associated with lower NRE may represent potentially useful germplasm for breeding for increased UTE. Present results indicate that traits that may confer a negative departure from the overall negative relationship between yield and grain N% are low NRE from photosynthetic organs (i.e. stay-green) and high PANU.

6.4.8 Quantifying the role of N accumulated at anthesis

Canopy N content at anthesis was allocated to the three conceptual N pools as proposed by Lemaire and Gastal (1997) on the basis of assumptions made from the study data. For all N treatments the SN pool accounted for the least canopy N at anthesis, overall at 28% with the percentage of SN decreasing with N supply. The PN pool accounted for 34% of canopy N, with the percentage relatively unchanged by N supply up to the N opt-trt thereafter decreasing due to more N in the RN pool. Finally the RN pool overall accounted for slightly more of the canopy N than the PN, overall at 37% with the percentage increasing sharply from 33% at the N zero-trt to 56% at the N max-trt.

For all three site-seasons the true stem accounted for the most SN, averaging across experiments at the N opt-trt at 21 kg ha^{-1} (40% of canopy SN). This was strongly related to its function as the main structural element of the wheat plant. Whilst the main photosynthetic organs leaf lamina and leaf sheath accounted for the most PN (67% and 24% of canopy PN at N opt-trt, respectively); the true stem and ear accounted for only small amounts of PN. However it is possible that the present estimates may represent an underestimate of ear PN, as the calculation was based on the planar area which has been shown to be considerably less than the total area of the sum of the ear components (Critchley, 2001). All crop components contained some RN; averaging across experiments at the N opt-trt the true stem contained the most (43%), then the ear (26%; although this was perhaps due to the inclusion of the developing grain in the 'chaff' at anthesis), and the leaf lamina and leaf sheath (18% and 13%, respectively).

Above the N opt-trt the leaf lamina and leaf sheath both increased in RN content, suggesting that the photosynthetic tissues continued to accumulate N, possibly in photosynthetically active enzymes such as Rubisco (Evans, 1989; Lawlor *et al*., 1987a) which has been suggested to have a storage role in wheat (Millard, 1988). The considerable quantity of RN located in the true stem in all experiments indicates that this crop component has a significant reserve N function, as previously observed by Triboi and Ollier (1991). True stem RN capacity may facilitate increased N uptake capacity during the pre-anthesis phase given the physical size and location of the organ (Jamieson,

personal communication), and provide non-photosynthetic N for grain-filling during the post-anthesis phase (Jamieson and Semenov, 2000).

N remobilised from the RN pool during the post-anthesis phase was termed *storage* N whilst that not remobilised was termed *accumulation* N (Millard, 1988). The majority of RN was remobilised in all experiments leaving only a small amount of N remaining in the straw at harvest at the fertilised treatments. Overall, averaged across experiments the true stem had the highest proportion of *storage* N (34%), then the ear (30%), leaf lamina (21%), and leaf sheath (15%). Therefore present results suggest that the true stem has a more important role in canopy N crop *storage* than the leaf lamina or leaf sheath. The variety effect on lamina *storage* N in TT06 was associated with Istabraq having higher lamina SLN but lower PN compared to the other varieties (associated with low LAI at anthesis whilst the lamina N content was similar), and potentially linked to later complete canopy senescence (i.e. stay-green effects) for this variety at the optimum and maximum N treatments, potentially leading to increased UTE.

Turning to consider *accumulation* N, overall for Istabraq in all experiments the true stem (77%) had considerably higher *accumulation* N than the other crop components; compared with the ear (23%), and the leaf sheath and leaf lamina (both 0%). Higher *accumulation* N as a result of higher N loading at anthesis would act to reduce the UTE in the fertilised crop, and therefore would not be associated with reducing the global canopy N without affecting yield. However, *accumulation* N through decreased NRE in the leaf lamina and leaf sheath would act to increase UTE (e.g. stay-green effect). Overall there was a trend for Savannah to have a higher amount of *accumulation* N in both seasons compared to the other varieties, and this *accumulation* N was mainly located in the true stem. This was associated with significantly higher N content at anthesis for Savannah, and whilst there was no varietal difference in true stem NRE, in TT06 there was a trend for Savannah to have lower true stem NRE than the other varieties. However this effect did not result in significantly lower UTE for Savannah. The other three varieties showed similar true stem *accumulation* N, again possibly the result of the narrow range of germplasm in this study.

6.4.9 Uncertainties in estimating crop N pools

The main assumption in the model was that only three crop N pools exist. Although two further N pools ('transport N' and 'non-photosynthetic metabolic N') have been identified, the amount of N in these pools is considered to be relatively small in comparison with the main N pools (Grindlay, 1997; Lemaire and Gastal, 1997; Critchley, 2001). Secondly, it was assumed that the PN content of the leaf sheath, green true stem and green ear tissues was maximal at the SLN which gave the breakpoint between RUE and SLN for the leaf lamina. Although there was insufficient data to calculate the breakpoints for individual components in the present study, there is evidence to suggest that the PN requirement of green tissues varies between organs (Field, 1983; Hirose and Werger, 1989; Grindlay *et al*., 1997); moreover that the green areas of the leaf sheath, true stem and ear is greater than the planar areas used in this study (Critchley, 2001). Finally, it was assumed that N in the RN pool is remobilised first in preference to the PN, with PN only being remobilised when the amount of N remobilised was greater than RN. However, it is likely that there would be some turnover between N in the two pools since they exist in the same tissue; e.g. Rubisco in the leaf lamina which is involved in both PN and RN pools, and has a high rate of dynamic turnover without apparent mobilisation from the component (Irving and Robinson, 2006). However despite these criticisms, present analyses provide a new quantitative framework that may be developed and validated further in future work.

6.4.10 Conclusions

The accumulation, partitioning and remobilisation of N between the crop components was found to be affected by N supply, but only small differences between varieties were observed. Results supported hypothesis (6) 'that crop components differ in their accumulation of reserve N, and the true stem has a more significant role in the accumulation of RN at anthesis than other crop components'. The capacity of the crop to take up and accumulate N may be related to the physical capacity of the components, particularly the true stem length (this study; Martre, 2006) and stem wall thickness. Consequently the amount of RN increased significantly with N supply up to the N opt-trt,

but only slightly there above at the N max-trt. Despite the high N content of the true stem at anthesis at all N treatments, the NRE from this crop component was typically low compared to the leaf lamina and leaf sheath and significant quantities of *accumulation* N were remaining at harvest in the fertilised crops, and therefore created potential inefficiency in UTE, although the high SN requirement of this organ is intrinsically linked to its function. Results supported hypothesis (8) 'that *accumulation* N creates inefficiencies in crop N use by reducing NRE and increasing straw N content, especially at high N availabilities', although only small varietal differences in NRE were observed in relation to hypothesis (10) 'that there are genetic differences in N remobilisation efficiencies of the plant organs (leaf laminae, leaf sheath and true stem) and their responses to N linked to patterns of senescence'.

Present results indicated that mobilisation of RN from true stem and leaf lamina occurred before PN from the photosynthetic tissues, thereby providing a buffer to canopy senescence, supporting the hypothesis (9) 'that *storage* N provides a buffer against premature redistribution of photosynthetic N and hence canopy senescence'. Studies in maize report similar findings (Beauchamp *et al*., 1976; Friedrich and Schrader, 1979). Experimental and modelling results have shown that grain N accumulation is mostly source determined (Martre *et al*., 2003), and results from this study indicate that the true stem is a significant source of N for grain filling at all N availabilities, and not just at low N supply as proposed in hypothesis (7) 'that *storage* N has an important physiological role in wheat crops, especially true stem *storage* N at low N availabilities' for N uptake, rate of N uptake and N utilisation. However, the true stem has also been shown to have the capacity to accumulate significant quantities of N in excess of that required by the grain (i.e. leading to *accumulation* N in the fertilised crops), and therefore a potential target trait for breeding for reduced canopy N content without compromising yield. Manipulating true stem RN capacity by breeding may therefore provide an avenue for increasing UTE. The next chapter aims to investigate further, through source-sink manipulations imposed in present experiments, the determinants of genetic variation in N relocation to the grain and NRE for the true stem and other plant organs at contrasting N levels.

7 N SOURCE-SINK MANIPULATION EXPERIMENTS

7.1 INTRODUCTION

From the analysis in chapter 6, reserve N has been quantified in all crop components at all N treatments at anthesis, and assigned to either *storage* or *accumulation* roles at harvest. Significant quantities of *accumulation* N were identified in the true stem at the optimal and maximum N treatments, the exact function of which is yet to be explained. The results reported in chapters 4 to 6 described the extent to which the true stem N responds to N supply and the varietal differences in responses. The extent to which the remobilisation of this RN can be increased in response to changes in source-sink balance was tested by imposing degraining and defoliation treatments as reported in the present chapter.

During the post-anthesis phase, the vegetative tissues become net exporters of N and therefore the major N source (i.e. the total non-structural crop N at anthesis) for the developing grain, which becomes a net importer of N and acts as the N sink. Manipulation of the post-anthesis source-sink ratio was achieved through either reducing the N source size or N sink size at around two to three weeks after anthesis. Reduction in the N source was achieved by removing by hand leaf lamina ('defoliation'); the leaf lamina has been shown to be the major contributor of N for grain filling (this study; Guitman *et al.*, 1991). Alternatively, reduction in the N sink was achieved by removing all the spikelets from one side of the ear ('degraining'). Consequent effects on the grain N content at harvest were examined and the N contribution to the grain of the respective crop components calculated.

The source-sink manipulation treatments imposed in the experiments tested: (i) whether the remobilisation of N to the grain is source or sink driven by examining 'C' and 'N' accumulation in the grain in response to an increase in source-sink ratio (degraining), and (ii) whether the amount of *storage* N in relation to *accumulation* N in the true stem is increased through a decrease in source: sink ratio (through defoliation). With regard to

(ii), the reduction in grain N in proportion to the loss in leaf lamina N would provide information on the potential capacity of true stem RN for remobilisation. The chapter concludes with a discussion of the findings in relation to testing the study hypothesis: (11) 'that remobilisation of N to the grain is source driven, and that source-sink manipulation treatments (through defoliation and de-graining) can promote the use of canopy RN' (in particular that contained in the true stem).

7.2 METHODOLOGY

7.2.1 Manipulation treatments

Manipulations were imposed after the end of flowering during early to mid grain filling; at GS61 +17d (TT06), +18d (TT07), and +22d (LC07), as at this point endosperm cell division and expansion has ended and potential grain size and grain structural N is fixed (Singh and Jenner, 1982; Calderini *et al*., 2001; Gooding *et al*., 2003). Manipulations therefore were intended to mainly affect storage protein accumulation in the developing starch endosperm (Gupta *et al*., 1996).

- Defoliation was achieved by the removal of 'leaf 3 and below' on each shoot; leaving only the upper two leaves which contribute the majority of leaf lamina photosynthetic capacity (Rawson *et al*., 1983).

- Degraining was achieved by the removal of half of the ear through removal of all the spikelets from one side of the ear, which affects all spikelet positions equally.

The two manipulation treatments (defoliation and degraining) were made to Istabraq at two N treatments (zero and optimum) in all three experiments, together with an un-manipulated control. The shoots for manipulation were chosen within specified areas in the plots of the main experiment; these areas were randomly selected and clearly marked. The manipulated and control areas were 30 cm row-lengths of either 3 adjacent rows (in the Terrington experiments) or 5 adjacent rows (in the Lincoln experiment), giving a total sample area of 0.108 m^2 or 0.225 m^2, respectively. A buffer was left around the

manipulated and control areas to minimise border effects or the influence of quadrat sampling in nearby areas of the plots.

All the shoots within the 'manipulated' area were counted and then manipulated. The plant material removed during manipulation, either leaf lamina (which was separated into green and dead material) or chaff, was dried to constant weight, and then weighed and the N content determined (as detailed in 3.5.5 and 3.5.7). At harvest both the control and the manipulated shoots were sampled for physiological analysis, N content and yield analysis (as detailed in 3.5.6).

7.2.2 Statistical analysis

The significance of the treatment effects was determined by ANOVA, for the main effects ('N treatment' and/or 'manipulation' and/or 'crop component') and their interactions (as detailed in 3.7.1). A cross-site season ANOVA was applied to determine the consistency of the main treatment effect across the three site-seasons ('experiment') and its interactions.

7.3 RESULTS

7.3.1 Shoot density and grains per ear

There was no effect of manipulation treatment on shoot density at harvest in all three experiments, and therefore results in this chapter are reported on a per shoot basis. There was no effect of defoliation treatment on the grains per ear (GPE) at harvest compared to the control in all three experiments, whilst degraining approximately halved the GPE.

7.3.2 Quantification of source-sink manipulation

7.3.2.1 Defoliation

The amount of potential grain N contribution from the green leaf lamina (i.e. from leaf lamina PN+RN) removed in the defoliation treatment was determined as 'total N content – structural N' (estimated using the assumptions in 6.3.10.1) (Table 7-1).

Table 7-1 Amount of leaf lamina photosynthetic N (PN) and reserve N (RN) removed (mg N shoot^{-1}), and proportion of leaf lamina or total canopy PN+RN removed, at two N treatments in TT06, TT07 and LC07, and mean across experiments.

N-trt	N removed	TT06	TT07	LC07	Mean
Zero	Amount (mg N shoot^{-1})	0.65	1.92	0.91	1.16
	Proportion of leaf lamina	0.09	0.23	0.17	0.16
	Proportion of total canopy	0.03	0.09	0.05	0.06
Opt	Amount (mg N shoot^{-1})	3.59	3.04	4.04	3.56
	Proportion of leaf lamina	0.25	0.17	0.21	0.21
	Proportion of total canopy	0.09	0.07	0.10	0.09

7.3.2.2 Degraining

The potential grain N sink capacity per shoot for each N treatment was estimated as 'grains ear^{-1} of control treatment' x 'N content grain^{-1} of degrained treatment' (equating to maximum grain N content) (Table 7-2). The reduction in potential grain N sink capacity in the degrained treatment was therefore determined from the difference with the control treatment. Averaged across the zero and optimum N treatments, degraining reduced the potential grain N sink capacity per shoot to 52% of the control treatment.

The degrained treatment also removed the potential grain N contribution from the chaff (see 7.3.2.1), determined as half of the non-structural chaff N at anthesis. For TT06, TT07 and LC07 this was calculated to be 3.77, 4.95 and 2.64 mg N shoot^{-1}, and 4.91, 7.49 and 2.74 mg N shoot^{-1} for the zero and optimum N treatments, respectively.

Table 7-2 Grain N sink capacity (mg N shoot^{-1}) for two manipulation treatments and control, at two N treatments in TT06, TT07 and LC07, and mean across experiments.

N-trt	Manipulation	TT06	TT07	LC07	Mean
Zero	Defoliation	24.7	50.1	44.4	39.7
	Control	26.5	44.0	43.8	38.1
	Degraining	12.7	25.5	22.7	20.3
Opt	Defoliation	48.9	56.2	60.3	55.1
	Control	50.6	52.2	60.4	54.4
	Degraining	23.9	27.8	32.3	28.0

7.3.2.3 Source-sink N balance

The amount of non-structural N (at anthesis) per grain for each N rate and manipulation treatment is shown in Table 7-3. There was an effect of N treatment in LC07 ($P<0.05$) and TT07 ($P=0.07$), of manipulation treatment in all three site-seasons ($P<0.01$), and an interaction between N and manipulation treatment in TT06 and LC07 ($P<0.05$). The cross site-season ANOVA showed an effect of N treatment, manipulation (M) and experiment ($P<0.05$), and interactions between N x M, and N x experiment combinations ($P<0.05$).

Averaging across experiments there was a trend for lower N source per grain in the defoliated treatment than the control ($P<0.08$) at 0.56 and 0.61 mg N grain^{-1}, respectively. In contrast, the source-sink balance in the degrained treatment was higher than the control ($P<0.001$) at 1.02 and 0.61 mg N grain^{-1}, respectively. The N treatment x defoliation interaction was likely due to defoliation in TT06 having no effect at the N zero-trt but causing decrease in source-sink balance at the N opt-trt. With respect to the control treatment, defoliation decreased the source-sink balance at the zero and optimum N treatments by 8% and 9% respectively, whereas degraining increased the source-sink balance by 55% and 72%, respectively.

Table 7-3 Source-sink N balance (mg non-structural N grain^{-1}) for two manipulation (M) treatments and control, at two N treatments in TT06, TT07 and LC07, and mean across experiments. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

N-trt	Manipulation	TT06	TT07	LC07	Mean
Zero	Defoliation	0.54	0.37	0.28	0.39
	Control	0.52	0.46	0.32	0.43
	Degraining	0.86	0.63	0.52	0.67
Opt	Defoliation	0.73	0.75	0.70	0.73
	Control	0.78	0.84	0.77	0.80
	Degraining	1.45	1.32	1.34	1.37
	SED N x M	0.169	0.147	0.074	0.098
	df N (P)	2 ns	2 ns	2 *	2 *
	df M (P)	8 ***	8 ***	7 ***	31 ***
	df N x M (P)	2.9 *	3.4 ns	2.3 ***	3.1 ***
	df Exp. (P)	-	-	-	31 ***

7.3.3 Individual grain dry weight

Grain weight (GW) at harvest was affected by N treatment in TT06 ($P<0.05$), and by manipulation treatment in TT07 and LC07 ($P<0.001$); there were no interactions in any experiment (Table 7-4). From the cross site-season analysis, there were effects of manipulation treatment and experiment ($P<0.001$), and interactions between N x M, N x experiment, and M x experiment combinations ($P<0.01$). In comparison with the control, overall defoliation caused a small decrease in GW, with the decrease being slightly larger at the N zero-trt (-2%; $P<0.05$) than at N opt-trt (-1%; ns). Degraining increased GW at both N treatments ($P<0.001$), with the response being smaller at the N zero-trt (6%) than at the N opt-trt (12%).

The responses in GW to defoliation were generally consistent across site-seasons in the range 1.5-1.7%. However the responses to degraining were smaller in TT06 (1.1%), than TT07 (14.0%) or LC07 (12.8%), indicating that either the removal of grain had little effect on the photosynthetic capacity or that the control GW was approaching the potential grain weight in TT06.

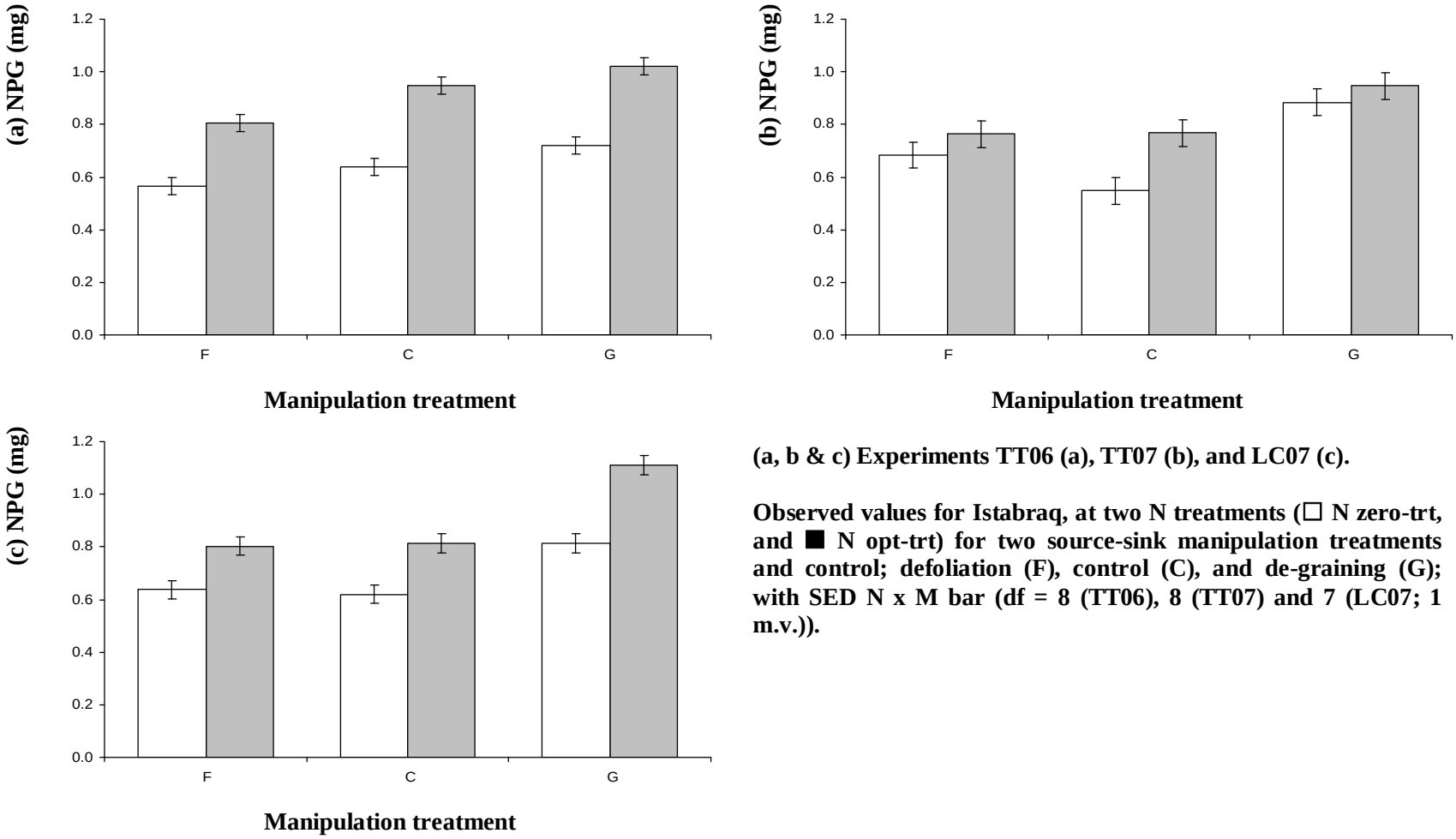
Table 7-4 Individual grain weight (mg) for two manipulation (M) treatments and control, at two N treatments at TT06, TT07 and LC07, and mean across experiments. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

N-trt	Manipulation	TT06	TT07	LC07	Mean
Zero	Defoliation	44.9	40.0	43.8	42.9
	Control	46.0	41.2	44.1	43.7
	Degraining	46.1	45.1	48.4	46.5
Opt	Defoliation	43.4	37.0	42.5	41.0
	Control	43.8	37.1	43.1	41.3
	Degraining	44.7	44.2	50.0	46.3
	SED N x M	0.64	1.35	1.06	0.70
	df N (P)	2 *	2 ns	2 ns	2 ns
	df M (P)	8 ns	8 ***	7 ***	31 ***
	df N x M (P)	9.3 ns	4.0 ns	4.4 ns	4.8 **
	df Exp. (P)	-	-	-	31 ***

7.3.4 Grain N content at harvest

Grain N content (NPG, the amount of N per grain) was affected by N treatment in TT06, and by manipulation in all three experiments (P<0.001); there was an N x M interaction in TT07 (P<0.10) likely due to the low grain N content in the control at the N zero-trt (Figure 7.1). The cross site-season analysis showed an effect of N and M (but not of experiment), and interactions between N x M, N x experiment, and M x experiment combinations (P<0.01).

Overall in comparison with the control, defoliation had a small effect which contrasted across N treatments, with an increase of 3% at the N zero-trt, and a decrease of 6% (P=0.054) at the N opt-trt. Degraining produced a larger response than defoliation, and increased NPG at the zero (34%) and optimum N (22%) treatments (P<0.001). In comparison with the N max-trt in the main experiment, degraining overall increased the NPG by 25% (at 0.82 and 1.03 mg grain^{-1}, respectively). The defoliation treatment had an inconsistent effect across site-seasons, decreasing NPG in TT06 (13%), increasing NPG in TT07 (10%), and increasing NPG at LC07 (1%). The effect of the degraining treatment was more consistent across site-seasons; TT07 had the greatest increase (39%), then LC07 (34%), and TT06 (10%).



(a, b & c) Experiments TT06 (a), TT07 (b), and LC07 (c).

Observed values for Istabraq, at two N treatments (☐ N zero-trt, and ■ N opt-trt) for two source-sink manipulation treatments and control; defoliation (F), control (C), and de-graining (G); with SED N x M bar (df = 8 (TT06), 8 (TT07) and 7 (LC07; 1 m.v.)).

Figure 7.1 (a, b & c) Effects of source-sink manipulation treatment on the grain nitrogen content (NPG) at harvest for Istabraq at two N treatments in TT06, TT07 and LC07.

7.3.4.1 Straw N content at harvest

7.3.4.2 True stem N content

True stem N content at harvest increased with N supply in LC07 ($P<0.001$) with a trend for increasing N content with N supply in TT06 and TT07; and by manipulation treatment in all three experiments ($P<0.05$); there were no interactions in any experiment (Figure 7.2). Cross-site season ANOVA showed an effect of N treatment, manipulation and experiment ($P<0.05$), and an M x experiment interaction ($P<0.05$). The effect of manipulation was consistent across TT06 and LC07. However TT07 showed a contrasting effect compared to the other two site-seasons, as lower shoot density in the manipulation treatments compared to the control increased the true stem N content per shoot. This likely contributed to the increase in true stem N content per shoot observed in the defoliation treatment compared to the control. The present results at TT07 should therefore be interpreted with caution.

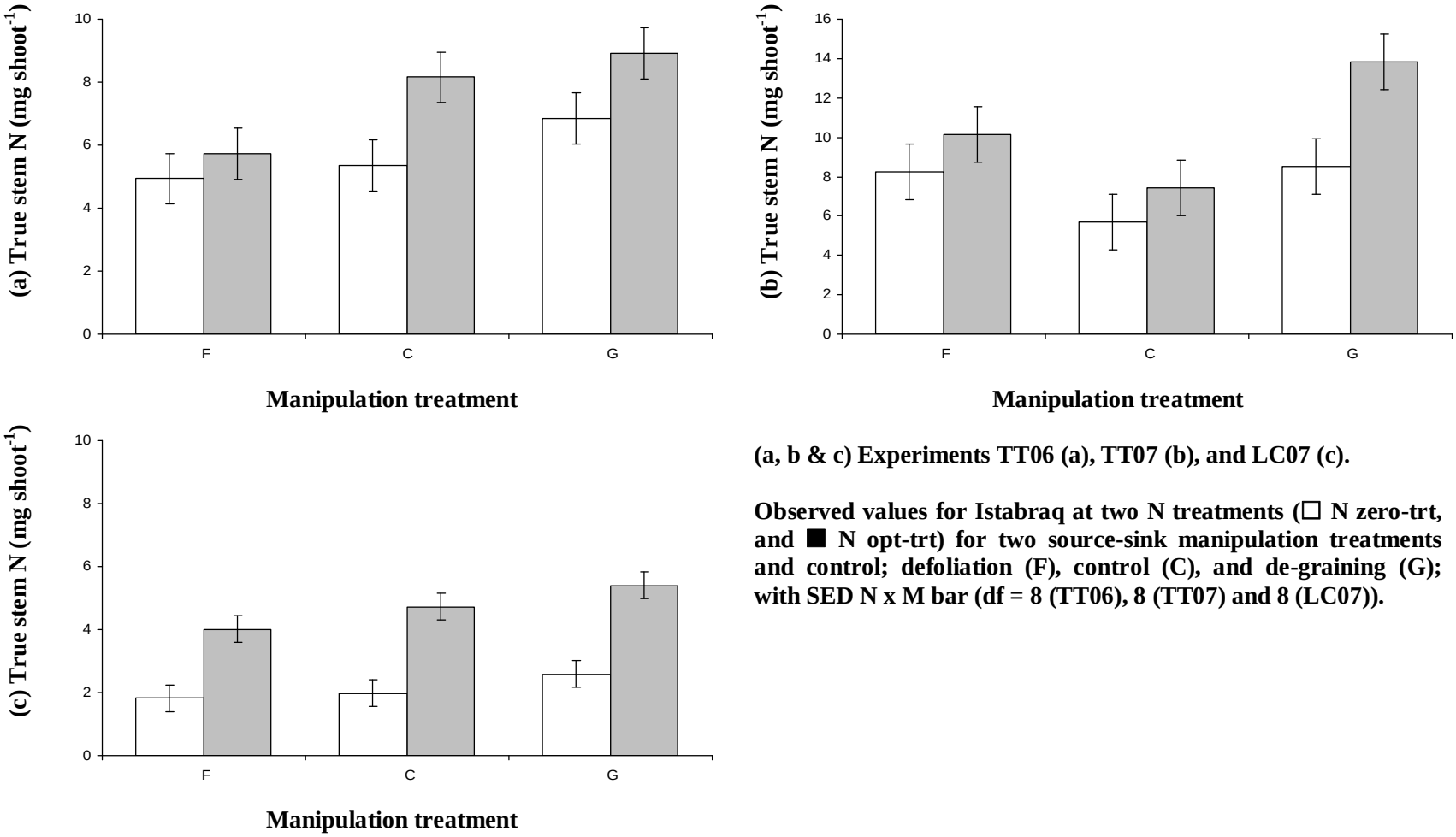
Averaging across TT06 and LC07, the defoliation treatment had a lower true stem N content at harvest compared to the control treatment (18%; $P<0.01$), with the response again greater at the N opt-trt than the N zero-trt (24 and 8%, respectively). Conversely the degrained treatment had higher true stem N content than the control treatment (17%; $P<0.05$), but with the response smaller at the N opt-trt than the N zero-trt (11% and 28%, respectively). Averaging across N treatments, the defoliation treatment decreased true stem N more in TT06 (21%) than LC07 (13%); and degraining increased the true stem N the more in LC07 (19%) than TT06 (17%). Turning to consider effects of manipulation in TT07, true stem N content was increased by both the defoliation (40%) and degraining (70%) treatments.

7.3.5 Leaf lamina, leaf sheath and chaff N content in LC07

In LC07 the N content of the individual straw components at harvest was measured in all manipulation treatments. This allowed further analysis of the relative effects of the manipulation treatments on the N content of the leaf sheath, true stem, leaf lamina and

chaff. In comparison with the control, for all straw components the main effects of N treatment and manipulation treatment were significant ($P<0.05$; except chaff $P<0.06$); the interactions were not significant (Figure 7.3). Generally, in the experiments the response to manipulation treatment was consistent across straw components, with defoliation decreasing the component N content and degraining increasing the N content.

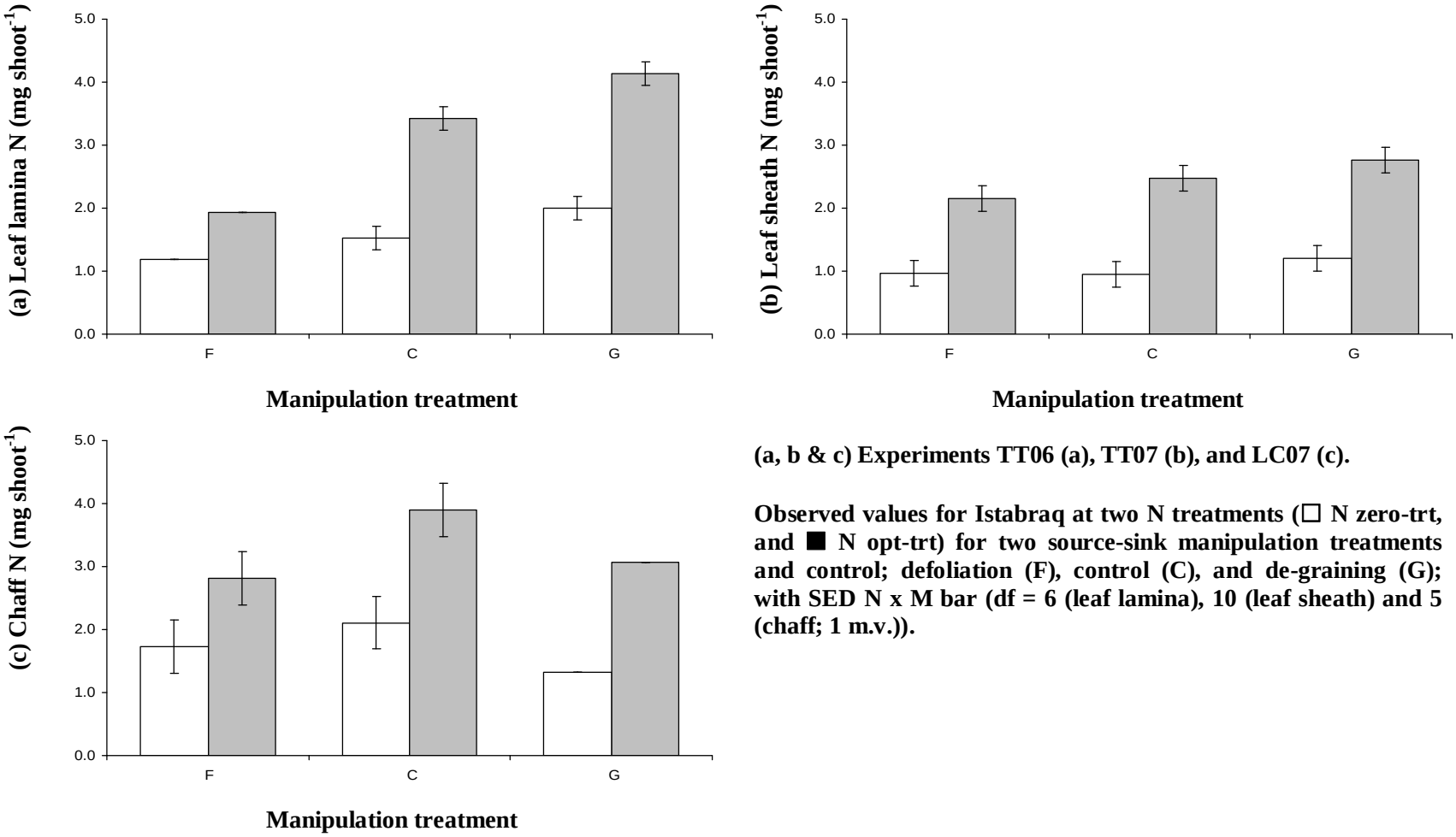
The leaf sheath N content at harvest was unchanged by defoliation at the N zero-trt, but there was a decrease at the N opt-trt (13%; ns); whilst degraining increased the leaf sheath N content at both the zero (25%) and optimum N (12%) treatments ($P<0.05$). Degraining also increased the leaf lamina N content at both the zero (31%) and optimum N (21%) treatments ($P<0.01$). Defoliation similarly decreased the chaff N content at both the zero (18%) and optimum N (28%) treatments ($P<0.06$).



(a, b & c) Experiments TT06 (a), TT07 (b), and LC07 (c).

Observed values for Istabraq at two N treatments (□ N zero-trt, and ■ N opt-trt) for two source-sink manipulation treatments and control; defoliation (F), control (C), and de-graining (G); with SED N x M bar (df = 8 (TT06), 8 (TT07) and 8 (LC07)).

Figure 7.2 (a, b & c) Effects of source-sink manipulation treatments on the true stem N content at harvest for Istabraq at two N treatments in TT06, TT07 and LC07.



(a, b & c) Experiments TT06 (a), TT07 (b), and LC07 (c).

Observed values for Istabraq at two N treatments (☐ N zero-trt, and ■ N opt-trt) for two source-sink manipulation treatments and control; defoliation (F), control (C), and de-graining (G); with SED N x M bar (df = 6 (leaf lamina), 10 (leaf sheath) and 5 (chaff; 1 m.v.)).

Figure 7.3 (a, b & c) Effects of source-sink manipulation treatments on the N content of the leaf lamina, leaf sheath and chaff at harvest for Istabraq at two N treatments in LC07.

7.3.6 Response of crop components and PANU

7.3.6.1 Amount of N remobilisation and PANU for crop components in LC07

The amount of remobilisation (NR) from the crop components (leaf lamina in degrained treatment; and leaf sheath, true stem, and chaff in the defoliated treatment) was calculated as detailed in 6.2.2. In order to compare NR across crop components and to calculate PANU per shoot, the amount of photosynthetic and reserve N removed in the leaf lamina by defoliation (7.3.2.1), and the amount of photosynthetic and reserve N removed in the chaff in the degrained treatment (7.3.2.2) was estimated (indicated by † and ‡ in Table 7-5). This was done assuming that the equivalent of the amount of PN and RN removed in the relevant plant component was all remobilised post-anthesis in the control treatment together with the assumptions of 6.3.10.1. PANU was calculated as the difference between the total NR and the grain N content per shoot (Table 7-5).

The amount of N remobilised to the grain increased with N supply for the leaf lamina, leaf sheath ($P<0.01$) and true stem (ns), and decreased for the chaff (ns), whereas PANU showed a non-significant trend to decrease with N supply. Responses to manipulation treatments were generally consistent across components; defoliation increased NR (but not significantly), whilst degraining decreased NR and PANU ($P<0.001$). NR showed a relatively greater response to degraining than to defoliation. In general the positive effect of defoliation on NR was relatively greater at the N opt-trt, whereas the negative effect of degraining on NR was relatively greater at the N zero-trt.

In comparison with the control, overall defoliation increased NR mainly from the chaff and true stem (by 15% and 9%, respectively; ns), with the leaf lamina and leaf sheath almost unchanged. There was a decrease in PANU with defoliation at the N opt-trt (-18%), but no change at the N zero-trt. Degraining significantly decreased NR from the leaf lamina (6%; $P<0.01$), with a trend for decreased NR from the true stem, leaf sheath and chaff (by 15, 7 and 5%, respectively). There was a considerable

decrease in PANU with degraining by 82% (P<0.001), with the effect greater at N opt-trt than at N zero-trt.

Table 7-5 The amount of N remobilised to the grain (NR) from crop components, post-anthesis N uptake (PANU), and grain N content (mg N shoot-^{1}) for two source-sink manipulation treatments and control, at two N treatments in LC07. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

N-trt	Manipulation	Lamina	Sheath	Tr. stem	Chaff	PANU	Grain
Zero	Defoliation	5.13 †	2.44	3.48	6.07	16.23	33.60
	Control	5.52	2.45	3.32	5.74	16.45	33.48
	Degraining	5.05	2.21	2.72	5.51 ‡	7.28	22.76
Opt	Defoliation	18.54 †	7.42	6.25	5.55	6.00	43.75
	Control	17.97	7.10	5.54	4.47	9.20	44.29
	Degraining	17.26	6.80	4.87	4.20 ‡	-0.79	32.33
	SED N x M	1.427	0.261	0.907	0.389	4.318	2.735
	df N (P)	2 *	2 **	2 ns	2 ns	2 ns	2 ns
	df M (P)	8 ***	8 ns	8 *	7 *	7 ***	7 ***
	df N x M (P)	2.0 ***	5.3 ns	2.7 ns	6.0 ns	2.1 *	2.2 ns

† NR calculated as measured NR in the defoliated shoot plus amount of PN+RN removed by defoliation; at 0.91 and 4.04 mg N shoot^{-1} for the zero and optimum N treatments, respectively.

‡ NR calculated as measured NR in the degrained shoot plus amount of PN+RN removed by degraining; at 2.64 and 2.74 mg N shoot^{-1} for the zero and optimum N treatments, respectively.

7.3.6.2 Amount of N remobilisation and N remobilisation efficiency for true stem at TT06, TT07 and LC07

True stem NR and NRE increased with N supply in all experiments. There was an effect of manipulation treatment for NR and NRE in all three experiments (P<0.05); there were no interactions in any experiment (Table 7-6). Cross site-season ANOVA showed for both NR and NRE a main effect of N treatment and manipulation treatment (P<0.05), and an interaction between N x experiment (P<0.07). For NRE there was an apparent interaction between M x experiment (P<0.05), but this was

probably due to a trend for lower shoot density in the manipulation treatments compared to the control in TT07 as referred to in 7.3.4.2.

In TT06 and LC07 defoliation increased true stem NR (P<0.06) and NRE (P<0.05) compared to the control, by 21% and 17%, respectively; the responses were smaller at the N zero-trt than at the N opt-trt (but not significantly). Degraining decreased both NR (P<0.07) and NRE (P<0.05) compared to the control, by 20% and 21%, respectively; the responses were larger at the N zero-trt than at the N opt-trt (ns). Overall both NR and NRE showed a greater response to the degraining than to defoliation. Turning to consider effects in TT07, observed responses were not reliable due differences in shoot density across manipulation and control treatments, although the pattern of NR and NRE for the manipulation treatments was consistent with TT06 and LC07.

Table 7-6 The amount of N remobilised to the grain (NR; mg shoot^{-1}) and N remobilisation efficiency (NRE; %) for the true stem for two source-sink manipulation treatments and control, at two N treatments in all three experiments, and mean across experiments. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

		NR (mg shoot^{-1})				NRE (%)			
N-trt	**Manipulation**	**TT06**	**TT07**	**LC07**	**Mean**	**TT06**	**TT07**	**LC07**	**Mean**
Zero	Defoliation	3.4	1.6	3.5	2.8	40	15	66	40
	Control	2.9	4.1	3.3	3.5	35	42	63	47
	Degraining	1.4	1.3	2.7	1.8	18	10	51	26
Opt	Defoliation	8.4	7.9	6.3	7.5	59	43	60	54
	Control	6.0	10.6	5.5	7.4	42	58	54	51
	Degraining	5.3	4.2	4.9	4.8	37	23	47	36
	SED N x M	1.45	2.40	0.91	0.73	7.7	15.9	6.9	4.7
	df N (P)	2 ns	2 ns	2 ns	2 **	2 ns	2 ns	2 ns	2 *
	df M (P)	8 **	8 **	8 *	32 **	8 **	8 **	8 **	32 ***
	df N x M (P)	2.9 ns	3.4 ns	2.7 ns	33 ns	5.3 ns	4.1 ns	3.6 ns	34 **
	df Exp. (P)	-	-	-	32 ns	-	-	-	32 ***

7.3.7 Proportion of grain N sink capacity filled at harvest

The amount of grain N per shoot at harvest (mg N shoot^{-1}; Table 7-5) for respective treatments was compared to the potential grain N sink capacity per shoot of the

control (Table 7-2) to give the proportion of the grain N sink capacity filled (Table 7-7). There was an effect of manipulation treatment in all three site-seasons (P<0.001), and an N x M interaction in LC07 (P<0.05). The cross site-season ANOVA showed an effect of manipulation treatment (P<0.001), and a M x experiment interaction (P<0.001); again likely due to lower grain NPG in the control than in the manipulation treatments in TT07 as a result of relatively higher shoot density than the control.

Overall relative to the control, the proportion of grain N sink filled at harvest was similar in the defoliation treatment at the zero and optimum N treatments (P<0.01); both at 79%; with no difference between N treatments or experiments. Compared to the control, the grain N sink filled in the degrained treatment was significantly lower (P<0.001); at 53% and 51% respectively, there was no difference between N treatments and experiments. However this indicates that the potential grain N sink capacity was completely filled in the degrained treatment given that approximately half the N sink capacity had been removed; determined as '1/proportion of grain N sink remaining x proportion of grain N sink filled' (adjustment in normalised values for the degrained treatment presented in brackets in Table 7-7).

Table 7-7 Proportion of potential grain N sink filled (%) for three manipulations at two N treatments in TT06, TT07 and LC07, and mean across experiments. Adjusted values for degrained treatment to account for N sink capacity removed are shown in brackets. Significance of the analysis is shown (Probability (P); ns, not significant, and *, ** and * significant at the 5, 1 and 0.1% probability level, respectively).**

N-trt	Manipulation	TT06	TT07	LC07	Mean
Zero	Defoliation	73	88	77	79
	Control	88	62	77	75
	Degraining	48	58	52	53
		(100.2)	(100.1)	(100.3)	(100.2)
Opt	Defoliation	76	88	72	79
	Control	93	81	73	82
	Degraining	47	53	54	51
		(99.5)	(99.5)	(101.0)	(100.0)
	SED N x M	5.1	10.2	5.4	4.2
	df N (P)	2 ns	2 ns	2 ns	2 ns
	df M (P)	8 ***	8 ***	7 ***	31 ***
	df N x M (P)	10 ns	4.9 ns	2.2 *	7.2 ns
	df Exp. (P)	-	-	-	31 ns

7.4 DISCUSSION

7.4.1 Determinants of grain DM accumulation

Defoliation of leaf 3 and below at around two weeks after anthesis resulted in a small non-significant decrease in grain weight (-1.3%). Loss of green area through defoliation has been reported to reduce assimilate supply during the grain filling period, and also to promote canopy senescence reducing the duration of grain filling (Simmons *et al*., 1982; Van Sanford, 1985). However, the relatively small effects in the present study indicated that probably the control was not limited by assimilate supply, and was either sink limited or close to sink limitation (Borrás *et al*., 2004; Reynolds *et al*., 2005); although the reduction in source was quite small. The response to defoliation was marginally greater at the N zero-trt compared to the N opt-trt (-1.8 and -0.7%, respectively), indicating the N zero-trt was possibly less sink-limited. Thus, the N zero-trt may have had a greater dependence on the lower leaves to supply assimilates through lower photosynthetic capacity of the top two leaves and greater light penetration to the lower leaves thereby increasing their proportion of total canopy assimilate production. Studies suggest that an increase in grain sink strength in the post-anthesis period through temporarily opening rows around ear emergence to increase light interception and grain number may stimulate photosynthesis in this period (Reynolds *et al*., 2005); this upregulation of photosynthesis was perhaps more likely in the N opt-trt which has been shown in chapter 5 to have higher SLN than required to maximise photosynthesis, and therefore a reserve photosynthetic capacity.

The degraining treatment significantly increased grain weight, as assimilate supply essentially doubled in relation to the grain sink size. Present results therefore suggest that the grain had not realised its potential weight in the control treatment, and responded to the increased assimilate availability, consistent with previous studies (e.g. Calderini and Reynolds, 2000). Although previous studies suggest that yield in wheat is typically sink limited (Borrás *et al*., 2004; Reynolds *et al*., 2005), the relatively small increases in grain weight observed may reflect a degree of co-limitation of grain filling (i.e. that grain growth is partially controlled by both source and sink), or that the crop had shifted from an initial state of relative source limitation before degraining to one of sink limitation afterwards (Bingham *et al*., 2007). However, the increase in grain growth was small in relation to the increase in source-

sink ratio especially in TT06, indicating that the capacity of the canopy to provide assimilates to the growing grains was generally adequate for Istabraq in these experiments. This is in agreement with the findings of other investigation in good, well managed growing conditions (Savin and Slafer, 1991; Richards, 1996). Additionally, the reduction in sink size may decrease the photosynthetic rate of the residual leaves through feedback inhibition (Miralles and Slafer, 2007). The response to degraining was greater at the N opt-trt than the N zero-trt, indicating this treatment was more source-limited possibly associated with higher potential grain sink capacity (through higher endosperm cell production during anthesis in response to higher potential assimilate supply; Brocklehurst, 1977).

7.4.2 Determinants of grain N accumulation

Recent experimental and modelling evidence has suggested that the deposition of N in the grain may be largely driven by the N supply available to the grain, and that grain N accumulation is overall source limited in wheat in non luxury N conditions (Ma *et al*., 1995; Martre *et al*., 2003), and at supra-optimal N availability may become co-limited by both supply and demand (Martre *et al*., 2006). On the other hand, the grain would appear to provide a strong sink for N with the ability to store all the potentially translocatable N (Borghi *et al*., 1986), up to an upper threshold of NPG possibly limited by the rate and capacity of protein synthesis in the grain (at around 1.1 mg N grain^{-1}; at the N max-trt in this study; and Jamieson, personal communication). The present experiments test between these two possibilities. If grain N content is driven by the availability of N from the vegetative sources, increasing the relative size of the N source available to the grain through the degraining treatment should result in a large increase in the grain N content per grain (Triboi and Triboi-Blondel, 2002; Martre *et al*., 2003).

Overall defoliation caused a small non-significant decrease in NPG by 2%. The leaf lamina are major contributors of RN for grain filling (this study; Guitman *et al*., 1991), and defoliation caused a reduction in the N source for remobilisation by 9%. Additionally defoliation possibly also reduced N uptake promoted by leaf lamina transpiration (Neales *et al*., 1963; Simpson *et al*., 1982; Cox *et al*., 1985a). However as the effect of defoliation on NPG was small in comparison with the amount of N

removed, this indicates an increased use of RN from the residual green area. The effect of defoliation was larger at the N opt-trt than at the N zero-trt, presumably due to the removal of a larger amount of leaf lamina RN (3.6 and 1.2 mg N shoot^{-1}, respectively) in relation to the grain N sink size.

Degraining effectively doubled the N supply per grain, and overall increased NPG by 27% ($P<0.001$). This indicates that the N storage capacity of the grain was not completely filled in the control treatment, and that grain N accumulation during the grain filling period was significantly source limited rather than being regulated by the activity of the grain (i.e. due to the synthesis and accumulation of storage proteins, such as gluten (Shewry *et al*., 2002) and gliadin proteins. This result is in agreement with previous studies, even when soil N was non-limiting (this study; Martre *et al*., 2003). Increasing the N source strength in relation to the grain sink has been observed to increase grain N concentration in previous studies. For example, in an ear-halving experiment Borghi *et al*. (1986) found that the grain protein concentration increased by 43% (from 11.8% to 16.9%). In the present study, the boost in NPG with degraining was greater at the N zero-trt than at the N opt-trt indicating that NPG in the N opt-trt was closer to the upper limit of grain N concentration whereby NGP eventually becomes sink limited. Reducing grain N demand through degraining may also decrease N relocation from the canopy and delay senescence (Martre *et al*., 2003). In the present study, the degrained treatment was observed to senesce last in all three experiments. This indicates that the patterns of N dynamics and senescence are linked during grain filling in wheat. Therefore slower remobilisation, possibly through manipulation of plant hormone signalling mechanisms and/or enzymes controlling catabolism of Rubisco, may be an avenue to increase UTE.

7.4.3 N supply to grain from straw components

Compared to the control, defoliation decreased the N content of all straw components as a consequence of increased N remobilisation. Plants compensated for the loss of the leaf lamina N through an increase in NR from the remaining vegetative organs, but not through PANU which decreased at the N opt-trt and remained unchanged at the N zero-trt. For LC07 the increase in NR was greater for the chaff and true stem (at 15% and 9%, respectively) than for the leaf lamina and leaf sheath which showed no

change. For all three experiments there was a consistent increase in true stem NR, and true stem NRE was significantly increased. This indicates that it is possible to mobilise the *accumulation* N in the true stem (quantified in chapter 6) as a source of grain N. Possibly this could be achieved genetically through an increase in activity of key enzymes controlling nitrate assimilation such as glutamine synthetase (Galais and Hirel, 2003). The response to defoliation for NR was lower at the zero than the optimum N treatment associated with the removal of a smaller proportion of leaf lamina N in the N zero-trt.

The degraining treatment increased the N content of all straw components at harvest through decreased NR. For LC07 the decrease in NR ranged from 15% for the true stem to 6 and 7% for the leaf lamina and leaf sheath, respectively, to 4% for the chaff. The decrease in true stem NR observed in LC07 was also seen in TT06, and indicated that this organ is an important source of N for grain filling. Overall the response to degraining was greater at the zero than the optimum N treatment for all components in LC07, indicating relatively larger total canopy N in relation to grain N demand. The largest response to degraining however was shown by PANU, overall decreasing by 82%. This large reduction in PANU suggested a prioritisation of grain N supply, with the N in residual vegetative components used in preference to root N uptake.

7.4.4 Grain N loading as a function of N sink capacity and source-sink N balance

The amount of grain N per shoot at harvest for each N treatment was compared to the potential grain N sink capacity in the control treatment in all three experiments. In TT06 defoliation decreased the percentage of grain N sink filled by 17%, possibly as a result of slightly fewer GPE (-5%; ns) but mainly as a result of lower NPG (-13%; $P<0.01$) through the removal of the leaf lamina grain N contribution. However, the relationship between the removal of potential grain N contribution and the decrease in grain N sink filling was inconsistent across experiments, and therefore may be combined with additional effects of reduction in N uptake promoted by leaf lamina transpiration (Cox *et al*., 1985a). This may have been especially so in TT06 when the second half of grain filling (July 2006) was notably hot and dry and leaf lamina transpiration rates would be depressed.

Degraining approximately halved the grain N sink capacity at manipulation, however at harvest the degrained treatment showed slightly higher than 50% filling of grain N sink capacity (53 and 51% at the zero and optimum N treatments, respectively); cf. control at (75 and 82%, respectively). Given that approximately half the N sink capacity had been removed in the degrained treatment, when adjusted for the reduced grain number, the proportion of the potential grain N sink capacity filled was approximately 100%; indicating that the potential grain N sink capacity was completely filled. Significantly increasing the source strength in relation to the sink capacity therefore resulted in significant additional N accumulation in the grain, likely increasing towards an upper threshold of NPG of around 1.1 to 1.2 mg N grain^{-1}, similar to that found by Gooding *et al*. (2003) and Triboi and Triboi-Blondel (2002). These findings indicate that the grain N loading is mainly source limited as increasing the N source capacity in relation to the grain N sink strength through degraining significantly increased proportion of grain N sink capacity filled; although some co-limitation with the grain sink capacity is evident, particularly in the N zero-trt.

7.4.5 Conclusions

The present study examined the post-anthesis N source-sink relationships and drivers for grain filling (i.e. whether grain N is limited by the source or the sink), and the extent to which the RN in the true stem is mobilised in response to increased grain N sink size. Overall the effect of degraining on grain and true stem responses was greater than that of defoliation, as degraining almost doubled N source supply per grain whereas defoliation only reduced the source supply by around 10% given that the quantity of leaf lamina removed was relatively small compared to the canopy grain N source. In an entirely source-driven system, increasing the grain N sink size in relation to the available N by defoliation would have had no effect on the amount of N remobilised from the remaining vegetative organs. However, present results showed that defoliation resulted in only a small decrease in grain N loading as a result of increased NR from the true stem and chaff (likely from RN identified in chapters 5 and 6) and this almost entirely compensated for the reduced leaf lamina N availability. The N concentration of these crop components decreased to below the minima

identified in chapter 6 that were equated with the SN requirement, indicating that the RN in the vegetative components was possibly higher than previously estimated.

Consequently there was little effect of defoliation on NGP, although a small decrease in the proportion of the potential grain N sink filled was observed in TT06. Increased NR with defoliation was greatest for the true stem and chaff, with the leaf lamina and leaf sheath showing little change. The true stem increased N contributed to the grain averaged across TT06 and LC07, by 20% compared to the control, and therefore provided an important buffer of remobilisable N to the grain. This was in excess of that identified in chapter 6. This additional *accumulation* N may have been in a more readily redistributed non-photosynthetically active form (i.e. not Rubisco), possibly in specific soluble storage proteins (Shewry *et al*., 1995). Potentially genetic variation in this true stem RN could therefore be used to increase the source supply of N to the grain as a means of maintaining grain protein composition at high yields in bread-making cultivars, or as a breeding trait for delaying leaf lamina senescence in feed wheat cultivars. Degraining significantly increased the NPG indicating that grain N accumulation in the control treatment was primarily source limited, and additional N supply increased the total quantity of protein per grain at harvest. However, overall the amount of N remobilised was lower than that of the control suggesting some co-limitation with grain sink capacity as NGP approaches an upper threshold (i.e. grain N accumulation limited by enzyme-limited grain protein synthesis capacity).

8 GENERAL DISCUSSION

In this chapter the major findings of the study are discussed in relation to the original hypotheses and relevant findings reported in the literature. The chapter considers present results on the crop requirement for N and quantification of crop N status with respect to the theoretical frameworks proposed by Sylvester-Bradley *et al*. (1990a), Justes *et al*. (1994) and Lemaire *et al*. (1989). Crop N loading up to anthesis and N unloading during the grain-filling phase are related to the major physiological processes influencing growth and yield formation, as well as to N partitioning, accumulation and remobilisation between the crop components. Potential agronomic and physiological traits for increased UTE presently identified are set out in relation to a feed-wheat ideotype with a reduced requirement for N fertiliser, and the potential for breeding new wheat varieties with increased UTE is discussed. This is followed by overall conclusions, a discussion of some methodological issues encountered during the study, and recommendations for future work.

8.1 INTRODUCTION

In intensive agricultural systems nitrogen is the most essential nutrient determining the yield of crops. Large amounts of fertiliser N are applied to maximise yields and it is a major input representing a significant expense. However, N fertilisers increase the risk of foliar diseases and lodging, and also indirectly cause pollution (Davies and Sylvester-Bradley, 1995). There are therefore increasing economic and environmental pressures to identify wheat cultivars which require less fertiliser N, whilst maintaining yields. Breeding wheat varieties tolerant of moderately low N supply will reduce production costs and minimize negative environmental impacts associated with use of N fertiliser applications, and with high protein grain in end-uses.

Consistent with previous studies, positive yield responses to N supply were observed associated with effects on both numerical (e.g. grains m^{-2}) and physiological (e.g. above-ground biomass) yield components. Modern high-yielding varieties have typically been selected under non-limiting fertiliser conditions to respond to high N inputs (Bänziger *et al*., 1997; Foulkes *et al*., 1998; Ortiz-Monasterio *et al*., 2001; Presterl *et al*., 2002). This has favoured genotypes that are efficient in N uptake when

N is abundant, but has provided little or no selection pressure to improve the efficiency of utilisation of acquired N. Selection under high N supply can mask efficiency differences among genotypes in ability to recover and utilise N to produce grain (Kamprath *et al*., 1982), and the highest ranking genotypes selected under high N may not perform relatively as well under limited N supply (Moll *et al*., 1982).

Recent genetic gains in NUE worldwide in wheat have been positively correlated with both N uptake and utilisation efficiencies (Hirel *et al*., 2007). Whilst high UPE is a desirable trait, particularly in reducing N leaching losses, present results show that UTE becomes increasingly important in determining NUE with increasing N supply; and that canopy N content becomes increasingly independent of root N uptake as N supply increases. Present results showed that, between the zero and optimum N treatments, the reduction in NUE was due to both lower UPE and UTE, whilst at the supra-optimal N treatments further reductions in NUE were mainly associated with lower UPE, and UTE was therefore more important in determining NUE. UTE was observed to be more important than UPE in explaining genetic variation in NUE with increased N supply in spring wheat in Mexico (Ortiz-Monasterio *et al*., 1997); and the limited evidence from the present study (due to the small number of cultivars tested) would generally support this observation. Genotypic variation for UPE and UTE has been demonstrated for winter wheat (Cox *et al*., 1985a; Van Sanford and MacKown, 1987; May *et al*., 1991, Le Gouis *et al*., 2000; and this study). However recent improvements in UTE have been mainly driven by increases in HI, and modern winter wheat cultivars in the UK are reported to be approaching the theoretical upper limit of ca. 0.62 (Foulkes *et al*., 2007) as estimated by Austin (1980). Therefore one of the most important aims for future breeding for lower N requirement is to increase biomass production under moderately low N supply while maintaining present values of HI (Calderini *et al*., 1999; Foulkes *et al*., 2007) by selection for increased biomass production per unit of crop N (BPE).

The overall aim of the present study was to examine whether the fertiliser N requirements of wheat crops are positively and quantitatively related to their capacity for pre-anthesis N accumulation and/or post-anthesis N remobilisation, and to improve understanding of the mechanisms underlying the relationship between these processes and N fertiliser requirement. Experiments in contrasting environments

investigated the physiological basis of variation in yield with N supply and variety, and their interaction, by examining effects of experimental treatments on the accumulation, partitioning and remobilisation of N in the canopy, particularly between the photosynthetic and non-photosynthetic plant organs. Overall a better understanding of the mechanisms underlying more efficient N recovery and conversion into grain dry matter of a crop will assist in identifying and prioritising traits that breeders can manipulate to raise NUE and lower fertiliser requirements in wheat crops, potentially providing tools for high-throughput screening in breeding programmes.

8.2 PHYSIOLOGICAL REQUIREMENT FOR N

Present results showed that the crop N concentration declined as the crop developed through the season, as was reported by Greenwood *et al*. (1980) and Grindlay (1997). Results overall fitted the critical N dilution curve model of Justes *et al*. (1994) for winter wheat grown in northern France. The crop N% required to produce the maximum aerial biomass at anthesis was calculated (N Nutrition Index =1; Lemaire *et al*., 1989) providing a basis for the quantification of N accumulation and partitioning in relation to crop N status and allowing discrimination between sub-optimal and supra-optimal N supply. In all three site-seasons, the amount of applied N required to produce a crop at anthesis with sufficient N to maximise biomass growth (i.e. NNI=1) was less than the N opt-trt and the fitted 'economic optimum N amount'. Therefore an excess of fertiliser N was applied at the N opt-trt (for Istabraq in the range 37-111 kg N ha^{-1}) for anthesis growth *per se* leading to 'luxury' N uptake by all crops across experiments and varieties.

For crops with NNI>1, the canopy at anthesis contains more N than is required to maximise growth, and N accumulates in the canopy comprising 'excess' N. The phenomenon of excess N accumulation within the canopy of crop plants has received relatively little attention. All, or a part, of the excess N can subsequently be remobilised and reused for growth or maintenance, providing flexibility in dealing with fluctuating supplies in periods of high demand during grain growth (Thornton and Millard, 1996). In the post-anthesis phase, N remobilised from the 'reserve' N pool which is remobilised to the grain was termed '*storage* N'. However, that which

remains in the straw at harvest is effectively non-functional has been termed '*accumulation* N', and this can create inefficiencies in the use of N fertiliser associated with additional nutrient demands during growth.

In the present study, crops supplied with abundant N demonstrated a capacity to take up more N than required for current growth and to accumulate excess N to luxury levels; overall at anthesis for Istabraq by 41 and 52 kg N ha^{-1} at the optimum and maximum N treatments, respectively. At the supra-optimal N treatments, NNI was >1 in all three site-seasons, and excess N uptake therefore resulted in N accumulation rather than additional growth. At the N opt-trt, most of this excess N for biomass growth at anthesis accumulated in the leaf lamina (43%), with the remainder predominately in true stem (27%) and leaf sheath (23%) rather than the ear (7%). In the post-anthesis phase, results showed that the majority of canopy N in the RN pool was remobilised (overall in the range 34-92 kg N ha^{-1}) contributing a considerable proportion of grain N. *Accumulation* N was identified in all three site-seasons, overall in the range 9-17 kg N ha^{-1}; and mostly located in the true stem (72-100% of total *accumulation* N). The functional importance of *storage* N indicates that it can occur even in N-deficient plants as a response to future increases in demand, whereas *accumulation* N cannot (Staswick, 1994): at the N zero-trt, *storage* N and *accumulation* N were in the ranges 16-35 kg N ha^{-1} and 0-3 kg N ha^{-1}, respectively.

8.2.1 Requirement for N in pre-anthesis phase

In each site-season the crop responded to N supply through enhanced crop N uptake, canopy green area, light interception and biomass production. Above-ground DM growth at the respective N treatments was broadly linearly related to radiation interception as reported by Monteith (1994). However, a negative departure from the linear relationship was observed at the N zero-trt, with reduced RUE associated with low leaf lamina N content. Above-ground N at anthesis was overall a function of N availability, and canopy N content increased with N supply to around the N opt-trt, thereafter increasing only slightly at the supra-optimal N treatments. The genotypic variation in AGN at anthesis was small in the present study and only apparent in one out of two seasons in the Terrington experiments. This was possibly since the cultivars were all modern, semi-dwarf, feed/biscuit wheats with similar release dates

and some common parentage (e.g. common parent of Riband for Atlanta, Istabraq and Savannah). In the GREEN Grain data set genetic variation in AGN at anthesis was observed in GGTT07 ($P<0.01$; 42 varieties, Elite UK and some 'global' varieties) but not in GGTT06 (40 varieties, Elite UK and some 'older' UK varieties), indicating that greater genetic variation is available within wider wheat germplasm than that represented by current UK varieties.

During the stem-elongation phase, there was little change in the proportion of AGN in each crop component with N supply, and varietal differences between Atlanta and the other three varieties were due to timing to anthesis in relation to sampling date (rather than intrinsic physiological effects). However, crops subjected to N deficiency had an increased proportion of N in the ear and a reduced proportion in other crop components, especially in the upper leaf lamina. Similar findings were reported by Vouillot and Devienne-Barret (1999). Thus, with increasing N supply up to the N opt-trt, relatively more N was partitioned to the leaf laminae, and the SLN increased up to 3.0 g m^{-2} (especially in the upper leaves) compared to the N content required to maximise photosynthesis which present results showed to be around 2.0 g m^{-2}. Estimation of the breakpoint of the relationship between SLN and RUE gave relatively consistent results across site-seasons in the range 1.97-2.13 g m^{-2} for SLN and 2.87-3.10 g MJ^{-1} for RUE (see 5.3.11.1); values similar to those reported by Field and Mooney (1986) and Sinclair and Horie (1989). Present findings showed that this 'optimum' SLN was obtained at relatively low N supply; in the range 58 kg N ha^{-1} (LC07) to 98 kg N ha^{-1} (TT06), whilst SLN in TT07 was overall lower than in the other site-seasons. At higher N availabilities considerable quantities of N accumulated in the leaf lamina as observed by Critchley (2001), with no observed increase in RUE. Similar findings for a lack of response of RUE to increasing N supply at high N levels were reported by Evans (1983) and Lawlor *et al*. (1987).

In all site-seasons there was a non-uniform vertical distribution of leaf lamina SLN, which can be quantified by the ratio of the extinction coefficient for N (K_N) to the extinction coefficient for light (K_L). In previous work in wheat K_N/K_L was close to 1 (Dreccer *et al*. 2000) and therefore the vertical distribution of N for canopy N photosynthesis per unit N was close to optimum (Anten and Werger, 1996; Pons and Anten, 2004). From the present data it was not possible to calculate K_N/K_L (due to

insufficient light data at leaf levels within the canopy), but the highest leaf lamina N concentrations at anthesis were observed in the upper leaves receiving the highest PAR, with both SLN and LI decreasing with depth in the canopy. The vertical distribution of N was therefore apparently broadly optimised. However, the N content of the upper leaves still most likely exceeded the requirement to maximise photosynthesis (Field, 1983). Highest SLN values were observed in LC07 which may have been a response to the higher light environment (i.e. higher N content required for light-saturated photosynthesis) and/or high N supply (i.e. increasing luxury uptake). It has been suggested that the majority of this accumulated N is in the form of Rubisco which appears to fill a dual role as both a photosynthetic enzyme and an N storage protein (Lawlor *et al*., 1989; Lemaire and Millard, 1999; Parry *et al*., 2003). Several studies (Pons *et al*., 1989; Grindlay *et al*., 1997) have shown that plants continue to re-adjust the canopy leaf N distribution in relation to the light environment during the season to maximise the carbon gain during growth, involving the movement of the non-structural N (i.e. PN and/or RN).

At the N opt-trt, a significant fraction of crop N was loaded in the leaf sheath and true stem at anthesis, representing ca. 18 and 27% of the total N, respectively, and this fraction was relatively unaffected by N supply. The function of the leaf sheath appears to be similar to the leaf lamina, with the majority of the functional N involved in photosynthesis and with a relatively small proportion of SN. Present results showed the true stem overall contained about 25% of the canopy N, representing a large proportion of canopy SN (0.41) and a considerable proportion of RN (0.41), but only a small proportion of PN (0.03). There has been very little research on the form and function of this true stem RN. In the stem-elongation phase, it may represent a transient canopy store, facilitating leaf expansion and elongation, and maintenance of RUE. Alternatively, it may have a role in the post-anthesis phase as an ‘essential canopy store’ for grain filling, maintaining green canopy area and/or photosynthetic efficiency by delaying senescence during N relocation to the grain, as observed in sorghum by Borrell and Hammer (2000).

The intrinsic capacity of the plant to take up and accumulate N during the stem-elongation phase is likely a function of the N-sink size (Jamieson and Semenov, 2000) and there often appears to be a finite ceiling for N uptake (Sylvester-Bradley and

Kindred, 2009) of around 4-5 kg N ha^{-1} d^{-1} (Jamieson and Semenov, 2000; and this study); there was a small varietal effect on rate of N uptake during the stem-extension phase observed in TT06 ($P<0.05$). The actual rate of N uptake may therefore be sink-limited (Jamieson, personal communication) related to the capacity of the canopy to accumulate RN (Martre *et al*., 2006). There was a significant difference in the amount of RN between the site-seasons, associated with environmental factors affecting crop N uptake. However, there was only small varietal variation in RN observed in the Terrington experiments which was associated with the varietal variation in the rate of N uptake. Present results showed most N accumulation in the upper leaf lamina, especially in LC07 with SLN >3.0 g N m^{-2}, and in the true stem. High RN capacity is associated with rapid N uptake and accumulation during periods of high N availability, thereby reducing N losses to the environment through immobilisation and then leaching, and acting as a transient reservoir of N during dynamic canopy growth. The improved recovery of fertiliser N with UK breeding observed from 1978 to 1994 (Foulkes *et al*., 1998) may be due to selection for genotypes with an increased RN potential in the stems and leaf sheaths. Overall, biomass production was closely related to N uptake up to the N opt-trt in the present study, generally supporting the contention of Sinclair and Jamieson (2006) that N accumulation at anthesis is a critical determinant of grain number per unit area and yield in wheat.

8.2.2 Requirement for N in the post-anthesis phase

Grain DM and N accumulation in the post-anthesis phase depend on the N uptake prior to anthesis and the ability to remobilise this N from the vegetative organs to the grain, and on continued N uptake by the roots (Dalling *et al*., 1976; Austin *et al*., 1977; Campbell *et al*., 1977). Canopy N content at anthesis is positively correlated with grain N content in wheat (Van Sanford and MacKown, 1987; Dhugga and Waines, 1989; Cooper and Blakeney, 1990) and is the predominant source of N for grain filling in wheat (Barbottin *et al*., 2005) and barley (Przulj and Momcilovic, 2001). The amount of N accumulated in the canopy components during the stem-elongation phase, to a large extent, determines the amount of unloading during grain filling (Jamieson and Semenov, 2000, and this study). However, PANU also contributes a significant amount of the grain N (Austin *et al*., 1977; Cox *et al*., 1985a); in the present study averaging across N treatments PANU was in the range

44-60 kg N ha^{-1}. The investigation of Papakosta and Gagianas (1991) showed that PANU was favoured by high soil N supply which subsequently reduced the amount of N remobilised.

Present results showed that large quantities of N were remobilised post-anthesis from the vegetative components (overall in the range 90-153 kg ha^{-1}). The plant organ that contributed most of the canopy N remobilisation was the leaf lamina (range 40-51%) and this organ also contributed the most to the grain N content (range 29-35%), as also reported in rice by Mae (1997). Genetic and environmental variation in the amount of N remobilised from the leaf lamina was positively associated with the specific leaf N at anthesis, which, in turn, was positively associated with the size of the PN and RN pools. However, the leaf sheath and true stem also provided a considerable part of the grain N source (range 10-14% and 9-17%, respectively). The amount of N remobilised from the leaf sheath responded similarly to the leaf lamina to N supply, but with slightly lower N remobilisation efficiency likely due to a higher SN content. The true stem had the lowest N remobilisation efficiency, with around 50% of the N remaining at harvest which increased with N supply, partly associated with more non-remobilisable SN. However, responses in defoliation experiments demonstrated that significant additional amounts of this true stem N could be remobilised when sink size was increased relative to the N source size. N remobilisation efficiency from all components was highest in situations of low N supply, consistent with results on winter wheat of Papakosta and Garianas (1991) in Greece, and Cox *et al*. (1986) in California. N remobilisation efficiency was relatively stable across genotypes in the present study. Small genetic variation in N remobilisation efficiency was also observed by Barbottin *et al*. (2005) using a larger range of winter wheat cultivars.

Nitrogen remobilisation was assumed to be from the RN and PN pools, as remobilisation of N in structural components of the canopy is limited (Pons and Percy, 1994) and SN was not presently considered to be remobilised to the grain. At harvest the SN pool contained the majority of the straw N, and although the actual amount of SN may have been slightly over-estimated (given that N% in the true stem was slightly lower in the defoliated treatment than the control in the source-sink manipulations treatments), the model assumptions overall were generally robust.

Based on the patterns of remobilisation observed in this study, the initial N source for grain filling appeared to be from the RN pool, since prolonged green area was observed during grain-filling despite significant N remobilisation from the tissues. Unloading appeared to occur earlier under low N supply, and canopy senescence was more rapid than at the optimum or maximum N treatments. True stem N remobilisation was highest in the first half of the post-anthesis phase, indicating that a considerable quantity of true stem RN may function as a buffer for unloading to the grain N, thereby possibly reducing unloading from PN in the leaf lamina and leaf sheath and delaying senescence. Senescence was thus observed to occur mainly in the second half of the grain-filling phase linked to mobilisation of PN. Findings from the source-sink manipulation treatments showed more rapid senescence and enhanced N remobilisation in the defoliation treatment and delayed senescence and reduced N remobilisation in the degraining treatment compared to the control. Previous studies have also linked the pattern of canopy senescence with N availability and remobilisation in wheat (Sarandon and Caldiz, 1990), and 'stay-green phenotypes' in durum wheat have been associated with prolonged photosynthetic capacity and increased yield (Spano *et al.*, 2003).

Although at all N treatments the rate of N mobilisation from leaf lamina was slightly higher in the first half of the post-anthesis phase, NR appeared to be drawn mostly from the RN pool in the first half of the phase and from PN pool in the second half of the phase. This is supported by observations presented in Figure 6-16 based on GAI data at mid grain-filling. Consistent with findings reported in previous investigations (Peoples and Dalling, 1988). In all three experiments, the timing of the leaf lamina and leaf sheath senescence was observed to be related to the level of N stress. At the optimum and supra-optimum N treatments, senescence occurred predominantly after mid-grain filling, whereas at low N supply senescence started before mid-grain filling and the RN declined earlier. At this point the photosynthetic tissues started to behave as source organs providing N for the formation of the grain (Sinclair and De Wit, 1975). Thereafter, the breakdown of the photosynthetic enzymes results in a decrease in leaf area index and SLN, thereby reducing photosynthetic activity and RUE (Gregory *et al.*, 1981) and restricting grain assimilate supply. Senescence has an important role in the N economy of cereals and the identification of mechanisms underlying 'stay-green' properties may offer scope to improve N economy in the

longer term. In this study differences in enhanced 'stay-green' across N treatment levels were associated with greater leaf N concentration at anthesis and greater PANU, and stay-green effects were observed in the optimal and supra-optimal N treatments compared to the N zero-trt. Similar results were also observed in sorghum (Borrell and Hammer, 2000). Data testing for varietal effects of stay-green were not available. However, genetic variation in the stay-green trait and associated QTLs have been identified elsewhere in wheat (Verma *et al*., 2004).

In the present study separation of the RN pool into *storage* N (which is not remobilised) and *accumulation* N (which is remobilised) was based on the assumptions relating to the crop N pool model discussed in chapter 6. Overall the amount of both *storage* N and *accumulation* N increased with N supply. Analysis of crop components showed that all RN in the leaf lamina and leaf sheath was remobilised (i.e. all RN was *storage* N), whereas the true stem still contained considerable quantities of RN at harvest (i.e. *accumulation* N). The true stem would therefore appear to provide a realistic physiological target for reducing canopy *accumulation* N. Responses to degraining experiments demonstrated that a significant quantity of this true stem *accumulation* N could be remobilised, in the region of 20% at both the zero and optimum N treatments (i.e. in the ranges 1.4-2.7 kg N ha^{-1} and 4.9-8.2 kg N ha^{-1}, respectively). An increase in true stem N remobilisation efficiency is therefore feasible, and could provide a mechanism of further buffering N relocation of PN and increasing grain yield via delayed canopy senescence for feed wheats (or increasing grain N% for bread wheats). This would offer an avenue for reducing canopy *accumulation* N and potentially reducing the crop fertiliser N requirement without reducing yield.

8.3 RELATIONSHIP BETWEEN CROP N REQUIREMENT AND N-UTILISATION EFFICIENCY

N-utilisation efficiency reflects the ability of the crop to convert N taken up from the soil into dry matter (BPE), and the partitioning of the dry matter to yield (HI). Breeding has improved UTE under non-limiting N supply (Calderini *et al*., 1995), typically through genetic gains in HI (e.g. Fischer and Wall, 1976). Several studies on

wheat have shown that the proportion of genetic variation in NUE accounted for by UTE increases with N supply (Van Sanford and Mackown, 1987; Ortiz-Monasterio *et al*., 1997; Le Gouis *et al*., 2000), and present results were generally in agreement with those findings. Results showed a decline in UTE with increasing N supply was the consequence of a decline in BPE, whilst HI was overall not significantly affected. Significant genetic effects on green canopy area per unit N uptake (CNR) at anthesis were observed in both Terrington experiments, although the varietal pattern was not consistent across seasons. Varietal differences in BPE at harvest were observed in TT07, associated with lower N uptake by Istabraq, and there was a trend for a varietal pattern in BPE across seasons (with Savannah and Istabraq higher than Atlanta and Claire). Genetic ranges in BPE at the N opt-trt in the present study (65-68 (TT06) and 59-64 (TT07)) were smaller than those reported in the GREEN Grain data set (76-95 (GGTT06) and 72-92 (GGTT07)) for a wider set of cultivars.

Biomass production efficiency depends upon the efficiency of the utilization of acquired nitrogen in constructing the photosynthetic machinery of the canopy and carrying out photosynthesis. Large amounts of N are required for leaf lamina and leaf sheath growth, with around 75% of total reduced N connected with photosynthesis (Field and Mooney, 1986). Data from the present study showed a significant effect of N supply on the amount N per unit green canopy area, increasing with N supply to the N opt-trt and thereafter increasing only slightly. This is consistent with the findings of Grindlay *et al*. (1993), and partly explains the effects of N observed on BPE and RUE. The amount of canopy N required to maximise growth at anthesis at NNI=1 was 140 kg ha^{-1} in the Terrington experiments and 189 kg ha^{-1} in the Lincoln experiment; the difference was possibly due to the higher light environment of Lincoln leading to a higher leaf lamina N requirement for light-saturated photosynthesis. The application of the NNI to present results also demonstrated that the optimum amount of N required for growth at anthesis was lower than that required to optimise yield.

8.4 DEVELOPING A WHEAT IDEOTYPE WITH PHYSIOLOGICAL TRAITS TO INCREASE N UTILISATION EFFICIENCY

The effects of N treatment and varieties on BPE observed in the present study can be associated with specific physiological canopy traits, indicating opportunities to develop a feed wheat ideotype with a higher UTE and a lower fertiliser N requirement, whilst maintaining yield most likely of lower grain N concentration.

Biomass production efficiency was observed to decrease with N supply consistently across varieties and site-seasons. Genetic differences in BPE were not observed at anthesis, and in only one of the two Terrington experiments at harvest, with the varietal trends different across site-seasons. However, the non-significant trends for differences in BPE observed amongst varieties at anthesis were generally associated with genetic differences in canopy traits. In summary, there was a positive association between AGN and AGDM amongst the four varieties observed in TT06, but not in TT07 although similar trends were apparent. The AGN at anthesis amongst the varieties was positively associated with the rate of N uptake during the stem-elongation phase (i.e. in TT06 at the N opt-trt Atlanta had the highest AGN and rate of N uptake, then Istabraq, Savannah and Claire) leading to higher N content of all crop components, particularly the leaf lamina and true stem. Varieties with high leaf lamina N content had lower GAI and higher SLN (except Atlanta), and as a consequence, low PN and high RN in this organ. High true stem N content was also associated with high true stem RN content, and there was a trend for a positive association between stem height and SN content across varieties.

Varietal differences observed in canopy traits during the post-anthesis phase were again small, but could be linked to differences in BPE and UTE at harvest. In TT06 UTE was positively associated with BPE amongst the four varieties (i.e. at the N opt-trt Atlanta has the highest BPE, then Savannah, Istabraq and Claire). This was mainly a consequence of an inverse relationship between yield and grain N concentration amongst varieties (while grain N content was similar). Overall, varieties with the highest RN at anthesis remobilised the most N to the grain, with the varietal pattern of true stem NRE similar to that of true stem *storage* N (i.e. in TT06 Atlanta and

Savannah had the highest true stem NRE and storage N, and Claire and Istabraq had the lowest). However, there was no clear association amongst varieties between leaf lamina NRE and/or PANU and canopy senescence variables (e.g. date of complete canopy senescence) possibly due to a lack of data for varieties at GS75.

8.4.1 Stem-elongation phase

The expansion of the green canopy area is N driven. More rapid uptake and assimilation of available N are therefore important mechanisms to increase green canopy area and radiation interception, especially in the first half of the stem-elongation phase. Present results confirmed observations from previous studies (Dhugga and Waines, 1989) that cultivars stopped accumulating N in the shoot with increasing N supply in non-limiting N conditions, due to an upper limit to uptake capacity which was possibly associated with canopy N sink size. It would therefore likely be advantageous for shoots under moderately low N supply to have high N uptake capacity consequently facilitating rapid canopy expansion. The capacity to accumulate N in the crop during periods when N supply exceeds the crop N requirement (i.e. higher maximum N uptake per day) would therefore confer an advantage to cultivars under moderately low N, and would increase fertiliser recovery in these crops leading to reduced N losses through leaching and denitrification. This is especially important so the crop can take up N quickly if it rapidly becomes available (e.g. under wet conditions). In the present study, the leaf lamina and true stem have been identified as having high RN function, and could be targeted to increase RN capacity and maximum rate of N uptake (kg N ha^{-1} day^{-1}), and thereby increase UPE.

At anthesis it is overall desirable in an N-efficient cultivar to increase the partitioning of crop N to the photosynthetic N in order to increase the ratio of PN and RN to SN in the canopy. There may be scope to increase the leaf lamina PN pool through larger canopies (Kull and Jarvis, 1995). However, this would also increase the SN required to produce and support the larger leaf lamina, and potentially lead to increased shading of lower leaves thereby reducing RUE. Alternatively, increased SLN would have little effect on the SN requirement of the canopy and would not impact on the canopy profile, but would increase the RN capacity of the leaf lamina. As RN accumulation in the leaf lamina is likely to be in the form of photosynthetically active

proteins such as Rubisco, this would also provide instantaneous increases in photosynthetic capacity should the light intensity increase. This is most important in the upper leaves, and contributes to the non-uniform vertical distribution of N observed in the green canopy at anthesis which is associated with higher canopy productivity. High SLN at anthesis would therefore be a trait to indicate high leaf lamina RN capacity, and could be rapidly screened in-field using leaf lamina spectral absorbance techniques (e.g. SPAD). However high SLN would also increase the 'metabolic cost' of maintaining PN, and may decrease net carbon gain (Hirose and Werger, 1987a). Therefore long-term increases to leaf lamina RN capacity could be achieved by increasing efficiency of photosynthetic enzymes (Reynolds *et al.*, 2000) thereby reducing the leaf N content required to maximise RUE (i.e. breakpoint between SLN and RUE).

The true stem also provided considerable RN capacity, and would be an opportunistic location for a dynamic RN pool that N could be rapidly translocated to growing tissues through the phloem transport systems of the shoot. A reduction in the SN requirement of the true stem could increase the proportion of RN in the true stem. This could potentially be achieved by reducing the stem-wall thickness or reducing stem height whilst maintaining stem wall thickness, as relatively little genetic variation was found in true stem N concentration at anthesis in this study (in the range 0.93-0.98% and 0.99-1.13% in TT06 and TT07, respectively). Thicker stems have a higher proportion of N in structural compounds (Puckridge and Donald, 1967) but stem thickness could be reduced without increasing lodging susceptibility (Berry *et al.*, 2004) possibly by increasing stem material strength, and consequently thinner stem walls may reduce the ratio of canopy N to green area. However, in the present study all cultivars were considered to be 'thin walled' and so present results could not be linked to this trait, and results linking true stem N content at anthesis with wall thickness from varieties identified as having either 'thin', 'medium' or 'thick' stem walls within the GREEN Grain study proved inconclusive.

It is also possible that a reduction in stem height may decrease the proportion of AGN as SN. Although there was only a small range of variation in stem length in the varieties in this study (overall in the range 623-692 mm), genetic variation in stem N per mm was observed in TT07 ($P<0.01$) and there was a trend for variety differences

in TT06. This variation was associated with stem height, as N uptake was similar across varieties; averaged across seasons at Terrington, shorter cultivars (Atlanta and Claire) had higher stem N per mm than taller cultivars (Istabraq and Savannah). Shorter cultivars may therefore increase the ratio of PN and RN to SN, and overall increase true stem RN capacity. Austin *et al*. (1977) found that modern, semi-dwarf cultivars accumulated less N by anthesis compared with older, taller lines, likely relating to a reduction in SN. However, a significant reduction in shoot height may affect the canopy light profile (thereby reducing light interception and harvest biomass), and make combine harvesting more difficult. Overall wall thickness and stem height traits may provide useful rapid-screening traits linked to CNR, with genetic variation amongst a wider range of genotypes linked to true stem RN capacity.

8.4.2 Grain-filling phase

At anthesis the canopy structure and N distribution patterns are typically arranged to maximise the whole-shoot net carbon gain. Prolonging the optimal N distribution of the green canopy at anthesis through the grain-filling phase will favour the increased production of photo-assimilates. Mobilisation of chloroplast N has a central role in leaf lamina metabolic activity and canopy senescence (Hörtensteiner and Feller, 2002), and grain N acquisition is linked to senescence patterns in durum wheat (Spano *et al*., 2003). Delayed senescence and 'stay-green' traits could therefore have an important role in increasing UTE and yield of wheat crops grown under moderate N supply. Present results indicated that during the first half of grain-filling, canopy RN buffered senescence by supplying N to the grain rather than N being drawn from the PN pool (thereby demonstrating a functional role as *storage* N). The capacity to translocate RN efficiently from the non-photosynthetic organs (i.e. true stem NRE) may delay or decrease the rate of N transfer from the PN pool and boost grain growth per unit canopy N.

High NRE was observed for the leaf lamina and leaf sheath (overall in the range 70-78% and 52-70%, respectively). However, true stem NRE was low (41-61%) for all varieties in all three site-seasons, with significant quantities of *accumulation* N at harvest. Unfertilised crops typically showed higher NRE from all components compared to well-fertilised crops, with straw N content increasing with N supply.

Overall the amount of *accumulation* N remaining in the true stem at harvest was higher at the N opt-trt compared to the N zero-trt (12 and 1 kg N ha^{-1}, respectively), and higher still at the N max-trt (17 kg ha^{-1}). Since the true stem contains the most RN amongst plant organs at anthesis and most *accumulation* N at harvest, increasing true stem NRE could further delay unloading from the PN pool during early to mid grain filling thereby slowing canopy senescence, reducing the amount of N remobilised from the leaf lamina and leaf sheath, and increasing yield under N-limited conditions.

From the present results, grain N demand during the second half of the grain-filling was satisfied mainly by PANU and from the PN pool. Continued PANU was observed until the end of the grain-filling period in this study. Andersson (2005) also found N uptake to physiological maturity in winter wheat, and in well-fertilised crops PANU was positively associated with 'stay-green' in sorghum (Borrell and Hammer, 2000). Increased longevity of the green canopy could be also achieved by reducing the grain N demand (which assumes some sink regulation of N unloading; Martre *et al*., 2006), thereby reducing or delaying N relocation from the PN pool. Selection for cultivars with low grain N concentration (i.e. feed wheats) through low grain storage protein content (i.e. glutenins and gliadins) may reduce the demand for N in the second half of grain-filling and thereby reduce extraction of N from the PN pool. Gliadins in particular are low in essential amino acids and have low nutritional value in livestock diets, and are therefore less important in feed wheat cultivars. This may in turn maintain or increase grain yield and consequently increase UTE, whilst reducing grain N content. Improving yields through higher starch per grain whilst maintaining NHI would dilute grain N content via intrinsically linked processes relating to N metabolism. Future increases in NHI are likely to be increasingly difficult to achieve as NHI is already high (0.70-0.80). However, such increases would overall be undesirable in feed wheats since they may have negative effects on UTE and yield through faster unloading of N from the green organs as discussed above.

8.5 APPLICATION OF PHYSIOLOGICAL TRAITS IN BREEDING FOR INCREASED N-UTILISATION EFFICIENCY

NUE, UPE and UTE are complex traits and relatively conservative, and their regulation is not so well understood but are unlikely to be controlled by easily identifiable genes. Generally, effects of variety in this study for UTE and underlying physiological traits were either small or non-significant. For instance, true stem RN has been identified as a key trait but only small varietal differences were found in experiments at Terrington (overall in the range 42-46 kg ha^{-1} and 28-39 kg ha^{-1} for TT06 and TT07, respectively), with varietal patterns inconsistent across site-seasons. This narrow genetic range for traits may have resulted from both the small range of genotypes presently tested and/or selection in UK breeding programmes to optimise fertiliser response under high N supply in modern elite feed wheat varieties. Genetic variation is required in breeding programmes for selection of key traits, and high trait heritability is also required for breeding improved cultivars. There may be greater genetic variability which could be exploited for breeding to improve UTE available within a wider range of current UK germplasm (e.g. as quantified within the GREEN Grain trials with UTE at the N opt-trt in the range 65-72 (GGTT06) and 70-83 (GGTT07)) or alternatively within a wider range of cultivars and/or wheat relatives worldwide; and further phenotyping for genetic diversity is required.

Another strategy to increase genetic variability would be through genetic modification of the activity of key enzymes involved in N assimilation. For example, NUE was positively associated with the activity of key enzyme traits involved in N assimilation and remobilisation of N to the grain in maize (Gallais and Hirel, 2003). Up-regulation of enzymes potentially controlling true stem NR, such as glutamine synthetase (Gallais and Hirel, 2003; Hirel *et al*., 2007) and alanine aminotransferase (Good *et al*., 2007), and signalling pathways connected with N status (Shewry, 2007) could offer opportunities for increasing UTE. Genetic studies associated with the use of molecular markers are a way of identifying 'quantitative trait loci' involved in the genetic variation of complex characters such as physiological traits related to UTE (Gallais and Hirel, 2003).

Co-mapping of whole-crop traits with genes encoding for key enzymes allow identification of 'candidate' genes, for which the favourable allele can be validated by transferral to a genotype with an unfavourable allele to test whether there is the expected effect (Gebbing *et al*., 1999). The development of markers for use in marker-assisted selection to selected favourable alleles could increase N uptake and loading to anthesis, and unloading to harvest, consequently increasing the ratio of *storage* N to *accumulation* N, but could also increase remobilisation of PN which could reduce yield. To phenotype genetic variation in key traits in mapping populations and amongst segregating populations in breeding programmes rapid-screening techniques are required (e.g. SPAD or spectral reflectance indices, e.g. NDVI; 'Normalised Difference Vegetation Index'), or if no screens are reliable (e.g. true stem RN) then it may be possible to develop and deploy molecular markers. Therefore future studies to develop high-throughput screens for target traits are a high priority for future research underpinning breeding progress in UTE.

8.6 OVERALL CONCLUSIONS

Referring in turn to the original hypotheses (1 to 12) stated in chapter 2, in summary this study has demonstrated that:

1. NUE decreased with increasing N supply similarly across site-seasons. Between the zero and optimum N treatments, the decrease was equally associated with declining UPE and UTE. However, above the N opt-trt only UPE continued to decline and UTE was therefore more important in determining NUE at high N supply. The main driver of lower UTE was BPE, although the response differed slightly between site-seasons. Varietal differences in the optimum amount of applied N were not observed in TT06 and TT07 (except for Atlanta), and in TT06 varietal differences in yield at this N opt resulted in differences in NUE. These differences in NUE amongst varieties in TT06 were due to differences in UTE, and there were strong genetic effects on UTE in both Terrington experiments. These were associated mainly with varietal differences in HI, but varietal differences in BPE in TT07 indicated the potential to breed for superior UTE with some consistency in varietal patterns in BPE across seasons (Savannah and Istabraq had higher BPE than Atlanta and Claire).

2. The present data fitted the critical N dilution curve for winter wheat (Justes *et al*., 1994), and the critical N concentration for maximum biomass at anthesis was achieved with relatively low amounts of applied N in both the Terrington and Lincoln experiments (114-147 kg ha^{-1} and 189 kg ha^{-1}, respectively). N uptake increased above these amounts with N supply to the N opt-trt (180-220 kg ha^{-1} and 300 kg ha^{-1}, respectively). CNR increased with N supply to the N opt-trt, and was overall in the range 30-37 and 46 kg N ha^{-1} of green area in the Terrington and Lincoln experiments, respectively. These values are in excess of the 30 kg N ha^{-1} green area requirement for canopy production in winter wheat suggested by Sylvester-Bradley *et al*. (1990a). Although higher values at LC07 were associated with a higher light environment, present results indicated that, in the well fertilised crops, N accumulation occurred at anthesis in excess of that required for structural and photosynthetic uses in all varieties and site-seasons.

3. In all site-seasons at anthesis, the crops accumulated significant amounts of excess N at the N opt-trt, increasing with N uptake to the N max-trt (overall increasing from 37 to 45 kg ha^{-1}, respectively). Averaging across experiments, the proportion of AGN as excess N increased disproportionately with N supply in TT06 and LC07; increasing from 0.16 at the N opt-trt to 0.23 at the N max-trt, but decreased in TT07 due to lower N uptake at the N max-trt. However, the proportion of AGN at anthesis as excess N varied between site-seasons: at the N opt-trt the proportion was higher in LC07 (0.21) than TT06 (0.12) or TT07 (0.10).

4. Crop N partitioning at anthesis was generally similar at all N treatments, with proportionally slightly more in the ear at the N zero-trt and a slightly more in the leaf lamina and leaf sheath at the N opt-trt. Only small varietal differences in N partitioning were observed in the present study, and N treatment x variety interactions were generally absent. Small varietal differences were mostly associated with flowering date with Atlanta reaching anthesis 3-4 days earlier than the other three varieties. The presently observed genetic differences may reflect that the cultivars were all modern, semi-dwarf, feed/biscuit wheats with similar release dates and therefore tended to be physiologically very similar. A study of

wider germplasm (e.g. non-UK varieties and/or wider relatives of wheat) may therefore be justified to find greater genetic variation in crop N partitioning traits.

5. Radiation-use efficiency was linearly related to SLN up to the breakpoint of the relationship between RUE and SLN in all three site-seasons. Thus, RUE was reduced through low leaf lamina N content at the unfertilised treatments. Estimation of the breakpoint of the relationship between SLN and RUE gave relatively consistent results across site-seasons in the range 1.97-2.13 g m^{-2} for SLN and 2.87-3.10 g MJ^{-1} for RUE; values similar to those reported by Field and Mooney (1986) and Sinclair and Horie (1989). However, overall little genetic variation in the key traits of SLN and RUE at anthesis was observed in the present study, due again to the similarity between varieties mentioned above.

6. The pattern of N allocation to crop N pools between crop components was consistent across N treatments. RN was found in all crop components at the N opt-trt in all three site-seasons, but was observed to be particularly located in the leaf lamina and the true stem (averaged across experiments at 19 and 45 kg N ha^{-1}, respectively). The response of the leaf lamina to N supply increased SLN to around 3 g N m^{-2} in the well fertilised treatments, in excess of the 2 g N m^{-2} observed to be required to maximise RUE, with RN observed only in the fertilised treatments. The true stem accumulated RN at all N treatments (i.e. even at the N zero-trt of 11 kg ha^{-1}), and at the optimum and maximum N treatment accumulated considerable quantities of RN (45 and 45 kg ha^{-1}, respectively). This indicated that the true stem plays an important functional role with regard to RN in wheat crops, particularly optimally fertilised crops.

7. Reserve N accumulation in the leaf lamina and leaf sheath was likely in the form of photosynthetically active proteins such as Rubisco (however, further investigations are required to test this further). The apparent dual function of this enzyme would allow both increased photosynthetic efficiency and leaf duration, and for Rubisco to act as a N storage protein. The true stem contained around 25% of the crop N content at anthesis; of which averaged across experiments at the N opt-trt, 66% was RN (31% SN and 3% PN). This indicated that N accumulation up to anthesis is a major function of the true stem. RN accumulation occurred in

the true stem even in the unfertilised crops (7-12 kg N ha^{-1}), but not in the leaf lamina or leaf sheath. Overall, 65 and 58% of this true stem was remobilised at the optimum and maximum N treatments, respectively (i.e. was *storage* N). In the present study true stem RN was shown to be important in maintaining canopy function during grain filling by buffering relocation of N to the grain from the PN pool during the first half of the grain-filling phase, thereby increasing photo-assimilate production and grain filling.

8. *Accumulation* N was identified as the RN which was remaining in the straw at harvest, thereby reducing overall crop NRE. This *accumulation* N has been shown to be non-essential for growth or grain production, and therefore creates inefficiencies in UTE by increasing crop fertiliser N demand without increasing grain yields. The amount of *accumulation* N increased with N supply, and was primarily located in the true stem. True stem *accumulation* N at the N opt-trt was in the range 9-14 kg ha^{-1} (representing 14-26% of true stem N content at anthesis, and approximately 15-19 kg ha^{-1} of applied fertiliser N – calculated using AFR data from the present study) and increased to the N max-trt in the range 11-25 kg ha^{-1} (representing 16-38% of true stem N content at anthesis, and approximately 18-35 kg ha^{-1} of applied fertiliser N). True stem NRE was considerably lower than the leaf lamina and leaf sheath, and decreased with N supply. Varietal differences in true stem *accumulation* N were observed in both Terrington experiments, associated with true stem N content at anthesis rather than true stem NRE. Although the varietal pattern of true stem *accumulation* N was not consistent across seasons, in both Terrington experiments the variety with the lowest true stem *accumulation* N also had the highest UTE (Atlanta in TT06, and Istabraq in TT07), and *vice versa*.

9. During the first half of the grain-filling period, N from the RN pool was remobilised more readily than PN to provide N for the grain. This RN remobilisation (particularly from the true stem) provided a buffer against remobilisation of PN from the photosynthetic tissues (i.e. leaf lamina and leaf sheath) and the majority of the green canopy area was retained up to mid-grain filling. Increased *storage* N was therefore associated with delayed canopy senescence and 'stay-green' effects and increased the production of photo-

assimilates for grain-filling. Data at GS75 were collected for Istabraq only and as a result variety differences could not be tested.

10. Reserve N in the leaf lamina and leaf sheath was effectively remobilised during the grain-filling period, leaving little or no *accumulation* N in these organs at harvest. However the true stem contained considerable quantities of *accumulation* N in the straw at harvest, which increased with N supply. Therefore increased NRE of RN in the true stem would increase the ratio of *storage* N to *accumulation* N in the canopy potentially leading to greater 'optimisation' of true stem N storage and increases in crop UTE. Defoliation experiments demonstrated that increased true stem NRE was possible through higher grain sink size relative to source size, representing an increase of around 20% N contribution which was consistent across N treatments.

11. Degraining resulted in a significant increase in grain N content (overall by 27%) compared to the control. This indicated that the grain N accumulation was significantly source limited, in agreement with previous studies (Martre *et al.*, 2003). Responses to degraining also showed that a reduction in grain N demand delayed canopy senescence and increased grain weight. Responses to defoliation demonstrated the potential to increase NR from canopy stores, mainly in the true stem, which could potentially be used to further buffer canopy senescence during grain-filling as an avenue to increase UTE.

12. The physiological traits associated with accumulation and partitioning of canopy N which have been correlated with increased UTE (through increased BPE) can be used to propose a feed wheat ideotype with reduced fertiliser N requirements whilst maintaining yields under moderate to low N supply:

During the stem-elongation phase to anthesis:

- High capacity to accumulate N in the true stem to allow rapid uptake of available N and increase the true stem RN pool at anthesis.

- Reduction in the accumulation of N in the leaf lamina and leaf sheath restricting accumulation of N above that required to maximise RUE (due to high metabolic costs of maintaining photosynthetic enzymes).
- Increased efficiency of photosynthetic enzymes (e.g. through Rubisco properties) to reduce the SLN at the breakpoint of the relationship between RUE and SLN whilst maintaining RUE.

During the grain-filling phase to harvest:

- Reduced N remobilisation and low N remobilisation efficiency from the leaf lamina and leaf sheath to maintain canopy green area (and 'stay-green' traits).
- Increased true stem N remobilisation and high remobilisation efficiency of RN to the grain in order to buffer remobilisation of PN from photosynthetic organs.
- Increased ratio of canopy *storage* N to *accumulation* N to reduce crop fertiliser N requirement and non-remobilised RN remaining in the straw at harvest.
- Low grain N content (especially of gliadins) to reduce the grain 'N demand' during the second half of the grain-filling phase thereby reducing or delaying the N relocation from the PN pool in the photosynthetic organs.

8.7 METHODOLOGICAL ISSUES ENCOUNTERED DURING THE STUDY

A major issue encountered in field experimentation investigating N-related traits is the strong genotype x N x environment interaction (Kramer, 1979; and this study), with a considerable proportion of the variability observed in experiments due to differences in meteorological conditions. The response of winter wheat to N fertiliser is notoriously variable (Sylvester-Bradley *et al*., 1982). However it is important that these studies were conducted in field situations as results obtained from greenhouse or growth-chamber experiments do not necessarily hold under field conditions (Dhugga and Waines, 1989), although GM work is possible in controlled environments. The field environment introduced considerable variation into the results, with typically significant effects of site and/or season. Large genotype x N x environment interactions make repeatable experimental evidence for genetic differences in tolerance of low N more difficult to obtain, and any genetic differences and the

heritability of genotypic variation more difficult to interpret (especially for traits of already low heritability), making it harder to select for traits in breeding programmes.

Several specific issues were encountered during the sampling regime: (1) The date of anthesis was observed to be consistently 3-4 days earlier for Atlanta than for the other varieties in the Terrington experiments. However, all varieties were sampled on the same calendar date for all growth stages in order to facilitate sampling and statistical analysis. Atlanta may therefore have been slightly more physiologically advanced (e.g. in patterns of N accumulation or remobilisation) leading to small apparent genetic differences. (2) There were plant establishment problems in both Terrington experiments, and although choice of quadrat sample areas at tillering attempted to avoid badly affected patches, there were some unavoidable resultant increases in variation of plant and shoot densities between samples. (3) The methods for in-field assessment of crop DM and N status were destructive and time consuming. This was inherent in the experimental design of the present study. However, more frequent sampling would have improved the accuracy of N loading and unloading patterns. Further development and calibration of canopy spectral reflectance techniques related to green area and biomass (e.g. NDVI) and N content (e.g. SPAD) which may permit rapid and repeated in-field measurement of the growth of crops in future studies seems justified. (4) Sample preparation, milling and analysis for N data was particularly time consuming and costly, and the use and development of 'Near Infra-Red' (NIR) analysis with calibrations for each crop component at specific growth stages to replace 'Dumas' N% analysis used in the present study would allow greater throughput of samples for N% determination at lower cost.

8.8 FUTURE WORK

Several approaches for further work can be proposed on either a short-term (5-10 years) or longer-term (10+ years) basis. Short-term objectives for achieving improved crop UTE are likely to be realised through crop breeding for specific physiological traits to drive improvements in grain yield per unit canopy N content; such as those identified in this study addressing crop N accumulation (e.g. true stem RN content at anthesis) and remobilisation (e.g. true stem NRE) and associated stay-green traits. However, there is a need for further studies to elucidate the mechanisms involved in

the N accumulation and N remobilisation processes, and to develop simple, high-throughput screens, well correlated with field expression of traits which could be applied as screening tools for NUE and UTE in breeding programmes. For example, monitoring the leaf N status using a chlorophyll meter (SPAD) and green area and biomass using spectral reflectance techniques (NDVI) at specific growth stages (see Babar *et al*., 2006); for a comprehensive review of monitoring using ground based sensors see Shanahan *et al*., 2008. These traits can be used to complement traditional breeding selection methods and to help inform choice of parental lines to cross with synergistic traits. Such screens could also be deployed in the UK variety evaluation systems to indicate suitabilities of new varieties to low fertiliser inputs.

The true stem has been shown to accumulate considerable quantities of N at anthesis. However, further study should examine the form and location of this N, and whether *storage* N and *accumulation* N are similar or differentiated. The genetic control of traits may not be independent, but linked to expression of other yield-related traits; e.g. decreasing stem N accumulation capacity may also reduce stem water-soluble carbohydrate storage (WSC) whilst WSC has been demonstrated to significantly increase grain yields (Blum, 1998; Shearman *et al*., 2005). Studies may continue to develop the relationships between N loading and unloading in respective plant organs: for example, would it be possible to identify varieties with high true stem RN and low leaf lamina RN or high true stem NRE and low leaf lamina NRE, or perhaps the processes are intrinsically linked across the plant organs.

The present study tested only a small range of genotypes within current commercial UK varieties, and therefore these results require confirmation with a larger range of genotypes. As relatively little variation was observed between the study varieties, there is a requirement for further experimentation with wider germplasm to find differences which are large enough to be exploited in breeding. In particular, new sources of germplasm may be found outside the Triticeae: wider relatives of wheat and synthetically derived wheats (e.g. Reynolds *et al*., 2007a). Comparison with other important crops may provide further insights into relationships; cereal crops such as barley and rice, and more exotic crops such as maize and sorghum, and may reveal traits which may be introgressed to winter wheat lines. For instance, wider screening may identify candidate genes for N dynamics and stay-green from other species, e.g.

maize (Rajcan and Tollenaar, 1999) and sorghum (Borrell *et al.*, 2001). Progress could also be made by analysing the existing genetic variability in wheat in different environments, and breeders might be encouraged to incorporate more levels of applied N (particularly low N conditions) into their future programmes to increase selection pressure for N-efficient cultivars which sustain yield levels with less fertiliser N use.

Further development of crop simulation models (such as SIRIUS; Jamieson and Semenov, 2000) to quantitatively link between organ and crop scale processes provides an approach for integrating our understanding of complex mechanisms controlling UTE as influenced by the weather, soil and crop management. The use of data from detailed physiological studies, such as data for N accumulation, partitioning and remobilisation parameters associated with crop N status from the present study, could be used to make the N allocation parameters in the models genotype-specific. This would add biological reality and increase the precision of models, and allow sensitivity analyses to test particular associations such as between *storage* N and yield and grain N%. With respect to the development of feed wheats with lower fertiliser requirement, improved crop models could help to simulate physiological responses (such as N partitioning and NR) to low N input conditions, and in doing so could reduce the need for costly and time-consuming field trials allowing multiple analyses of physiological parameters thereby facilitating the selection of useful traits in breeding programmes.

Longer-term objectives are likely to be achieved through further understanding of the metabolic control processes to optimise photosynthetic efficiency and N, the N requirement of canopy PN pools, and to improve crop RUE (e.g. through manipulation of Rubisco properties and the breakpoint between SLN and RUE). Substantial genetic gain in NUE in wheat may be achieved if breeders are able to identify specific novel genes for UTE and, through marker-assisted selection, backcross the genes into elite UK varieties. A better understanding of the metabolic and genetic control of N assimilation and remobilisation and the control of canopy senescence should be included in future work. In particular, improved understanding in wheat of enzymes such as cytosolic glutamine synthetase (GS1) and glutamate dehydrogenase (GDH) (see Lea and Ireland, 1999) which may link genetic variation

in NRE to specific genetic characters. Further, future structural modification of specific key enzymes, such as the photosynthetic enzyme Rubisco, may enhance photosynthetic efficiency, producing cultivars with potentially faster growth rates and which use canopy N more efficiently (see Reynolds *et al*., 2000). Current genome sequencing and mapping projects may provide useful data to approach these targets within the next decade (Hirel *et al*., 2007).

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10 APPENDICES

10.1 Appendix I: Field experiment management

Table 10-1 Farm operations throughout the crop cycle at Terrington 2005/06.

Application	Product	Date	Rate	Units/ha
Molluscicides	Mini pellets	21/10/2005	8.000	kg
Molluscicides	Mini pellets	26/10/2005	8.000	kg
Molluscicides	Mini pellets	04/11/2005	6.000	kg
Insecticides	Cypermethrin 100	18/11/2005	0.267	l
Herbicides	IPU 500	18/11/2005	2.000	l
Herbicides	Panther	18/11/2005	1.000	l
Fertiliser	Kieserite	03/03/2006	75.000	kg
Growth regulators	Chlormequat 720	13/04/2006	2.000	l
Herbicides	Starane	26/04/2006	1.000	l
Herbicides	Topic 240 EC	27/04/2006	0.267	l
Fungicides	BRAVO 500	27/04/2006	1.000	l
Fungicides	Proline	27/04/2006	0.533	l
Fungicides	Tern	27/04/2006	0.467	l
Adjuvants	Agral	12/05/2006	0.067	l
Growth regulators	Terpal	12/05/2006	0.733	l
Fungicides	BRAVO 500	25/05/2006	1.000	l
Fungicides	Comet 200	25/05/2006	0.533	l
Fungicides	Opus	25/06/2006	0.733	l
Insecticides	Dursban Wg	06/06/2006	0.533	kg
Fungicides	Amistar	13/06/2006	0.267	l
Fungicides	Opus	14/06/2006	0.333	l

Operation	Type	Date
Cultivation	Plough	16/08/2005
Cultivation	Power Harrow	09/09/2005
Cultivation	Power Harrow	10/10/2005
Cultivation	Drill	11/10/2005
Cultivation	Fertiliser application	06/03/2006
Cultivation	Fertiliser application	25/04/2006
Cultivation	Fertiliser application	11/05/2006
Cultivation	Combine	10/08/2006

Table 10-2 Farm operations throughout the crop cycle at Terrington 2006/07.

Application	Product	Date	Rate	Units/ha
Herbicides	Glyphos	08/10/2006	4.000	l
Molluscicides	Mini pellets	30/10/2006	8.000	kg
Molluscicides	Huron	14/11/2006	5.000	kg
Molluscicides	Huron	04/12/2006	5.000	kg
Insecticides	Cypermethrin100	15/12/2006	0.244	l
Herbicides	Ipu 500	14/12/2006	4.000	l
Fertiliser	Keiserite	26/03/2007	75.000	kg
Fertiliser	Phosphate, Tsp	26/03/2007	326.000	kg
Herbicides	Swipe-P	06/04/2007	4.489	l
Growth regulators	Chlormequat 720	10/04/2007	2.244	l
Fungicides	Bravo 500	23/04/2007	1.000	l
Fungicides	Opus	23/04/2007	0.511	l
Herbicides	Starane	23/04/2007	1.000	l
Fungicides	Bravo 500	21/05/2007	1.000	l
Fungicides	Comet 200	21/05/2007	0.489	l
Fungicides	Opus	21/05/2007	0.756	l
Fungicides	Comet 200	01/06/2007	0.244	l
Fungicides	Opus	01/06/2007	0.289	l

Operation	Type	Date
Cultivation	Plough	01/08/2006
Cultivation	Power Harrow	07/08/2006
Cultivation	Power Harrow	30/10/2006
Cultivation	Drill	30/10/2006
Cultivation	Fertiliser application	26/03/2007
Cultivation	Fertiliser application	24/04/2007
Cultivation	Fertiliser application	09/05/2007
Cultivation	Combine	12/08/2007

Table 10-3 Farm operations throughout the crop cycle at Lincoln 2006/07.

Application	Product	Date	Rate	Units/ha
Herbicide	Roundup	04/05/2006	4	l
Herbicide	Granstar	04/05/2006	15	g
Herbicide	Glean	25/08/2006	15	g
Herbicide	Combine	25/08/2006	1.25	l
Fungicides	Opus	20/10/2006	1	l
Growth regulators	Cycocel	31/10/2006	1.5	l
Fungicides	Folicur SC	31/10/2006	0.44	l
Adjuvants	Agral	31/10/2006	0.025	l
Fungicides	Cereous	16/11/2006	0.5	l
Insecticides	Karate	16/11/2006	0.03	l
Fungicides	Opus	05/12/2006	1	l
Fungicides	Cereous	04/01/2007	0.5	l

Operation	Type	Date
Cultivation	Plough	10/05/2006
Cultivation	Power Harrow	07/06/2006
Cultivation	Drill	08/06/2006
Cultivation	Fertiliser application	06/10/2006
Irrigation	Irrigation - 5ml	06/10/2006
Cultivation	Fertiliser application	06/11/2006
Irrigation	Irrigation - 20ml	06/11/2006
Irrigation	Irrigation - 25ml	24/11/2006

10.2 Appendix II: Field trial plans

Key:

N treatment (N) (1) N zero-trt; (4) N opt-trt; (6) N max-trt

Variety (V) (1) Istabraq; (2) Atlanta; (3) Claire; (4) Savannah

Buffer row (G)

	G	G	1a	1b	2a	2b	3a	3b	4a	4b	G	5a	5b	6a	6b	7a	7b	8a	8b	G	9a	9b	10a	10b	11a	11b	12a	12b	G	G	
N			4	4	4	4	4	4	4	4		1	1	1	1	1	1	1	1		6	6	6	6	6	6	6	6			
V			2	2	3	3	1	1	4	4		3	3	1	1	4	4	2	2		4	4	1	1	2	2	3	3			
	G	G	13a	13b	14a	14b	15a	15b	16a	16b	G	17a	17b	18a	18b	19a	19b	20a	20b	G	21a	21b	22a	22b	23a	23b	24a	24b	G	G	Rep 1
N			3	3	3	3	3	3	3	3		2	2	2	2	2	2	2	2		5	5	5	5	5	5	5	5			
V			2	2	4	4	1	1	3	3		1	1	2	2	4	4	3	3		3	3	2	2	4	4	1	1			
	G	G	25a	25b	26a	26b	27a	27b	28a	28b	G	29a	29b	30a	30b	31a	31b	32a	32b	G	33a	33b	34a	34b	35a	35b	36a	36b	G	G	
N			3	3	3	3	3	3	3	3		2	2	2	2	2	2	2	2		1	1	1	1	1	1	1	1			
V			4	4	3	3	2	2	1	1		3	3	2	2	1	1	4	4		1	1	3	3	2	2	4	4			
	G	G	37a	37b	38a	38b	39a	39b	40a	40b	G	41a	41b	42a	42b	43a	43b	44a	44b	G	45a	45b	46a	46b	47a	47b	48a	48b	G	G	Rep 2
N			4	4	4	4	4	4	4	4		6	6	6	6	6	6	6	6		5	5	5	5	5	5	5	5			
V			3	3	4	4	2	2	1	1		4	4	2	2	3	3	1	1		4	4	1	1	2	2	3	3			
	G	G	49a	49b	50a	50b	51a	51b	52a	52b	G	53a	53b	54a	54b	55a	55b	56a	56b	G	57a	57b	58a	58b	59a	59b	60a	60b	G	G	
N			6	6	6	6	6	6	6	6		5	5	5	5	5	5	5	5		2	2	2	2	2	2	2	2			
V			1	1	2	2	4	4	3	3		4	4	2	2	3	3	1	1		3	3	4	4	2	2	1	1			
	G	G	61a	61b	62a	62b	63a	63b	64a	64b	G	65a	65b	66a	66b	67a	67b	68a	68b	G	69a	69b	70a	70b	71a	71b	72a	72b	G	G	Rep 3
N			1	1	1	1	1	1	1	1		3	3	3	3	3	3	3	3		4	4	4	4	4	4	4	4			
V			2	2	1	1	4	4	3	3		4	4	1	1	3	3	2	2		3	3	1	1	4	4	2	2			

Figure 10.1 Field trial plan for TT06.

	G	G	1a	1b	2a	2b	3a	3b	4a	4b	G	5a	5b	6a	6b	7a	7b	8a	8b	G	9a	9b	10a	10b	11a	11b	12a	12b	G	G	
N			5	5	5	5	5	5	5	5		4	4	4	4	4	4	4	4		6	6	6	6	6	6	6	6			
V			4	4	2	2	3	3	1	1		3	3	2	2	4	4	1	1		1	1	3	3	4	4	2	2			
	G	G	13a	13b	14a	14b	15a	15b	16a	16b	G	17a	17b	18a	18b	19a	19b	20a	20b	G	21a	21b	22a	22b	23a	23b	24a	24b	G	G	Rep 1
N			1	1	1	1	1	1	1	1		2	2	2	2	2	2	2	2		3	3	3	3	3	3	3	3			
V			1	1	3	3	4	4	2	2		4	4	2	2	3	3	1	1		1	1	3	3	2	2	4	4			
	G	G	25a	25b	26a	26b	27a	27b	28a	28b	G	29a	29b	30a	30b	31a	31b	32a	32b	G	33a	33b	34a	34b	35a	35b	36a	36b	G	G	
N			3	3	3	3	3	3	3	3		5	5	5	5	5	5	5	5		1	1	1	1	1	1	1	1			
V			2	2	1	1	4	4	3	3		1	1	4	4	3	3	2	2		4	4	2	2	3	3	1	1			
	G	G	37a	37b	38a	38b	39a	39b	40a	40b	G	41a	41b	42a	42b	43a	43b	44a	44b	G	45a	45b	46a	46b	47a	47b	48a	48b	G	G	Rep 2
N			6	6	6	6	6	6	6	6		2	2	2	2	2	2	2	2		4	4	4	4	4	4	4	4			
V			1	1	3	3	4	4	2	2		3	3	1	1	2	2	4	4		3	3	2	2	1	1	4	4			
	G	G	49a	49b	50a	50b	51a	51b	52a	52b	G	53a	53b	54a	54b	55a	55b	56a	56b	G	57a	57b	58a	58b	59a	59b	60a	60b	G	G	
N			6	6	6	6	6	6	6	6		4	4	4	4	4	4	4	4		3	3	3	3	3	3	3	3			
V			3	3	4	4	1	1	2	2		3	3	2	2	4	4	1	1		3	3	2	2	4	4	1	1			
	G	G	61a	61b	62a	62b	63a	63b	64a	64b	G	65a	65b	66a	66b	67a	67b	68a	68b	G	69a	69b	70a	70b	71a	71b	72a	72b	G	G	Rep 3
N			2	2	2	2	2	2	2	2		1	1	1	1	1	1	1	1		5	5	5	5	5	5	5	5			
V			2	2	3	3	4	4	1	1		1	1	3	3	2	2	4	4		3	3	2	2	1	1	4	4			

Figure 10.2 Field trial plan for TT06.

	G	G	G	1	2	3	4	5	6	7	8	9	10	11	12	13	14	G	G	G
N				2	1	4	5	7	3	6	3	5	7	1	6	4	2			
	G	G	G	15	16	17	18	19	20	21	22	23	24	25	26	27	28	G	G	G
N				5	6	2	7	3	1	4	7	4	5	2	6	3	1			
	G	G	G	29	30	31	32	33	34	35	36	37	38	39	40	41	42	G	G	G
N				3	1	5	6	2	4	7	5	4	6	3	7	2	1			

Figure 10.3 Field trial plan for LC07.

10.3 Appendix III: GENSTAT Outputs

GENSTAT example output for: (a) ANOVA and (b) regression analysis (for HI), (c) LEXP function, (d) N opt estimation (for yield), and (e) broken stick analysis (for AGN). All examples calculated using data from TT06.

10.3.1 ANOVA

```
671  "General Analysis of Variance."
672  BLOCK block/Nplot
673  TREATMENTS POL(applied_N;3)*variety
674  COVARIATE "No Covariate"
675  ANOVA [PRINT=aovtable,information,means,residuals,%cv; FACT=32; CONTRASTS=7; FPROB=yes;\
676   PSE=diff,lsd,means; LSDLEVEL=5] HI
Analysis of variance

Variate: HI
```

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
block stratum	2	8.55E-05	4.28E-05	0.1	
block.Nplot stratum					
applied_N	5	0.0443	0.00886	21.32	<.001
Lin	1	0.028722	0.028722	69.12	<.001
Quad	1	0.014683	0.014683	35.33	<.001
Cub	1	3.77E-05	3.77E-05	0.09	0.77
Deviations	2	0.000858	0.000429	1.03	0.391
Residual	10	0.004155	0.000416	2.78	
block.Nplot.*Units* stratum					
Variety	3	0.014477	0.004826	32.24	<.001
applied_N.variety	15	0.002868	0.000191	1.28	0.266
Lin.variety	3	0.000754	0.000251	1.68	0.189
Quad.variety	3	0.000722	0.000241	1.61	0.204
Cub.variety	3	0.000961	0.000321	2.14	0.112
Deviations	6	0.00043	7.17E-05	0.48	0.82
Residual	36	0.005389	0.00015		
Total	71	0.071274			

Tables of means
Variate: HI
Grand mean 0.4888

applied_N	0	70	150	220	290	370
	0.4421	0.4693	0.5048	0.5092	0.5068	0.5005

variety	1	2	3	4
	0.477	0.5115	0.4766	0.49

applied_N	variety	1	2	3	4
0		0.4349	0.461	0.4264	0.4461
70		0.4657	0.4921	0.449	0.4704
150		0.4943	0.5215	0.4967	0.5068
220		0.4935	0.5256	0.5022	0.5155
290		0.4793	0.5371	0.4996	0.5113
370		0.4947	0.5319	0.486	0.4896

Standard errors of means

Table	applied_N	variety	applied_N variety
rep.	12	18	3
e.s.e.	0.00588	0.00288	0.00849
d.f.	10	36	32.69
Except when comparing means with the same level(s) of applied_N			0.00706
d.f.			36

Standard errors of differences of means

Table	applied_N	variety	applied_N variety
rep.	12	18	3
s.e.d.	0.00832	0.00408	0.012
d.f.	10	36	32.69
Except when comparing means with the same level(s) of applied_N			0.00999
d.f.			36

Least significant differences of means (5% level)

Table	applied_N	variety	applied_N variety
rep.	12	18	3
l.s.d.	0.01854	0.00827	0.02443
d.f.	10	36	32.69
Except when comparing means with the same level(s) of applied_N			0.02026
d.f.			36

Stratum standard errors and coefficients of variation

Variate: HI

Stratum	d.f.	s.e.	cv%
Block	2	0.00133	0.3
block.Nplot	10	0.01019	2.1
block.Nplot.*Units*	36	0.01223	2.5

10.3.2 Polynomial regression analysis

```
755  "Polynomial Regression"
 756  MODEL HI
 757  TERMS POL(applied_N;2)*variety
 760  ADD [PRINT=model,summary,estimates,accumulated; CONSTANT=estimate; FPROB=yes; TPROB=yes;\
 761   FACT=9] variety
```

Regression analysis

Response variate:	HI
Fitted terms:	Constant + applied_N + variety
Submodels:	POL(applied_N; 2)

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.	F pr.
Regression	5	0.05788	0.011576	57.05	<.001
Residual	66	0.01339	0.000203		
Total	71	0.07127	0.001004		
Change	-3	-0.01448	0.004826	23.78	<.001

Percentage variance accounted for 79.8

Standard error of observations is estimated to be 0.0142.

Message: the following units have large standardized residuals.

Unit	Response	Residual
20	0.4219	-2.89

Estimates of parameters

Parameter	estimate	s.e.	t(66)	t pr.
Constant	0.42929	0.0047	91.38	<.001
applied_N Lin	0.000541	4.69E-05	11.54	<.001
applied_N Quad	-1E-06	1.22E-07	-8.51	<.001
variety 2	0.03449	0.00475	7.26	<.001
variety 3	-0.00041	0.00475	-0.09	0.932
variety 4	0.01291	0.00475	2.72	0.008

Parameters for factors are differences compared with the reference level:

Factor	Reference level
variety	1

Accumulated analysis of variance

Change	d.f.	s.s.	m.s.	v.r.	F pr.
+ POL(applied_N; 2)	2	0.043404	0.021702	106.95	<.001
+ variety	3	0.014477	0.004826	23.78	<.001
Residual	66	0.013392	0.000203		
Total	71	0.071274	0.001004		

10.3.3 Linear plus exponential function

```
737  "Line plus exponential (addition of linear trend)"
 738  MODEL %85%DW_yield_t_ha
 739  TERMS applied_N*variety
 740  FITCURVE [PRINT=model,summary,estimates,accumulated; CURVE=lexponential; SENSE=left;\
 741   CONSTANT=estimate; FPROB=yes] applied_N
Warning 1, code OP 19, statement 1 on line 741

Command: FITCURVE [PRINT=model,summary,estimates,accumulated; CURVE=lexponential
The asymptote has been reversed. The curve will be fitted with SENSE=right.

Nonlinear regression analysis
                Response variate:      %85%DW_yield_t_ha
                Explanatory:           applied_N
                Grouping factor:       variety, constant parameters separate
                Fitted Curve:          A + B*(R**X) + C*X
                Constraints:           R < 1

Summary of analysis
Source          d.f.            s.s.          m.s.          v.r.      F pr.
Regression         6          320.89       53.4811         72.04      <.001
Residual          65           48.26        0.7424
Total             71          369.14        5.1992
Change            -3          -14.99        4.9973          6.73      <.001

Percentage variance accounted for 85.7
Standard error of observations is estimated to be 0.862.

Message: the following units have large standardized residuals.
                Unit          Response      Residual
                  61            11.152          2.89

Estimates of parameters
Parameter       estimate          s.e.
R                 0.9943        0.00206
B                -12.89
C                -0.01608
A variety 1       20.64
A variety 2       21.55
A variety 3       20.34
A variety 4       21.09

Accumulated analysis of variance
Change          d.f.            s.s.          m.s.          v.r.      F pr.
Applied_N          3        305.8945      101.9648        137.34      <.001
Variety            3         14.9919        4.9973          6.73      <.001
Residual          65         48.2569        0.7424
Total             71        369.1433        5.1992
```

10.3.4 N fertiliser optimum amount estimation

```
201  RCHECK [RMETHOD=deviance; GRAPHICS=high] residual; composite
 202  RGRAPH [GRAPHICS=high]
 203  expression e[1];value=!e(nopt=((log(0.003-'C')\
 204  -LOG('B'* LOG('R')))/LOG('R')))
 205  rfunction[calc=e[1]] nopt
```

Estimates of functions of parameters

Estimates and standard errors

Parameter	estimate	s.e.
Nopt	236.3	14.4

10.3.5 Broken stick analysis

Fit of two-straight-line model

Nonlinear regression analysis

Response variate:	AGN_kg_ha
Nonlinear parameters:	Breakpoint_X
Model calculations:	Twolines[1], Twolines[2], Twolines[3]
Fitted terms:	Breakpoint_Y, Slope_1, Slope_2

Summary of analysis

Source	d.f.	s.s.	m.s.	v.r.
Regression	4	1597377	399344.4	523.6
Residual	14	10678	762.7	
Total	18	1608055	89336.4	

Percentage variance accounted for 90.9

Standard error of observations is estimated to be 27.6.

Message: the following units have large standardized residuals.

Unit	Response	Residual
56	296.2	-2.08

Estimates of parameters

Parameter	Estimate	s.e.
Breakpoint_X	179.7	24.9
* Linear		
Breakpoint_Y	342	21.4
Slope_1	1.178	0.15
Slope_2	0.105	0.15

X value at intersection of lines

X value 179.73, approximate s.e. 24.94

95% confidence interval (113.7, 245.0)

10.4 Appendix IV: Model parameters for fitted curves

Equations for curves:

Linear $y = m x + c$

Quadratic $y = (a x^2) + (m x) + c$

Cubic $y = (b x^3) + (a x^2) + (m x) + c$

10.4.1 Chapter 4: General crop growth

Table 10-4 Model parameters for curves ($y = (a x^2) + (m x) + c$) for ear population density.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	1.11	-0.00181	385.1	55.7 (56.6)
	Atlanta	1.11	-0.00181	409.7	
	Claire	1.11	-0.00181	443.7	
	Savannah	1.11	-0.00181	416.3	
TT07	Istabraq, Claire & Savannah	0.91	-0.00157	328.6	45.5 (50.7)
	Atlanta	1.07	-0.00272	318.8	44.6 (37.9)
LC07	Istabraq	1.57	-0.00207	250.1	92.5 (31.0)

Table 10-5 Model parameters for curves ($y = (a x^2) + (m x) + c$) for grains per ear.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	0.0942	-1.78E-04	37.07	66.1 (3.87)
	Atlanta	0.0942	-1.78E-04	42.01	
	Claire	0.0942	-1.78E-04	33.85	
	Savannah	0.0942	-1.78E-04	34.07	
TT07	Istabraq, Claire & Savannah	0.0567	-1.42E-04	51.02	10.6 (4.86)
	Atlanta	0.0716	-1.31E-04	53.29	37.9 (4.17)
LC07	Istabraq	-	-	-	-

Table 10-6 Model parameters for curves (TT06 and LC07 ($y = m x + c$) , and TT07 ($y = (a x^2) + (m x) + c$)) for individual grain weight.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	-0.0130	-	46.94	66.1 (1.93)
	Atlanta	-0.0130	-	43.76	
	Claire	-0.0130	-	44.37	
	Savannah	-0.0130	-	49.18	
TT07	Istabraq	-0.0361	8.07E-05	40.82	61.8 (1.73)
	Claire	0.0101	-4.43E-05	37.55	
	Savannah	-0.0433	6.70E-05	40.82	
	Atlanta	-0.0441	7.41E-05	44.21	
LC07	Istabraq	-0.00389	-	43.88	23.0 (1.23)

Table 10-7 Model parameters for curves ($y = (a x^2) + (m x) + c$) for N-use efficiency.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	-0.171	1.57E-04	64.57	89.3 (5.04)
	Atlanta	-0.171	1.57E-04	68.64	
	Claire	-0.171	1.57E-04	63.68	
	Savannah	-0.171	1.57E-04	66.87	
TT07	All	-0.262	3.55E-04	68.91	95.3 (3.75)
LC07	Istabraq	-0.284	2.99E-04	91.43	94.4 (6.25)

Table 10-8 Model parameters for curves (TT06 and TT07 ($y = m x + c$) , and LC07 ($y = (b x^3) + (a x^2) + (m x) + c$)) for N-uptake efficiency.

Exp.	Variety	m	a	b	c	% variance accounted (SE)
TT06	Istabraq	-0.00143	-	-	1.3368	65.1 (0.134)
TT07	Istabraq	-0.00239	-	-	1.4054	85.8 (0.115)
LC07	Istabraq	-0.00679	2.16E-05	-2.38E-08	1.6057	91.0 (0.099)

Table 10-9 Model parameters for curves (TT06 and TT07 ($y = (a x^2) + (m x) + c$) , and LC07 ($y = (b x^3) + (a x^2) + (m x) + c$)) for N-utilisation efficiency.

Exp.	Variety	m	a	b	c	% variance Accounted (SE)
TT06	Istabraq	-0.0980	1.42E-04	-	48.41	86.4 (2.45)
TT07	Istabraq	-0.1311	2.63E-04	-	48.49	81.1 (2.77)
LC07	Istabraq	0.0007	-3.65E-04	5.31E-07	57.98	96.3 (2.05)

Table 10-10 Model parameters for curves ($y = (a x^2) + (m x) + c$) for above-ground dry mass.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	0.05569	-1.02E-04	15.895	74.4 (1.57)
TT07	Istabraq, Claire & Savannah	0.03937	-7.60E-05	13.427	57.4 (1.53)
	Atlanta	0.06930	-1.66E-04	11.965	74.5 (1.43)
LC07	Istabraq	0.08049	-1.01E-04	11.322	92.2 (1.74)

Table 10-11 Model parameters for curves (TT07 and LC07 ($y = m x + c$) , and TT06 ($y = (a x^2) + (m x) + c$)) for harvest index.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	5.41E-04	-1.04E-06	0.429	79.8 (0.0142)
	Atlanta	5.41E-04	-1.04E-06	0.464	
	Claire	5.41E-04	-1.04E-06	0.429	
	Savannah	5.41E-04	-1.04E-06	0.442	
TT07	Istabraq	2.08E-05	-	0.518	22.4 (0.0204)
	Claire	2.08E-05	-	0.530	
	Savannah	2.08E-05	-	0.515	
	Atlanta	2.08E-05	-	0.532	
LC07	Istabraq	-1.38E-04	-	0.534	53.1 (0.0232)

Table 10-12 Model parameters for curves ($y = (a x^2) + (m x) + c$) for biomass production efficiency.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	-0.2956	4.73E-04	110.1	92.6 (4.82)
TT07	Istabraq	-0.2593	5.51E-04	94.7	83.6 (4.70)
LC07	Istabraq	-0.1179	6.34E-05	112.8	86.0 (6.36)

10.4.2 Chapter 5: Pre-anthesis growth phase

Table 10-13 Model parameters for curves ($y = (a x^2) + (m x) + c$) for fertile shoot density.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	1.218	-0.00233	420.3	43.0 (64.8)
	Atlanta	1.218	-0.00233	438.4	
	Claire	1.218	-0.00233	467.8	
	Savannah	1.218	-0.00233	431.5	
TT07	Istabraq	-	-	-	-
LC07	Istabraq	1.424	-0.00174	260.9	92.1 (32.0)

Table 10-14 Model parameters for curves ($y = (a x^2) + (m x) + c$) for green area index.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	0.01707	-3.01E-05	4.49	74.8 (0.532)
	Atlanta	0.01707	-3.01E-05	5.39	
	Claire	0.01707	-3.01E-05	4.97	
	Savannah	0.01707	-3.01E-05	4.80	
TT07	Istabraq	-	-	-	-
LC07	Istabraq	0.01711	-1.94E-05	1.83	92.7 (0.402)

Table 10-15 Model parameters for curves ($y = (a x^2) + (m x) + c$) for canopy N requirement.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	0.0904	-1.38E-04	23.99	75.7 (3.11)
	Atlanta	0.0904	-1.38E-04	20.42	
	Claire	0.0904	-1.38E-04	20.95	
	Savannah	0.0904	-1.38E-04	22.55	
TT07	Istabraq	0.0892	-1.75E-04	17.96	60.5 (4.39)
	Claire	0.0892	-1.75E-04	21.54	
	Savannah	0.0892	-1.75E-04	24.40	
	Atlanta	0.0892	-1.75E-04	25.17	
LC07	Istabraq	0.0966	-9.64E-05	25.23	90.3 (3.01)

Table 10-16 Model parameters for curves ($y = (a x^2) + (m x) + c$) for specific leaf N for all leaf layers.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	0.006453	-1.10E-05	1.604	72.3 (0.217)
	Atlanta	0.006453	-1.10E-05	1.314	
	Claire	0.006453	-1.10E-05	1.389	
	Savannah	0.006453	-1.10E-05	1.439	
TT07	Istabraq	0.007340	-1.63E-05	0.952	57.7 (0.333)
	Claire	0.007340	-1.63E-05	1.114	
	Savannah	0.007340	-1.63E-05	1.466	
	Atlanta	0.007340	-1.63E-05	1.434	
LC07	Istabraq	0.008032	-8.88E-06	1.534	90.6 (0.222)

Table 10-17 Model parameters for curves ($y = (a x^2) + (m x) + c$) for specific leaf N for individual leaf layers.

Exp.	Leaf layer	m	a	c	% variance accounted (SE)
TT06	Flag	0.007029	-1.14E-05	1.935	84.3 (0.254)
	L2	0.007029	-1.14E-05	1.422	
	L3&rem	0.007029	-1.14E-05	0.862	
TT07	Flag	0.008085	-1.91E-05	1.006	60.8 (0.234)
	L2	0.008085	-1.91E-05	1.058	
	L3&rem	0.008085	-1.91E-05	0.875	
LC07	Flag	0.008252	-9.14E-06	2.319	91.9 (0.267)
	L2	0.008252	-9.14E-06	1.778	
	L3&rem	0.008252	-9.14E-06	0.963	

Table 10-18 Model parameters for curves ($y = (a x^2) + (m x) + c$) for above-ground dry mass.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	0.02822	-6.37E-05	13.866	26.4 (1.87)
	Atlanta	0.02822	-6.37E-05	14.791	
	Claire	0.02822	-6.37E-05	13.247	
	Savannah	0.02822	-6.37E-05	13.520	
TT07	Istabraq	-	-	-	-
LC07	Istabraq	0.03265	-4.08E-05	6.907	81.2 (1.17)

Table 10-19 Model parameters for curves ($y = (a x^2) + (m x) + c$) for biomass production efficiency.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	-0.3151	5.08E-04	99.91	88.0 (6.53)
TT07	Istabraq	-0.2892	5.39E-04	86.64	89.9 (5.66)
LC07	Istabraq	-0.3334	4.30E-04	106.20	95.9 (5.00)

Table 10-20 Model parameters for curves ($y = (a x^2) + (m x) + c$) for N nutrition index.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	0.003829	-5.66E-06	0.561	90.5 (0.0765)
	Atlanta	0.003829	-5.66E-06	0.638	
	Claire	0.003829	-5.66E-06	0.570	
	Savannah	0.003829	-5.66E-06	0.573	
TT07	Istabraq	0.004046	-7.69E-06	0.578	88.4 (0.0835)
LC07	Istabraq	0.004304	-4.80E-06	0.359	97.5 (0.0597)

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Table 10-21 Model parameters for curves ($y = m x + c$) for N harvest index.

Exp.	Variety	m	a	c	% variance accounted (SE)
TT06	Istabraq	-	-	-	-
TT07	Istabraq	-	-	-	-
LC07	Istabraq	-3.63E-04	-	0.8481	88.7 (0.0235)