Impact of plants upon soil structural genesis and dynamics

By

AURELIE BACQ-LABREUIL

A thesis submitted to the University of Nottingham for a degree of

Doctor of Philosophy

School of Biosciences

In collaboration with: Rothamsted Research, Harpenden

October 2018

Abstract

Soils are a fundamental component of terrestrial ecosystems, and support a myriad of functions via interactions between their physical and biological properties, mediated by soil structure. Soil structure is dynamic, and modified by biotic factors including plant roots. The mechanisms involved include enmeshment, exudation and rhizodeposition of C-rich materials, which adhere soil particles and serve as substrate for microbes - which in turn can further structure the soil. The overall aim of this study was to determine the effects of vascular plants upon soil structural genesis. These interactions were determined in different contexts, using aggregate size-distribution profiling and X-ray Computed Tomography to visualise and quantify soil structure *in situ*. Using long-term field studies (50 y) on a sandy and a clay soil, it was shown that the degree of plant presence in a soil had substantial effects upon its structure. Perennial plants (grassland) significantly increased porosity, pore size diversity and pore connectivity, compared to bare fallow soil which decreased these characteristics. The addition of organic manure to an arable soil had essentially the same effect upon structure as grassland management, revealing the profound effect of addition of manure upon soil structure. Moreover, an initially bare-fallow soil apparently required at least 10 years since conversion to show a partial recovery of soil structure due to the presence of plants. Further investigation in controlled pot experiments revealed that contrasting soil textures (sandy *vs.* clayey) induced differential effects of plants upon soil structural genesis, both in terms of aggregate size distribution and *in situ* soil structural properties. Furthermore, it was found that different plant species growing in the same sandy-loam soil had differing effects on soil structural

genesis and microbial community phenotype, relatable to their contrasting root architectures. The key overall conclusion is that plant effects upon soil structure are context dependent, contingent upon the inherent cohesiveness of the soil texture, the plant species involved, and time. The concept of ‘optimal pore-architecture’ was developed from these observations of the differential impact of plants depending on soil texture: plant responses appear to be modulated by the initial state of soil structure and inherent soil properties.

Acknowledgments

First of all, I would like to thank my principal supervisor Prof. Karl Ritz who managed to deal with my franglish, taught me a lot of things about writing and science in general, and with whom I learnt so much about the mysteries of soil. I also would like to thank my other supervisors Prof. Sacha Mooney, Dr. Andy Neal and Prof. John Crawford for their advice, professional support, encouragement and guidance throughout my PhD.

I also would like to thank the team from Rothamsted: Elsy Akkari, Dr. Xiaoxian Zhang, Dr. Marc Redmile-Gordon, and Cormac McAuliffe for their assistance and the collaborative work. Plus thanks to the Hounsfield team Dr. Brian Atkinson and Dr. Craig Sturrock for their guidance and their teaching regarding the X-ray Computed Tomography facility, and especially to Brian who helped me to pack my columns, brainstorm about ideas, and with my English writing. I would like to express a special gratitude to Dr. Zhou Hu who trained me on QuantIm and gave me the possibility to understand the complexity of soil structure. I also would like to express my gratitude to the technical staff who help me to make this project possible: Jim Craigon for his statistical wisdom, John Corrie, Saul Vazquez Reina for their support in Gateway's laboratory, Stephanie Ellis for the PLFA training, Andrew Jackson, Julietta Marquez for their induction to plant science, Dr. Alison Tidy for her microscope training, and the IT team, especially Chris Woodrow for his help with setting up QuantIm.

I would like to also acknowledge the financial support from University of Nottingham (School of Biosciences) which allowed me to carry out this research; and the financial support of British Society of Soil Science to enable attendance at regional and national meetings and international conferences, where I gained a lot of knowledge and contacts.

Profound thanks also to my PhD friends Jasmine Burr-Hersey and Sebastian Blunk who were always there to support me, help me to improve my writing and presentation skills, and with whom we brainstormed a lot and shared so many ideas. You have made my experience so much better. I also would like to thank my partner and friends who always supported and encouraged me, and everyone who had contributed to help me arrive where I am today. Thank you to the team of Agri-Esprit, who were the first people to tell me that I should consider to do a PhD.

Finally last and but not least, I would like to express my profound gratitude to my parents and my brother who have always supported me in my life choices even through very difficult moments.

Table of contents

List of figures

Chapter 1: General introduction

1.1. Introduction

Soils are arguably the most complex and fundamental ecosystems on Earth. They are essential to support the myriad of functions they deliver, including: water provision, purification and storage; providing food, fibres and fuel; climate regulation; nutrient cycling; carbon sequestrations; habitat for soil organisms, etc. (Fig. 1.1.; Rabot *et al.*, 2018). These functions are in essence provided by the system by virtue of the structural and biological properties of soils. Heterogeneous arrangements of aggregates and voids (called pores) define the physical soil structure. The different arrangements of the aggregates depend on the inherent texture of the soil and impact the size distribution of the pore system (Dexter 2004).

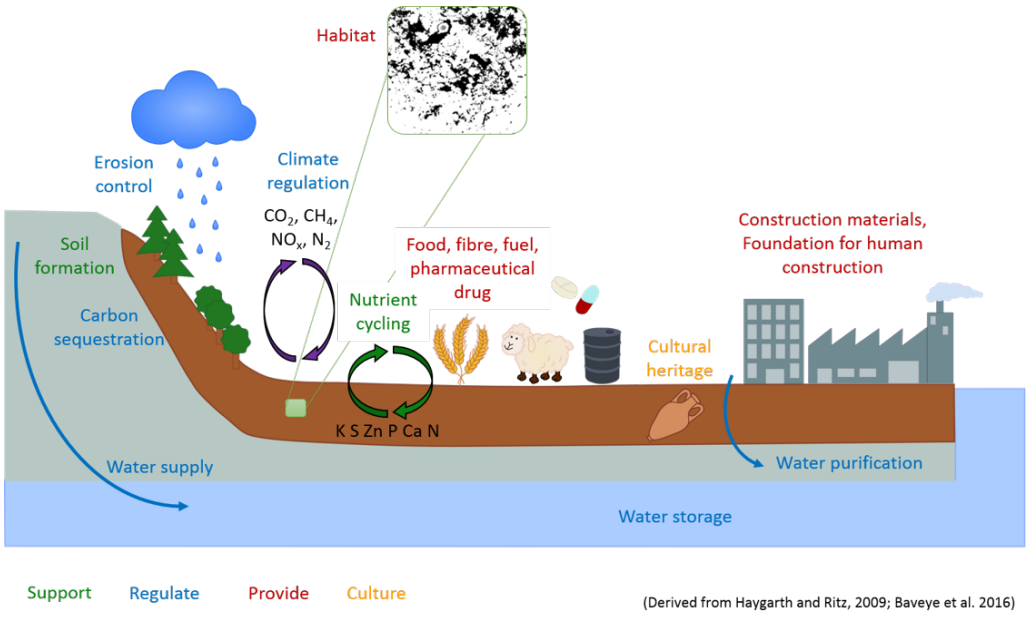


Figure 1.1.: Fundamental functions provided by the soil system, figure derived from Baveye et al. (2016) and Haygarth and Ritz (2009)

Aggregates are formed from different proportions of primary particles (classified as sand, silt, clay depending on their size, from largest to smallest), which profoundly impacts the characteristics of a soil. For example, a soil with a greater proportion of sand grains is classified as coarse soil, in comparison, soil containing a greater proportion of silt and clay is classified as finer soil (Dexter 2004). The increasing proportion of clay and silt particles generally increases the proportion of fine pores within the aggregates (van Breemen 1993).

Clay particles play a critical role in the process of aggregation due to their electrical charges (Tisdall and Oades 1982; Oades 1984; Dexter 1988). Clay particles form persistent bonds with soil organic matter via cation bridges with di- and trivalent metal cations present in the soil (Tisdall and Oades, 1982). The main abiotic agents in the formation and stabilisation of aggregates are clay content and organic matter (Tisdall and Oades 1982; Le Bissonnais 1996; Chenu *et al.* 2000; Bronick and Lal 2005). The proportion of clay in the soil drives abiotic aggregation. A soil with a larger proportion of sand and a low proportion of clay remains loose and unaggregated, compared to a soil with a greater proportion of clay which has a greater aggregation (Blake *et al.* 2003). Moreover, the ability of clay particles to shrink and swell augments structural development and resilience of the soil after wet and dry cycles (Shepherd and Walsh 2002).

Soil structure is also defined by its pore system characteristics. The gross porosity is the ratio of the air-space volume by the total volume of the system. Soil porosity is important in terms of water infiltration, nutrient cycles and root penetration (Carter 2001; Bronick and Lal 2005; Whalley *et al.* 2005). The

pore size distribution and pore-connectivity are important also to regulate water retention and flow, organic matter decomposition and root growth. Especially, pore sizes between 0.2 to 30 μm are critical for the water retention (Tisdall and Oades 1982), and pore sizes larger than 30 μm are critical for the aeration of the soil (Dexter 1988).

Furthermore, soil structure is dynamic and sensitive to modifications by natural (such as plants and organisms, wet and dry cycles, etc.) and anthropogenic (such as tillage) factors. In this chapter, the action of living organisms and plants upon soil structural dynamics is rehearsed and developed into a larger concept.

1.2. Effects of biotic activity on genesis and dynamics of soil structure

The biota is a key factor in the genesis and dynamics of soil structure. Soil structural modification requires actions mediated by the organisms and plants, which themselves require energy. The biotic activity drives the structural genesis of the soil via different functions: movement of soil particles and organic matter, soil particles enmeshment and production of extracellular polymeric substances (EPS). The secretion of EPS binds the soil particles together and also with the organisms secreting (e.g. plants, fungi and bacteria) which create a micro-environment in their vicinity. The different organisms contained within the soil play one or many of these functions and contribute to the genesis of structure.

The soil is a dynamic system, continuously modified by the biota. The modification of soil structure is possible via the interaction of the ‘ecosystem engineers’ typically macro- and meso-fauna. The modes of action here are by ingestion the soil and organic materials, and its subsequent excretion (e.g.

earthworms, mites) or moving the soil particles outside of their bodies during burrowing (e.g. earthworms, mammals, birds). The physical protection of smaller organisms is modified which can decrease the access to the substrate which includes prey in the case of predators. There are considered to be two types of ecosystem engineers: (i) 'extended phenotype engineers' (Jones *et al.* 1994, 1997), organisms which create structures that directly affect the fitness of individuals or the colony; and (ii) 'accidental engineers', which create biogenic structures that have no direct effect on them (Jouquet *et al.* 2006).

1.2.1. Macro-organisms

1.2.1.1. Soft-bodied invertebrates - Earthworms

Earthworms are considered as the main group of soil engineers in temperate soils (Lavelle 2001), and are commonly considered as accidental engineers. Earthworms are classified by their ecological roles from a functional perspective (Lee 1985; Lavelle *et al.* 1987): (i) epigeic: live in the surface-litter layers and feed on associated organic matter; (ii) endogeic: live predominantly belowground and ingest the breadth of soil materials as they burrow through the matrix (Edwards and Lofty 1977); (iii) anecic: produce vertical burrows from below ground to the soil surface, feeding on surface-litter and incorporating in the soil system via vertical burrows (Felten and Emmerling 2009).

Due to their ecological roles, the different categories of earthworm impact soil structure differently. For example, anecic earthworms create large-size vertical macropores (Capowiez *et al.* 2015). The presence of anecic earthworms thus typically increases water infiltration within the soil (Jouquet *et al.* 2012). Such

worms are sensitive to soil disturbance. After a ploughing event, the presence of anecic earthworms decreased and the presence of endogenic earthworms is increased in a tilled plot and the volume burrows were at the same level, after 5 months (Pelosi *et al.* 2017). Therefore, in a conventional tillage-based agricultural system, the endogenic earthworms would likely be the predominant group and in a no-till system, it would be the anecic earthworms. Moreover, the burrows facilitate gas transport from the surface through the soil. To create their burrows, anecic earthworms ingest the soil matrix and the surface-litter. The formation of the organic matter is done by the intimate mixing of the surface-litter with the soil matrix in the earthworm gut (Brown *et al.* 2000). Earthworms regulate soil organic matter across four scales of time and space (Lavelle 1997; Jouquet *et al.* 2006). First scale, short term digestion-association processes (hours to few days) is the digestion of the soil organic matter in the gut of invertebrates or around the earthworms by the microbial community. Second scale, the intermediate phase creating fresh biogenic structure (few days to weeks): the microbial activation occurs during the gut transit or the mechanical mixing of the organo-mineral materials. Third scale, longer term of stabilized biogenic structures (months to years) are formed by the incorporation of organic matter into the cast leading to an increase of the aggregate sizes within the cast edges which stabilised the biogenic structures and also by the high density vegetation and litter cover which prevent the destruction of the biogenic structures (Decaëns 2000). The biogenic structures are highly compact structures and are the main components of the macro-aggregates which determine soil hydraulic properties and erosion resistance (Blanchart *et al.* 1999; Chauvel *et al.* 1999). And finally, the fourth scale, soil

profiles (years to centuries): formation of the soil horizon by combining of the biogenic structures with other structures.

Therefore, due to their activity, earthworms are highly involved in the creation of soil macro and micro-porosity, the incorporation of organic matter, nutrient cycle and the water infiltration (Ehlers 1975; Shipitalo and Bayon Le 2004; Bottinelli *et al.* 2010; Fischer *et al.* 2014).

1.2.1.2. Hard-bodied invertebrates – ants

Ants are considered as extended phenotype engineers, as they modify soil structure to distinctly construct nests and food storage (Lavelle *et al.* 1992; Paton *et al.* 1995; Lavelle 1997), but ants are not as studied as the earthworms (Bottinelli *et al.* 2015). To develop the nest, ants transport soil from the belowground to the soil surface (Richards 2009; Bartlett and Ritz 2011). The construction of biogenic structures leads to colony development by constructing a defending location for the queen and her broods, to build food storage and reduce the problems link to weather variation (Sudd and Franks 1987; Jouquet *et al.* 2006).

The modification of the soil structure by ants create new aggregates and pore systems which increase the water infiltration, the storage of nutrient and water. The foraging activity increases the porosity within the nest, therefore, reduces the bulk density of the location of their nest (Cammeraat and Risch 2008; Jouquet *et al.* 2011). The biopores greater than 1 mm are known to increase the aeration, the drainage and also the other organisms within the soil (Oades 1984), which is similar to the properties of the ant channels (Lobry de Bruyn 1999). In case of a compacted soil, the presence of ants would be important for

the decompaction of the soil: as the ants move soil aggregates or sand grains to construct nests (Hole 1981) and decrease the porosity at the location of the nest. Ants incorporate as well mineral and plant materials, food remains and excreta into their construction bound together by saliva (Jouquet *et al.* 2006). The capacity to add decomposed organic matter to the nest is increasing the nutrient richness of the location, which could increase the growth of plant roots or micro-organisms in the vicinity of the nests. The resilience of a soil is the capacity of a soil system to recover from a destructive event; therefore, ants could be important for the resilience of compacted soil. Moreover, ant nests can be unstable, not long-lived and not compacted (Lobry de Bruyn 1999), so only after few months, the ants can change their location and leave the nest structure behind leading to a dispersal of the nutrient. This nutrient enrichment of the nest location can enhance microbial community and plant development. Moreover, ants are also described as seed-dispersal agent by moving seeds into their nest during the construction (Richards 2009). Seeds can benefit from the enrich-environment of the nest, to germinate and have resources to develop (see section 1.4). The impact of ant nests on water infiltration is uncertain, some studies stated that nests increased the water infiltration where other shown the opposite (discussed in Lobry de Bruyn, 1999).

1.2.2. Micro-organisms

1.2.2.1. Fungi

Fungal hyphae grow through fissures and pores and entangling the mass of clay, forming aggregates at a lower scale than roots (Fig. 1.2.; Tisdall and Oades 1982; Dorioz *et al.* 1993). Fungi are able to move clay particle and

orient clay particles around their hyphae (Dorioz *et al.* 1993). Fungal hyphae have a greater spatial impact than the bacteria and are involved in stabilisation of macro-aggregates (>250 µm; Chenu and Cosentino, 2011; Miller and Jastrow, 1990) and especially the water-stable micro-aggregates (Caesar-TonThat and Cochran 2000; Bossuyt *et al.* 2001; Helfrich *et al.* 2008). Saprophytic fungi also increase the aggregation process via the secretion of insoluble extracellular polysaccharides acting as a binding agent. These substances are heat resistant suggesting that they are not composed of large proteins (Caesar-TonThat and Cochran 2000).

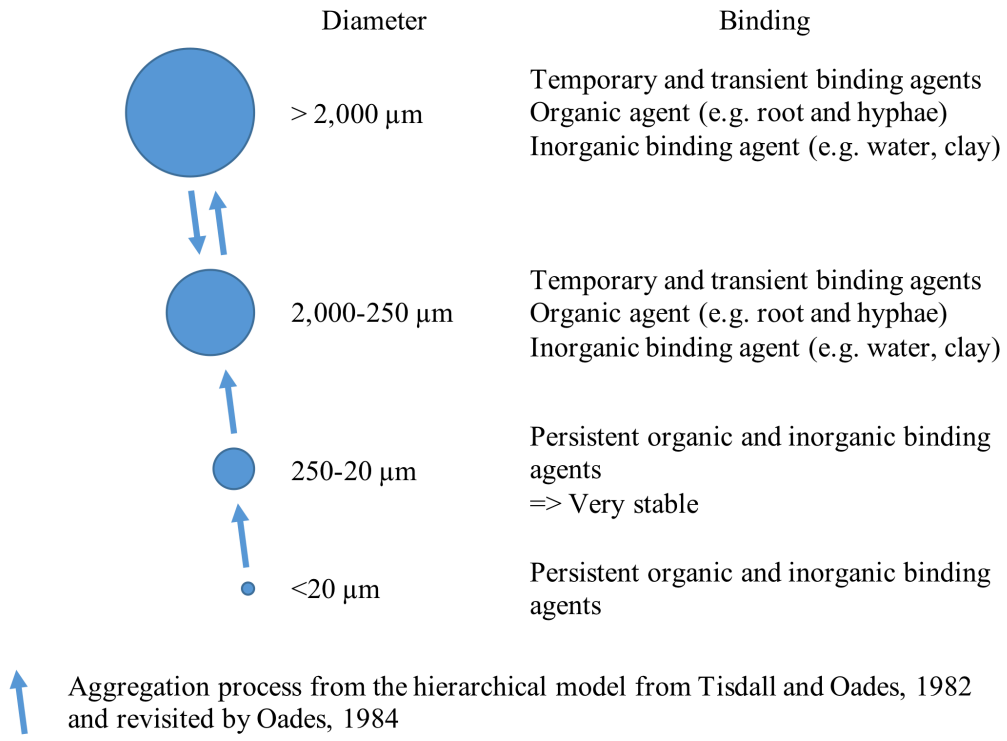


Figure 1.2.: Conceptualisation of the hierarchical model of soil aggregate formation as proposed by Tisdall and Oades (1982) and Oades (1984)

Arbuscular mycorrhizal (AM) fungi represents 20 – 30% of the microbial biomass in a grassland (Miller and Fitzsimons 2011). AM fungi form symbiotic associations with the majority of species of land plants (Fitter *et al.* 2000),

using the carbon produced by the plants and in return, exchanging nutrients with the roots. Fungi have the ability to translocate carbon and nutrient to enhance apical growth of fungal hyphae, which allow them to forage beyond pores between aggregates, to link soil regions with differing nutrient content, and to forage for nutrients while exploiting resources (Miller and Fitzsimons 2011).

In the rhizosphere soil, the presence of fungi increased significantly over 20 days, leading to an increase of the production of hydrophobic substances and porosity of the soil (Feeney *et al.* 2006). Fungi produce hydrophobic substances to insulate their hyphae and minimize desiccation (Wright and Upadhyaya 1998; Ritz and Young 2004). This production of hydrophobic substances binds soil particles, which enhanced aggregate stability via influencing the ability of the soil to shrink and swell (Feeney *et al.* 2006). A greater aggregate stability reduces the risk of erosion during rainfall events. Another study showed similar results, except the porosity decreased in the presence of fungi, but the aggregate stability was increased by fungal presence (Martin *et al.* 2012). Martin *et al.* (2012) also showed that the pore size distribution was modified: via a larger proportion of smaller pore sizes in the presence of fungi, which corroborates another study (Daynes *et al.* 2013). For both studies, the bulk density was different, Feeney *et al.* (2006) study worked with a soil at 1.3 g cm^{-3} compared to Martin *et al.* (2012) study working with a soil at 1.1 g cm^{-3} bulk density. These differences observed by the two authors might be a result of the bulk density variation between their experiments (see section 1.3.2 soil structure affects on fungi).

The essential role of the fungal hyphae for the stabilisation of macro-aggregate leads to the classification of the fungi as a part of the extended composite phenotype. Fungi are producing hydrophobic and EPS, to protect themselves and the surrounding neighbours against external factors (such as drought, rainfall). By producing and developing through the soil matrix, their hyphae impact the soil structure, thus their surrounding environments.

1.2.2.2. Bacteria

In comparison to the impact of fungi upon soil structure, the impact of bacteria on the soil structure is more spatially limited to their surroundings. Bacteria produce EPS to protect themselves against wet and drying cycles, starvation, toxicity, predators and attach on the surrounding particles (such as clay, sand grain) and also to organic matter to create microenvironments (Haynes *et al.* 1991; Dorioz *et al.* 1993; Holden 2011). The penetration of EPS is limited to the immediate environment of the bacteria (approximately a distance less than 1 µm), but the presence of bacteria enhances the formation of micro-aggregate (2 – 20 µm; Dorioz *et al.*, 1993; Tisdall and Oades, 1982). Bacteria are able, as the fungi, to realign clay particles in their surroundings via the secretion of the extra polysaccharides (Chenu and Stotzky 2002; Chenu and Cosentino 2011). The extra polysaccharides can be hydrophobic as well as hydrophilic substances which confers different functions for the microbes (Or *et al.* 2007; Chenu and Cosentino 2011). The hydrophilic substances hold water in their structure, thus in a drought event, microbes have still access to water supplies. At the microscale, the bacteria can be considered as a part of the extended composite phenotype, because of their extra polysaccharide productions which

bind the soil particles together. However, this production is stabilising micro-aggregates only.

1.2.3. Plant roots

Plant roots can be considered as 'ecosystem engineers' for restoring degraded plant communities (reviewed in Cameron, 2010), however, this concept can be extended to soil structure as well. Soil is the fundamental growth medium for plant roots to develop, to uptake water and nutrients needed to grow. Roots are also important for the physical stability of the plant by providing an anchor to a depth of few centimetre to several metre (van Breemen 1993). The actions of the root can be classified into five classes: (i) root penetration, (ii) hydraulic modification, (iii) rhizodeposition, (iv) root decomposition, (v) physical entanglement of soil particles (Six *et al.* 2004).

Plant roots growing in the soil via existing pores or by making new channels by elongating and pushing with their tip the soil matrix (Jin *et al.* 2013). During growth roots impact their surroundings in different ways. Plants enhanced the water drainage in the rhizosphere which might be a consequence of the modification of the pore connectivity in the rhizosphere (Whalley *et al.* 2005). Therefore, plant roots have a direct impact on soil structure in their immediate vicinity. For example, a study showed that plants growing in a sandy loam soil decreased the porosity in the rhizosphere compared to the one growing in the clay loam soil which increased the porosity (Helliwell *et al.* 2017). Plants are able to increase the porosity in a clay loam by extracting water from the rhizosphere which leads to a shrinkage of the clay particle and therefore increase the porosity (Reid and Goss 1982; Six *et al.* 2004).

The modification of the surrounding area of the root via physical entanglement of soil particles and aggregates tends to create a specific environment surrounding the root vicinity (approximately 50 – 200 μm; Dorioz *et al.*, 1993). By enmeshing the soil particles, plant roots enhance the stability of the soil aggregates, which is also reinforced by the exudation of mucilage (extra polysaccharide; Fig. 1.2.; Chenu and Cosentino, 2011; Morel *et al.*, 1991; Tisdall and Oades, 1982). Furthermore, mucilage is responsible for the increased affinity of the rhizosphere soil for water (Whalley *et al.* 2005): via reducing the surface tension of the water which facilitates the water and nutrient uptakes from smaller pores than would be inaccessible to the roots (Read *et al.* 2003). The increase of soil cover, which leads to a greater aggregate stability, improves the soil resistance to the risk of erosion during rainfall events, run-off rates, salinity, and the nitrate leaching while key nutrients are restored (Altieri 1999; Whitmore and Schröder 2007; Wang *et al.* 2011; George *et al.* 2012).

Plant roots via their growth modify the soil structure in their vicinity by enmeshing soil particles and by producing soil mucilage which modify the inherent properties of the surrounded soil. Therefore, plants can be seen as a component of the extended composite phenotype and playing a role in the formation of macro-aggregate (>2 000 μm), and macro-environment in their vicinity (Fig. 1.2.; Tisdall and Oades 1982). This optimisation of their surrounding environment leads to a better access to the water and nutrient supply to plants but also to the microbial community associated with plants. Furthermore, in natural systems, plants are the main actors for carbon input (and hence energy) into the soil. This carbon input is implemented by the roots

via excretion of mucilage, cell sloughing and eventual death and decomposition. Faunal grazing of roots also contributes. The carbon released in the soil by plants is used by the microbial community and macro-organisms to function, thus plants are key factors for the input of energy within the soil systems.

1.3. Effects of soil structure on biotic activity

1.3.1. Macro-organisms

Soil structure can be altered by the compaction of the soil for example which can have a detrimental impact on the soil living organism (Batey 2009). Earthworms are affected by the compaction of the soil visible by the reduced number of burrows and organisms within the soil profile (Capowiez *et al.* 2012; Müller-Inkmann *et al.* 2013; Bottinelli *et al.* 2015; Pelosi *et al.* 2017). However, earthworms are rarely present in soil that has either very fine or very coarse particles (Lee 1985). Therefore, the textural characteristics influences the presence of earthworms.

Other species can be affected by the loss of structure due their ability to move through the soil profile via the existing pore network. The loss of porosity and maybe pore sizes can lead to a decrease of the population for these species. For example, arthropod populations are sensitive to the loss of soil structure (via a decrease of aggregate stability and compaction), by the decrease of the population number after a compaction events in both laboratory and field conditions (Whalley *et al.* 1995; Sapkota *et al.* 2012; van Klink *et al.* 2015). This reduction might be due because the movements of micro-arthropods are limited by the existing pore space (Lee and Foster 1991). Therefore, following

a compaction event, the meso- and macro-pores can be lost, reducing the movement of the micro-arthropods through the pore systems.

1.3.2. Micro-organisms

1.3.2.1. Fungi

Soil properties can have impact on fungal growth. For example, a study showed that one species of fungi was sensitive to the bulk density with the optimal growth for the bulk density of 1.4 gm cm^{-3} (Harris *et al.* 2003). The increased surface of the soil matrix at this bulk density promoted the best fungal development, postulated to be due to a greater access to nutrient and water present at the surface of the surrounding of soil particles. However, at a greater bulk density the fungal development was limited. Fungi are aerobic organisms, the presence of oxygen is necessary for its growth, thus in an environment with reduced air-filled porosity, fungal growth is decreased (Ritz and Young 2004).

Moreover, Feeney *et al.* (2006) showed that the presence of fungi increased the porosity, and Martin *et al.* (2012) contradicted this results by observing a decrease of the porosity (see section 1.2.2). Both studies had a similar soil texture (sandy loam) but had different bulk densities, 1.3 gm cm^{-3} and 1.1 gm cm^{-3}, respectively (Feeney *et al.* 2006; Martin *et al.* 2012). For the lower bulk density, the presence of fungi decreased the porosity while a greater bulk density fungi increased the porosity. Thus, depending on the initial state of pore structure, the impact of the fungi was different.

Furthermore, the distribution of the pore sizes and the porosity of the soil play a role in water retention (see section 1.1), for example, a greater proportion of

small pores (approximately 0.2 – 30 μm) is critical for water retention (Tisdall and Oades 1982; Dexter 1988; Bronick and Lal 2005). Martin *et al.* (2012) observed that the presence of fungi increased the proportion of the smaller pores, which might be involved in the retention of water. Thus, a greater retention of water might be beneficial for living organisms especially in low bulk density or coarse soil where water flow through the soil profile freely. This might enhance the fitness of fungi but also other organisms within the soil (such as plants, bacteria).

1.3.2.2. Bacteria

The composition of the soil texture impacts the survival of the bacteria especially in drought events, especially the clay content. Clay minerals can retain water between the particles due to charges of clay particles. This water might be partially accessible by bacteria through the EPS produced by the bacteria, which can improve the survival of the bacteria in case of a drought event (Chenu and Stotzky 2002). In sand, the production of EPS by *Pseudomonas* increased in response to desiccation (Roberson and Firestone 1992). The absence of clay minerals to store water might have triggered the response of the bacterial community via the increase of production of EPS in order to protect the colonies, which decreased the drying rate of the sand. Moreover, the spatial heterogeneity of the resources invokes a heterogeneous spatial distribution of the bacteria within the soil (Ettema and Wardle 2002). This spatial heterogeneity of the resources is due to the soil structure, and the modifications induced by the soil organisms (explained in section 1.2). The porosity, pore connectivity and pore size diversity can control the accessibility

of sources for the different organisms. For example, in a very porous and connected soil, soil organisms might have access to different sources which might be accessible physically i.e. moving toward the sources or via diffusion of nutrients through water film. In comparison, in a low porous and connected pore network, the accessibility of the resources might be limited due to the difficulty of diffusion, and sources that can be physically removed and unaccessible. Therefore, accessibility of sources is dependent on the inherent soil structure.

Furthermore, the loss of carbon inputs impacts on the microbial community evolution. Long-term experiments revealed that in bare fallow soil, the organic carbon content, the soil nitrogen and the proportion of fungal community was decreased drastically compared to the grassland treatment after more than 50 years under the same management (Hirsch *et al.* 2009). From this experiment, there was no modification of the bacterial community structure amongst the three treatments: bare fallow, arable and grassland (Neal *et al.* 2017). However, the genotype for gene coding specific functions was modified between the treatments. This study revealed that in the bare fallow soil, there was a greater proportion of exo-enzyme and outer-membrane enzyme present in the genotype of the bacterial community for the gene coding for the assimilation of phosphate (Neal *et al.* 2017). This difference in proportion of gene coding for different functions was related to the carbon content of the soil and the pH. Soil structure in this case was not reported, but it could be hypothesised that the soil structure from the bare fallow is different from the grassland which can be one of the factors that modifies the genotype profile.

1.3.3. Plant roots

The alteration of the soil structure impacts the development of plants. Soil strength limits root elongations much more than water, i.e. with the increase of soil strength, root elongation is reduced (Jin *et al.* 2013). Roots penetrate and exploit strong soil but it costs them a lot of energy, therefore limiting their growth, and hence crop-yield (Masle and Passioura 1987; Whalley *et al.* 1995; Bengough *et al.* 2006). Morphologically, plant roots growing through a compacted layer of soil have a wider diameter compared to plant that grown in a less compacted soil (Materechera *et al.* 1992; Lipiec *et al.* 2003; Chen and Weil 2010; Alameda and Villar 2012; Tracy *et al.* 2012). However, the presence of a coarse soil representing a low soil strength, roots are thicker compared to roots that grow into finer soil (Helliwell *et al.* 2017). Moreover, in coarse soil, roots grow deeper in order to reach more water as coarse soil are usually free drain i.e. there is a low retention of water in the upper layer of the soil, and roots require to grow deeper to reach water sources (Blake *et al.* 2003). The nature of the soil modifies also the water retention of the soil. If the soil is too wet, the growth of the root is limited due to insufficient oxygen diffusion to the root tip resulting in hypoxia (Blackwell and Wells 1983).
The strength of the soil impacts the root growth and also the development of lateral roots. A growth strategy of lateral roots in a clay soil had been shown by Valentine *et al.* (2012): laterals proliferate through existing pore channels in their quest for water and nutrients. This observation has been confirmed by Helliwell *et al.* (2017), who showed that lateral roots in clay are initiated at a site with a decreased porosity. Also, plant roots growing in a sandy loam soil exhibited a thicker primary root with less secondary roots than plants growing

in a clay loam (Helliwell *et al.* 2017). Moreover, plants, which grow against the edge of an air-filled gap, develop laterals that grow preferentially through the soil profile than across the air-filled gap (Morris *et al.* 2017). Thus, root growth strategies are dependent on the surrounding environments, i.e. the soil structure, the presence or absence of available nutrient and water. Access to water and nutrients by plants requires physical contact between plants, water and nutrients. The sources of nutrient and water are usually present at the surface of the pore via water-film. Therefore, the access of resources is limited to roots growing across air-filled pores compare to roots that grow on the surface of pores. Laterals are developed to access a larger amount of sources throughout the soil profile. Therefore, their root growth strategies are to grow toward potential sources without consuming a lot of energy which would be required to grow, for example, across an air-filled gap or through a compacted layer (compacted and absence of soil; Helliwell *et al.*, 2017; Morris *et al.*, 2017; Valentine *et al.*, 2012).

1.4. Self-organisation of the soil system

1.4.1. Concept of self-organisation

The definition of a self-organised system is defined as a closed system that spontaneously modifies itself via endogenous effects without any effect of exogenous impacts (Young and Crawford 2004; Crawford *et al.* 2012; Lavelle *et al.* 2016). In the context of soil systems, the endogenous factors encompass soil properties and soil biota (Fig. 1.3.). The soil properties can be defined by soil structure, texture, bulk density, soil chemistry, water and organic matter content and all the characteristics form the initial state of soil properties. Soil biota (including macro- and micro-organisms and plant) interact with the soil

properties (see sections 1.2 and 1.3) which is responsible for the notional self-organisation property of the soil system (Crawford *et al.* 2012; Lavelle *et al.* 2016). These interactions lead to a modification of the soil system which impacts the different soil functions including nutrient cycling, organic matter regulation and water/gas diffusion (Fig. 1.3.). External factors, such as human impacts, weather conditions (rainfall, wind, temperature), and light exposure (i.e. day and night hours), can impact on the soil system but are not included in the concept of self-organisation (Fig. 1.3.).

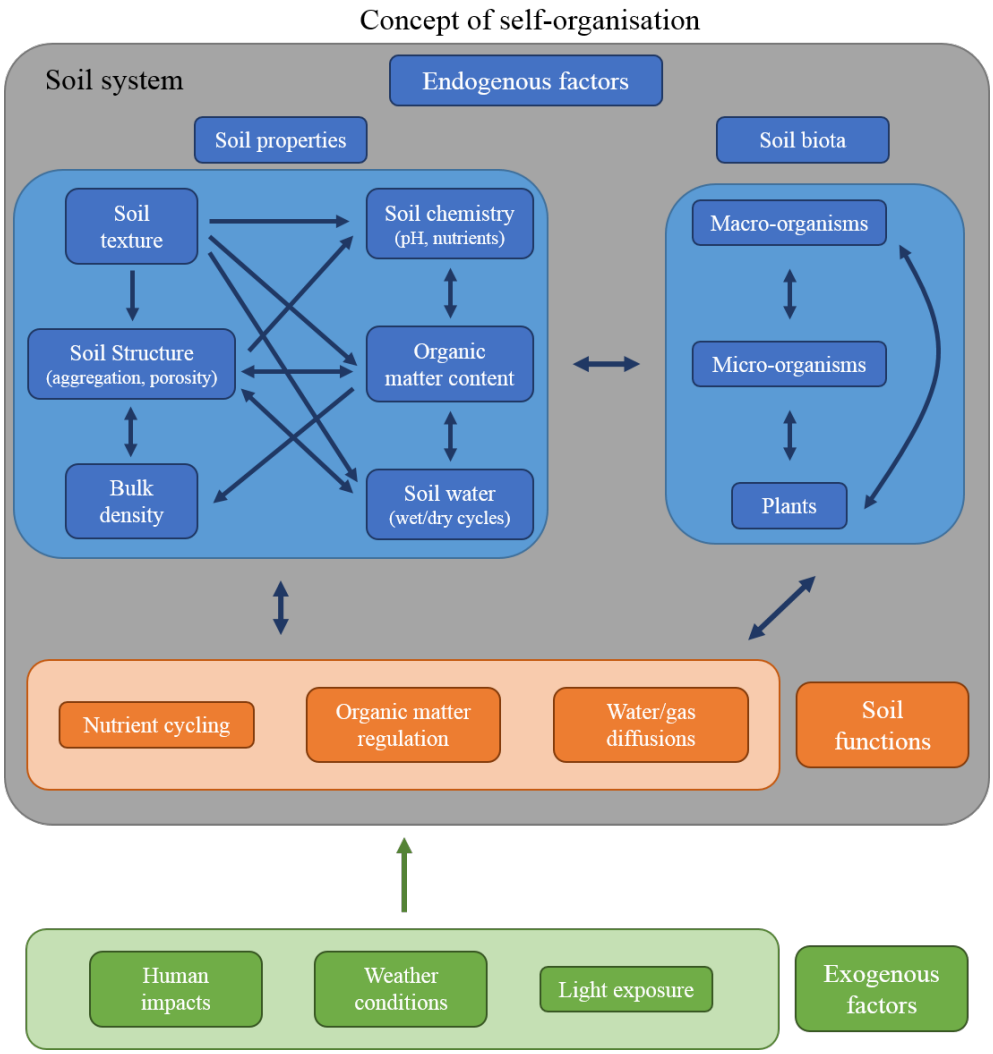


Figure 1.3.: Simplified concept of self-organisation within the soil system with the endogenous factors composing the soil system and their impacts on soil functions and potential exogenous factors that can disturb the soil system.

1.4.2. Self-organisation in the context of soil

The active actors of the self-organisation in soil are the biota - without life, the soil system is not able to modify itself spontaneously (Young and Crawford 2004; Crawford *et al.* 2012; Lavelle *et al.* 2016). In section 1.2, the effect of biota upon soil structural genesis or dynamics can be seen as 'feed-forward' process, in that biota activity usually induce the modification of soil properties which is fundamental for the spontaneous changes in soil characteristics. On the other hand, the effect of soil properties upon biota can be associated as 'feed-back' processes due to the different adaptation to the biota to the soil properties (see section 1.3).

Moreover, the biota provide energy to the soil system which enables it to be dynamic. Plants are a fundamental actor that provide energy via the translocation of carbon produced by photosynthesis from leaves to roots and then to the soil. Plants debris as well (such as leaves, shoot, and roots) can be used by the living organisms as energy. These different types of carbon are used by the living organisms to function within the soil profile. Thus, plants have a fundamental impact on soil environment (see section 1.2.3), and on rhizosphere organisms, due to their functions: development of roots to anchor in soil (van Breemen 1993) and uptake of water and nutrients (Gish and Jury 1983; Clode *et al.* 2009; Bao *et al.* 2014). This impact of plants upon soil properties can however be of significance to the functioning of other soil organisms (see section 1.2). The modification of the surrounding environment results usually from a specific function possessed by a species which is beneficial for its fitness, but has knock-on effects on the fitness of other community members, in turn affecting the overall environment in which all

organisms are operating. This is essentially the concept of the ‘extended phenotype’ (Dawkins 1982). The extended phenotype is defined as the species behaviour or function, that is beneficial to its fitness, and which has a direct or indirect impact on the surrounding ecosystem. This concept was expanded to the soil community because within the soil population, multiple organisms impact the soil properties which impact the entire community (see sections 1.2.1, 1.2.2.1 and 1.2.3). This is called ‘the extended composite phenotype’ encompassing the role of multiple organisms impacting the same environment (Phillips 2009, 2016). In this concept, the organism sizes range from the microbes to macro-organisms with the impact not proportional to the size of the organisms (Phillips 2016), which create a multi-scale interaction within the soil profile between the different organisms (Young and Crawford 2004; Crawford *et al.* 2012; Lavelle *et al.* 2016).

Interactions between macro-organisms (e.g. earthworms), plant roots and the micro-organisms with the soil structure are important to understand the uneven distribution of the organisms and the carbon within soil. The spatial distribution of the organisms leads to a spatial heterogeneity of the resources and creates micro-habitats (Ettema and Wardle 2002). The distribution of the micro-habitats modifies the soil structure in the vicinity of the community. The modification of the soil structure leads also to a modification of soil processes, for example, water infiltration and carbon sequestration or decomposition, discussed in the next section.

1.4.3. Consequences of the self-organisation upon soil functions

The modification of habitats within the soil profile can have drastic consequences upon soil functions. Indeed, the biota impact the soil structure i.e. modify pore architectures which affect soil functions. Here, two examples considered, water movement and carbon cycling.

1.4.3.1. Water transport

Water infiltration is governed by the inherent soil structure, essentially at the soil surface. Preferential pathways enhance the rate of water infiltration by promoting a pathway which bypasses a large area of the pore networks. This mechanism is referred as preferential flow (Hendrickx and Flury 2001). The orientation and the distribution of the macropores within the soil impact the water flow and retention of the system. Especially, the vertical orientation and well-connected macropores induce a greater water infiltration through the pore network leading to a high degree of preferential flow (Luo *et al.* 2010), notably induced by the presence of earthworms from anecic species (Fischer *et al.* 2014). Therefore, the presence of earthworms can have a significant impact on the water infiltration through the soil profile. Furthermore, the presence of plants impacts the distribution of pores via the different root strategies depending on the root architecture. Hydraulic conductivity is modified according to the nature of the root architecture influencing the soil structure. For example, tap rooted species increase the compaction around their vicinity (Burr-Hersey *et al.* 2017), which reduces the diffusion of water from the surrounding soil to the roots. However, more fibrous rooted species had a greater porosity and diversity of pore sizes (Burr-Hersey *et al.* 2017; Helliwell

et al. 2017) enhancing water advection. Fischer *et al.* (2014) showed that plant functional groups can impact hydraulic conductivity differently, e.g. legumes increased but grasses decreased the infiltration capacity of a field.

Therefore, the modification of the water dynamic within the soil profile is a consequence of the self-organisation, due to the differential impact of organism (including earthworms, plant species) upon soil structural dynamics.

1.4.3.2. Carbon sequestration vs decomposition

Organic matter (OM) originates from the decomposition of plant materials and the dead organisms in the soil. OM is source and sink for plant nutrients and provides energy to the micro-organisms (Carter 2001). OM is present in the soil under different forms (Carter 2001; Gaunt *et al.* 2001): (i) organo-mineral, OM associated with soil mineral (such as clay and silt); (ii) intra-aggregate, OM incorporated by micro- and macro-aggregates; (iii) free OM; and (iv) soluble OM.

The decomposition state depends on abiotic and biotic factors. The pore size distribution is fundamental in the decomposition of OM: a greater presence of pores and pore-connectivity lead to greater decomposition rate of the intra-aggregate OM (Carter 2001; Kravchenko *et al.* 2015). Pore size diversity can be regulated by the presence of biota (see section 1.2). For example, the creation of new pore sizes and new connections between pores can enhance the decomposition of intra-aggregate OM (Carter 2001; Kravchenko *et al.* 2015). Furthermore, the adsorption of OM to mineral (such as clay and silt) renders OM inaccessible for decomposition for both, shorter and longer time periods (Carter 2001; Kristiansen *et al.* 2006). The availability of the OM within soil

profile is dependent on endogenous factors: soil texture (especially clay content) and hydraulic properties; and exogenous factor: climate (Carter 2001; Shepherd and Walsh 2002). The modification of the soil structure by the biota might also enhance the OM decomposition via opening new connection and physically move the OM or the decomposers to the source. This implies the presence of organisms capable of displacing soil particles (such as earthworms) or creating new pores (such as plants and fungi).

Therefore, the capacity of the soil system to sequester or to decompose carbon relies on the activity of the soil biota, thus it is a consequence of the self-organisation of soil system.

1.5. Research focus, aims and hypotheses

1.5.1. Research focus

The concept of self-organisation of the soil system is complex, and a simple 'proof' of it is intractable. However, it is fundamentally underwritten by the concept that soil organisms generate soil structure (genesis) and modulate such structure by their actions (dynamics) (Fig. 1.3. simplified concept of self-organisation). This is the primary focus of this study, and specifically the research focuses on the effects of plants upon soil structure (Fig. 1.4.). This encompasses interactions between soil texture, structure, plant roots and the microbial community. Plants were chosen because they are the actors which provide primary energy to the soil system (see section 1.4.2). The research therefore mainly focuses on 'feed-forward' processes, i.e. the action of biota upon soil structure i.e. on pore characteristics (including porosity, pore size diversity and pore connectivity; Fig. 1.5.), with some consideration of functional consequences.

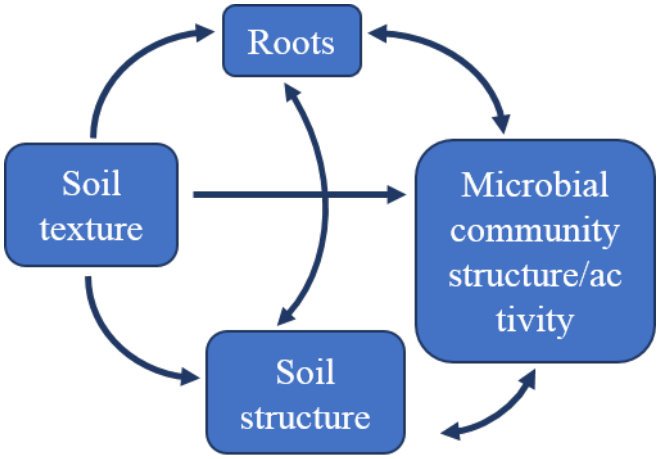


Figure 1.4.: Conceptualisation of key inter-relationships between soil based factors in soil structural genesis and dynamics, and self-organising processes. See text for further explanation.

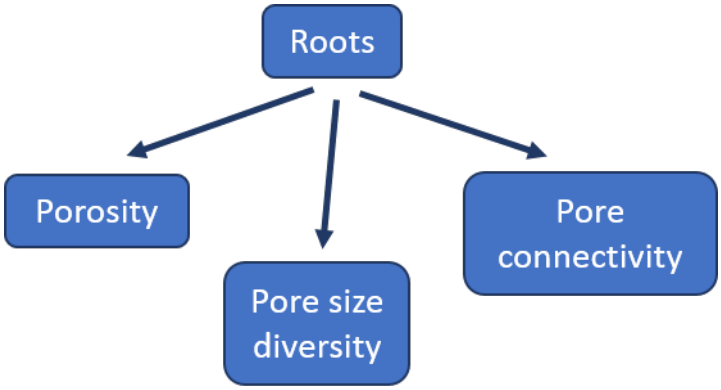


Figure 1.5.: Concept of the impact of roots upon soil structure: roots impact soil structure via increasing porosity, pore size diversity and pore connectivity. See text for further explanation

1.5.2. Research aims and hypotheses

The overall aim of this project was to understand plant impacts upon soil structural genesis and subsequent dynamics.

The main research question is essentially:

"Do plant roots and soil structure have a symbiotic relationship?"

Accordingly, the following principal hypotheses were set:

H1: The presence of a plant results in a greater porosity and pore size diversity due to the action of plant roots and a greater presence of plants invoke a greater impact upon soil structural characteristics.

This is addressed in Chapter 2

H2: The presence of plants exerts a rapid recovery of a compromised soil via a significant impact upon soil structural characteristics.

This is addressed in Chapter 3

H3: Plant roots have contrasting effects upon soil structural genesis depending on soil textures.

This is addressed in Chapter 4

H4: Four cover crops species have a differential impact upon soil structural genesis and microbial community phenotype.

This is addressed in Chapter 5

The first two hypotheses were tested in the context of long-term field experiments, and the second two in shorter-term pot-based studies.

This chapter is published as a research paper in Geoderma (2018) 332, 73-83.

Chapter 2: Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture

Aurelie Bacq-Labreuil[a], John Crawford[b], Sacha J. Mooney[a], Andrew L. Neal[b], Elsy Akkari[b], Cormac McAuliffe[b], Xiaoxian Zhang[b], Marc Redmile-Gordon[b,1], Karl Ritz[a*]

[a] *Division of Agriculture & Environmental Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK*

[b] *Sustainable Agriculture Science, Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK*

*Author contributions: A.B.L. led and conducted the experimental work, analysed the experimental results, drafted the manuscript and coordinated the revisions. E.A. and M.R.G. sampled the cores from the field at Rothamsted. C.M and X.Z conducted the model of the permeability using the scanned images. K.R., S.J.M., J.C., A.N. and M.R.G. contributed advice on the analyses. All authors contributed to the revision of the manuscript. K.R. and S.J.M. supervised the overall project. All authors give final approval for publication.

Geoderma 332 (2018) 73–83

Contents lists available at ScienceDirect

Geoderma

journal homepage: www.elsevier.com/locate/geoderma

Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture

Aurelie Bacq-Labreuil[a,*], John Crawford[b], Sacha J. Mooney[a], Andrew L. Neal[b], Elsy Akkari[b], Cormac McAuliffe[b], Xiaoxian Zhang[b], Marc Redmile-Gordon[b,1], Karl Ritz[a]

[a] Division of Agriculture & Environmental Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK
[b] Sustainable Agriculture Science, Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK

ARTICLE INFO

Handling editor: Yvan Capowiez

Keywords:
X-ray CT
Cropping systems
3D image analysis
Porosity
Pore size distribution
Pore connectivity

ABSTRACT

Soil delivers fundamental ecosystem functions via interactions between physical and biological processes mediated by soil structure. The structure of soil is also dynamic and modified by natural factors and management intervention. The aim of this study was to investigate the effects of different cropping systems on soil structure at contrasting spatial scales. Three systems were studied in replicated plot field experiments involving varying degrees of plant-derived inputs to the soil, viz. perennial (grassland), annual (arable), and no-plant control (bare fallow), associated with two contrasting soil textures (clayey and sandy). We hypothesized the presence of plants results in a greater range (diversity) of pore sizes and that perennial cropping systems invoke greater structural heterogeneity. Accordingly, the nature of the pore systems was visualised and quantified in 3D by X-ray Computed Tomography at the mm and μm scale. Plants did not affect the porosity of clay soil at the mm scale, but at the μm scale, annual and perennial plant cover resulted in significantly increased porosity, a wider range of pore sizes and greater connectivity compared to bare fallow soil. However, the opposite occurred in the sandy soil, where plants decreased the porosity and pore connectivity at the mm scale but had no significant structural effect at the μm scale. These data reveal profound effects of different agricultural management systems upon soil structural modification, which are strongly modulated by the extent of plant presence and also contingent on the inherent texture of the soil.

1. Introduction

Soil structure is dynamic and subject to modification by natural and anthropogenic actions, such as wetting-drying cycles and freeze-thaw action. These processes re-structure the soil with potential consequences for physical and biological processes (Rabot et al., 2018). Water flow and gas diffusion are both affected by the porous architecture (Naveed et al., 2016). The nature and magnitude of soil microbial activity are affected by the air-water balance in soil and the availability of nutrients, and microbial communities are strongly affected by their microenvironment in soil (Chenu, 1993; Helliwell et al., 2014). Soil microbes, along with plant roots, are implicated in aggregation processes via gluing and enmeshing activity (Tisdall and Oades, 1982). Microbial communities can contribute to aggregate stability and therefore help prevent de-structuring of soil structure (Chenu and Cosentino, 2011; Dorioz et al., 1993; Oades, 1993). This, in turn, might lead to the capacity of soils to adapt to changing environmental circumstances (Crawford et al., 2012; Feeney et al., 2006).

Tillage practices have a significant direct influence impact on soil structure, often increasing the macro-porosity of conventionally managed soils (Ambert-Sanchez et al., 2016). Conventional tillage can also result in depletion of nutrients and organic carbon within the soil (Coleman et al., 1997) and a decline in aggregated structure (Watts et al., 2001). Studies of a long-term (40+ years) field experiment at Rothamsted Research (Harpenden, UK) in which grassland was converted to arable and bare fallow managements has resulted in a decline of soil organic carbon and nitrogen (Gregory et al., 2016) and a decrease in microbial abundance under the different managements (Hirsch et al., 2009). These studies focused on soil biological and chemical properties (such as microbiota, pH, organic carbon). However,

* Corresponding author.
E-mail addresses: aurelie.bacq-labreuil@nottingham.ac.uk (A. Bacq-Labreuil), john.crawford@rothamsted.ac.uk (J. Crawford), sacha.mooney@nottingham.ac.uk (S.J. Mooney), andy.neal@rothamsted.ac.uk (A.L. Neal), elsy.akkari@rothamsted.ac.uk (E. Akkari), xiaoxian.zhang@rothamsted.ac.uk (X. Zhang), marc.redmile-gordon@naturalengland.org.uk (M. Redmile-Gordon), karl.ritz@nottingham.ac.uk (K. Ritz).
[1] Current address: Catchment Sensitive Farming, Natural England, Guildbourne House, Worthing, BN11 1LD, UK.

https://doi.org/10.1016/j.geoderma.2018.07.002
Received 16 February 2018; Received in revised form 28 June 2018; Accepted 2 July 2018

there is no information on the structure of the pore networks of the soils under these long-term managements.

The aim of this study was to identify any effects of different cropping managements associated with contrasting degrees of plant presence on soil structure in the context of two soil textural classes. Three long-term field cropping systems were studied: grassland (perennial plant), arable (annual plant) and bare fallow (no plant). We hypothesized that cropping management influences the inherent soil structural properties by (i) the presence of plants resulting in greater soil porosity and range of soil pore diameters due to root action; and (ii) a more persistent presence of plants invokes greater porosity and structural heterogeneity, apparent as a wider range of pore sizes in perennial systems. Structural properties of the soils were determined at two spatial scales of sample and resolution, i.e. 'core' scale (435 cm^3 at 40 µm resolution) and 'aggregate' scale (circa 4 mm^3 at 1.5 µm resolution). To establish the functional consequences of such structures for water flow in the soils, we also estimated their saturated hydraulic conductivity at both scales using a pore-scale modelling approach.

2. Materials and methods

2.1. Soils

Soil cores were collected in October 2015 at Rothamsted Research (Hertfordshire, UK) from two complementary long-term experiments: Highfield Ley-Arable experiment (LATLONG 51.8103N, -0.3748E), on a silty-clay loam textured soil developed on clay-with-flints over Eocene London Clay (Batcome series) and classified as a Chromic Luvisol by FAO criteria (hereafter referred to as clay soil, Table 1); and Woburn Ley-Arable experiment (LATLONG 52.0009N, -0.6137E), on a well-draining, sandy loam soil of the Cottenham Series (Hodge et al., 1984), classified as a Cambric Arenosol (FAO), (referred to as sandy soil, Table 1). The replication of the treatments was uneven and based on the inherent experimental plot design. Four cylindrical cores (68 mm diameter × 120 mm height) of the grassland and arable treatments and three replicate cores of the bare fallow treatment were extracted for the clay soil from the surface down to the height of the columns minus 1 cm (110 mm). Four cores of the grassland, arable with manure (25 t ha^{-1} $annum^{-1}$; hereafter referred to as arable manure) and bare fallow treatments and five replicate cores of the arable with inorganic fertiliser (10 kg N ha^{-1} y^{-1}; hereafter referred to as arable inorganic) were collected for the sandy soil. For both soil types, the arable treatment was under conventional tillage, ploughed to a depth of 23 cm, once a year. The arable fields for the clay and sandy soil was last ploughed respectively in September and October 2014 before sampling. The fallow plots were rotavated in June 2015 for the clay soil and tined in April 2015 for the sandy soil before sampling. All replicates were independent and derived from separate plots. All treatments had been maintained for at least 50 years. After sampling, cores were stored at 4 °C prior to further analysis.

2.2. X-ray computed tomography (CT)

Soil cores were scanned using a Phoenix v|tome|x M scanner (GE Measurement and Control solution, Wunstorf, Germany), set at 160 kV, a current of 180 µA, detector sensitivity of 200% and at a pixel/voxel resolution of 40 µm (resultant voxel volume = 64,000 $µm^3$). A total of 2900 projection images were taken at 250 ms per image using an averaging of 1 image and skip of 0. Total scan time per core was 24 min. After scanning, each core was dismantled, and the soil passed through a sieve series of 4, 2 and 0.71 mm. Three randomly-selected aggregates retained between the 2 and 0.71 mm sieves per core were scanned using a Phoenix Nanotom® (GE Measurement and Control solution, Wunstorf, Germany) set at 90 kV, a current of 65 µA and at a base resolution of 1.51 µm (resultant voxel volume = 3.44 $µm^3$). A total of 1440 projection images were taken at 500 ms period using an averaging of 3 images and skip of 2. The total scan time per sample was 69 min.

Reconstruction of all scanned images was processed using Phoenix datos|x2 rec reconstruction software. Scanned images were optimised to correct for any movement of the sample during the scan and noise was reduced using the beam hardening correction algorithm, set at 8. As a multi-scan routine was performed on the core samples, VG StudioMax® 2.2 was used to merge the top and bottom scans to obtain a single 3D volume for the complete core. For both core and aggregate samples, image sequences were extracted (dimensions described below) for image analysis. Core samples were scanned at the prevailing water content following sampling (approximately field capacity). Soil aggregates were derived from these cores following air-drying overnight and the moisture content recorded. The soil was passed through 4, 2 and 0.71 mm mesh size sieves while subjected to horizontal shaking for 3 min at 300 rotations min^{-1}. Twenty aggregates were randomly selected from between the 2 and 0.71 mm sieves, and conserved in sealed containers in the dark at room temperature.

2.3. Image analysis

Initial image analysis was performed using ImageJ (Schneider et al., 2012). For both soil cores and aggregates, a uniform region of interest (ROI) was defined for each sample; 40 × 40 × 40 mm and 0.981 × 0.725 × 0.604 mm respectively. Core ROIs were positioned centrally to limit inclusion of cracks or large stones created during the sampling process. Cubic ROIs for aggregates were not possible because of their variable geometry, so the largest ROI accommodated by all aggregates was chosen. The coordinates of these regions were adapted for each image volume/sequence. The image pre-processing consisted of: (i) cropping to the ROI; (ii) enhancing the contrast/brightness to 0.35%; (iii) application of a 2-pixel radius median filter; (iv) converting the image format to 8-bit; (v) saving the new image volume. Stones were segmented from the ROI volume in VG StudioMax® 2.2 using the surface determination tool.

All images were thresholded using the bin bi-level threshold approach by Vogel and Kretzschmar (1996) using the open source software QuantIm (http://www.quantim.ufz.de/). Each image within the image sequence has a single threshold value, to determine the

Table 1
Summary physical and chemical data of Highfield Ley-Arable experiment soils.

Treatment	Density[a]/g cm^{-3}	pH[a] (H_2O)/-log(g[H^+]L^{-1})	Organic carbon[a]/mg g^{-1} soil	Free organic carbon[b]/µg g^{-1} soil	Intra-aggregate organic carbon[b]/µg g^{-1} soil	Nitrogen[a]/µg g^{-1} soil	NaOH-EDTA extractable phosphorus[c]/µg g^{-1} soil
Fallow	1.30–1.45	5.1	0.8	150	380	100	235
Arable	1.30–1.45	5.8	1.3	370	490	150	517
Grassland	0.99	6.0	3.9	4690	3010	390	662

[a] Gregory et al., 2016.
[b] Hirsch et al., 2009.
[c] Neal et al., 2017.

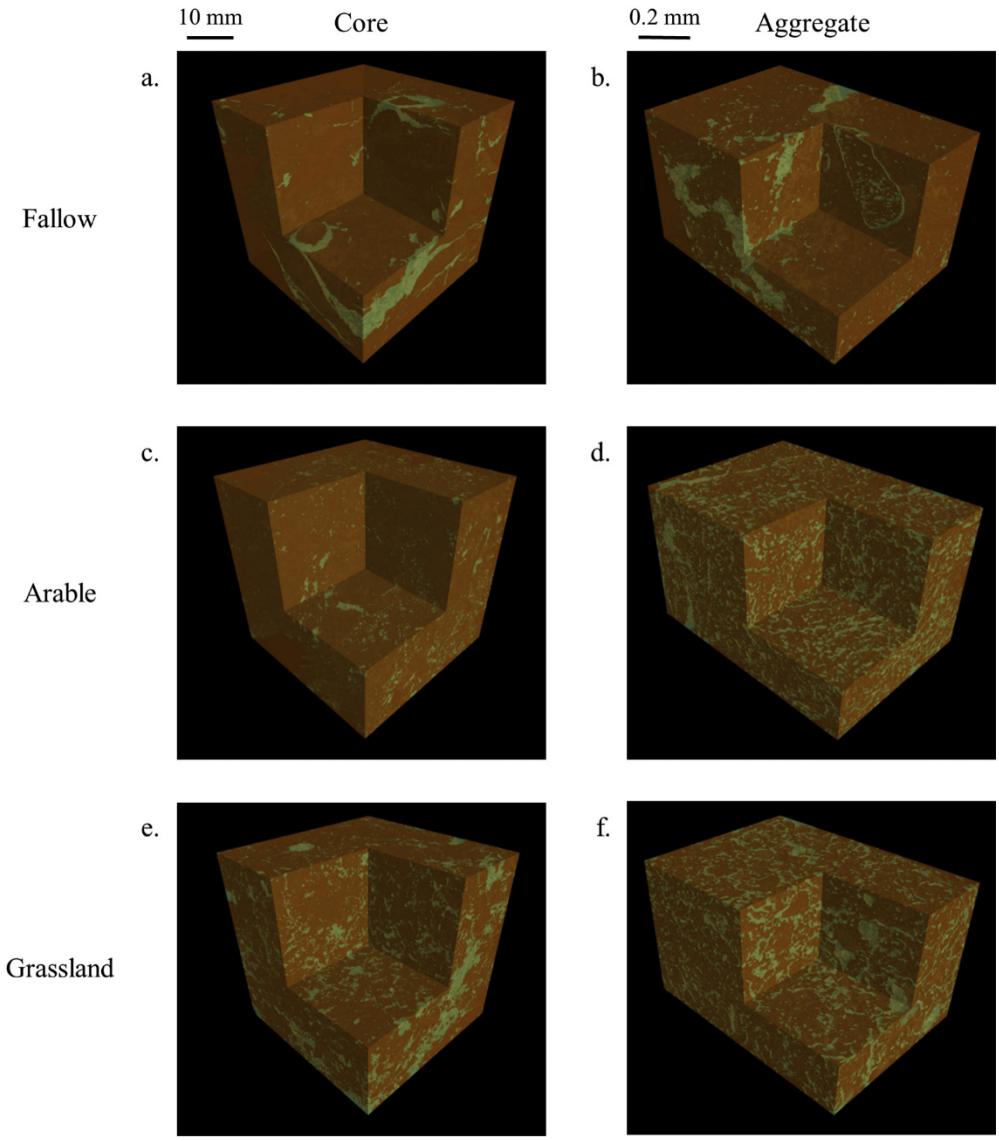


Fig. 1. 3D representation of clay soils under different cropping systems visualised at core (40 µm resolution; a, c, e) and aggregate (1.5 µm resolution; b, d, f) scales, displayed as thresholded images denoting pore (green) or solid (brown) phases. (a, b) bare fallow; (c, d) arable; (e, f) grassland. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

prescribed initial threshold values (T_1 and T_2), the Li-threshold algorithm in ImageJ was applied to 20 images randomly selected within the image sequence. The threshold values (T_1 and T_2) were attributed depending on extremes values obtained in the 20 images selected. Porosity, pore size distribution, pore connectivity and surface density determined according to Vogel et al. (2010). Here, total porosity refers to percentage of pores $>$ 0.05 mm at the core scale and $>$ 1.8 µm at the aggregate scale, as per the segmented data. Pore size distribution was expressed as the proportion of each pore size as the percentage volume normalised to the total ROI volume, and also as the proportion of each pore size as the cumulative percentage volume normalised to the total pore volume. The pore size diameter was determined using a maximum opening diameter based on a numerical sphere algorithm as described by Vogel et al. (2010). Pore connectivity is derived from the Euler number and normalised by the total volume. The more negative the Euler number, the more connected the pore system. Increase of sphere opening diameter results in loss of smaller pores or pore-irregularities due to irregular-shape pores. The surface density represents the proportion of transition from pore to solid which is the ratio of the pore surface area (mm^2) divided by the pore volume (mm^3). After the spherical opening, the numerical representation of the pore surface includes a combination of the pore-solid interface and a numerical surface placed in the middle of irregular-shaped pores (Vogel et al., 2010).

2.4. Hydraulic conductivity of aggregates and inter-aggregate pores

It is not straightforward to measure saturated hydraulic conductivity (K_{sat}) of individual aggregates. To facilitate direct comparison of K_{sat} for all sample classes, we numerically calculated the ability of inter- and intra-aggregate pores to conduct water based on the pore-scale velocity of water flow through the pore geometry. This was simulated using the lattice Boltzmann model developed previously (Zhang et al., 2005, 2016; Zhang and Lv, 2007). The details of the

model and how the permeability of each image was calculated are given in Appendix A.1. Supplementary materials. The computational demand of the ROI exceeded the power of the computer facility we could access, and we were therefore unable to directly simulate water flow in the ROI. Instead, we selected several sub-volumes, denoted as volume of interest (VOI), from each ROI to simulate water flow through them and calculated their permeability. The model used a sparse matrix algorithm to store only the pore voxels, and hence the maximum size of the VOI the model can process depended on the number of pore voxels (or porosity) within it. For each ROI image, we first calculated its porosity and then divided it into a number of equal sub-volumes ensuring that the number of pore voxels in each sub-volume did not exceed the maximum pore voxels the model can deal with. Pre-analysis of all images revealed that the pore geometry in the aggregates was a relatively uniform 0.5 mm, we thus used one VOI ($375 * 375 * 375$ μm) to represent one ROI. The pore geometry in the core was highly heterogeneous, and we randomly selected three samples from the sub-volumes into which the original ROI was divided to simulate water flow and calculate their permeability.

2.5. Statistical analysis

Analysis of variance (ANOVA) was performed on all primary variables using a split-plot design with cropping management and size classes of pores as factors. The relationship between the measured porosity and calculated saturated permeability was also explored using ANOVA to test for equality of slopes of $\log_{10}$-transformed data within the modelled VOI. All analyses were conducted using Genstat v 17.1 (VSN International Ltd. 2014).

3. Results

3.1. Effect of management on clay soil pore structure

3.1.1. 3D image assessment

Fig. 1 illustrates selected 3D representations of pore architecture from the clay soil cores (Fig. 1a, c, e) and aggregates (Fig. 1b, d, f) displayed as segmented images. For the soil cores, there was a clear decrease in the number of stones respectively fallow = arable > grass (Fig. A1, Table A1). Moreover, arable soils contained larger pores (> 1 mm) than the other treatments (Fig. 1c), especially at the interface with stone material (Fig.1c). The bare fallow core generally had smaller pores (0.25–1 mm; Fig. 1a). Grass cores had a wider range of pore sizes and contained more root and organic material (Fig. 2a, A1e). A similar observation was made for soil aggregate images as the bare fallow aggregates comprised mostly small pores despite the presence of a few, larger pores (Fig. 1b, A1b), the arable aggregates appeared to only have smaller sized pores (Fig. 1d, A1d), and grass aggregates again showed the widest range of pore sizes (Fig. 1f, A1f).

3.1.2. 3D characteristic analysis

There were significant differences in porosity characteristics for the clay soil under each management system, which contrasted in nature between core and aggregate scales (Table 2; Fig. 1). At the core scale, total pore volume was not significantly different between treatments (Table 2). At the aggregate scale, total pore volume was significantly different under all three treatments, with a distinct ranking of grassland > arable > fallow, and a two-fold difference between grassland and fallow (Table 2).

The pore size distribution normalised to the total ROI volume showed, at the core scale, for the arable treatment an equivalent proportion of all pore sizes < 1.12 mm, with a smaller proportion of pore sizes < 0.25 mm compared to grassland and fallow treatments (Fig. A2a). The proportion of pores > 1.12 mm was greater for the grassland and the arable treatments compared to fallow treatment (Fig. A2a). Cumulative pore size distributions were similar in their non-linear character under grassland and fallow at the core scale, showing a greater proportion of pore sizes < 0.25 mm than the arable soil (i.e. > 50% of pore volume; Fig. 2a). However, the proportion of pore sizes with a diameter > 1.12 mm was significantly greater under grassland and arable than fallow ($P < 0.001$; Fig. 2a). In contrast, under the arable treatment, the cumulative pore size distribution was linear between 0.05 and 1.04 mm (Fig. 2a), indicating that the pores in this range were uniformly distributed. At the aggregate scale, the proportion of pore sizes normalised to the total ROI volume increased significantly with the increasing pore sizes up to 5.97 μm for all treatments with a greater proportion of these pore sizes for grassland and arable treatments. For the pore sizes between 9.26 and 20.91 μm, the proportion of the pore sizes decreased for all treatment, with a substantial decrease for the arable compared to the grassland. There were no significant differences beyond this pore size, except for the pore sizes > 42 μm with the proportion of pores ranking from arable < fallow < grassland (Fig. A2b). The cumulative pore size distributions for pores < 9.26 μm under arable and fallow treatments were not significantly different, but both were significantly greater than grassland (Fig. 2b). For pores > 11.8 μm, the relationship was reversed and distributions under grassland and fallow were not significantly different, but arable had a significantly smaller proportion of larger pores (Fig. 2b).

At the core scale, the pore-surface density was significantly different under all three treatments for pore sizes < 0.095 mm, with ranking of arable < fallow < grassland (Fig. 2c), with a two-fold difference between grassland and arable. For the pore sizes < 0.25 mm, the surface density declined similarly under grassland and arable and there was no difference beyond the pore size 0.31 mm under all three treatments ($P < 0.001$; Fig. 2c). In contrast, at the aggregate scale, the surface density relating to the smallest pore sizes (1.86 μm) was significantly reduced under fallow compared to arable and grassland, which were not significantly different ($P < 0.001$; Fig. 2d). However, for the pore sizes between the pores 5.97 μm and 11.8 μm, the surface density decreased more drastically under the arable treatment compared to the grassland, both converging towards the fallow. The surface density was not significantly different for pores > 14.9 μm under all treatments (Fig. 2d).

There were no significant differences in pore connectivity between any of the treatments at the core scale ($P > 0.05$; Fig. 2e). At the aggregate scale, the connectivity was significantly greater under grassland and arable treatments than fallow with respect to pores < 5.97 μm, with no differences beyond this (Fig. 2f). There was no change in pore connectivity within aggregates under fallow across the size range measured (Fig. 2f).

At the core scale, soil porosity within the VOI used for permeability simulation was linearly related to porosity of ROI ($P < 0.001$), but was on average 73% greater ($P < 0.001$; data not shown), indicating that the pore geometry in ROI was highly heterogeneous at centimetre scale. Within VOI, porosity was significantly greater under grassland than fallow, with arable intermediate but not significantly different from either (Table 4). Simulated permeability mirrored these trends, and was circa two-fold greater for grassland than fallow (Table 3). There was a significant positive power-law relationship between porosity and permeability in the case of fallow and arable, and marginally so for grassland (Fig. A3a). Across all three treatments there was no significant difference between the regression coefficients for the power-law relationships (overall mean 1.12 ± 0.30; Table 4). At the aggregate scale, the porosity of VOIs and ROIs of aggregates was not different ($P < 0.001$; data not shown), revealing that at 0.3–0.5 mm scale the aggregates were relatively uniform. Here, mean porosity was significantly different between all treatments in the rank order of grassland > arable > fallow (Table 3). Modelled permeability in grassland treatments was double that in arable and fallow, which were not significantly different from each other (Table 3). At this scale, there was a significant positive power-law relationship between porosity and

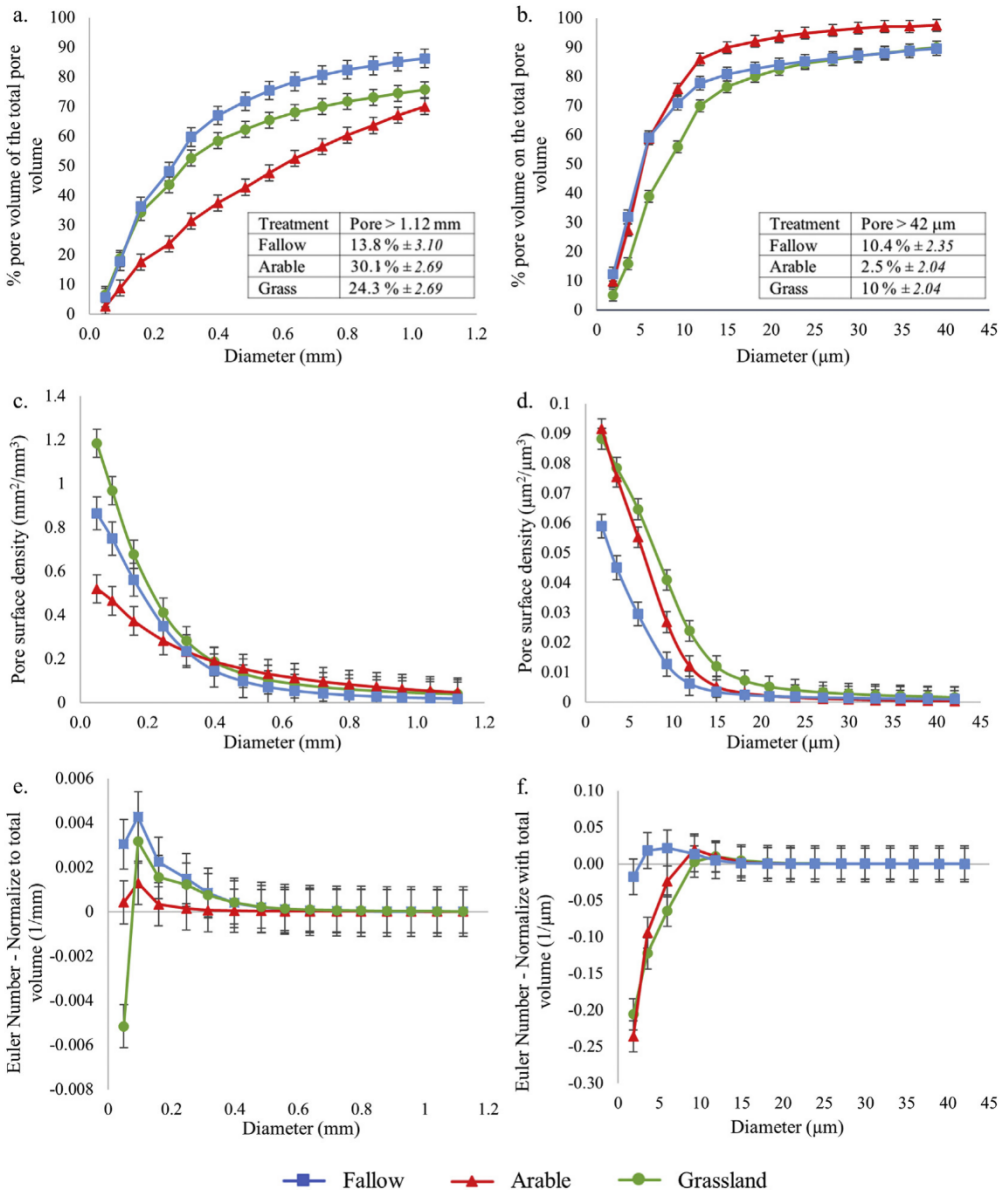


Fig. 2. Soil pore characteristics of clay soils under different cropping systems at core (40 µm resolution; a, c) and aggregate (1.5 µm resolution; b, d, e) scales: (a, b) cumulative pore distribution of cores; (c, d) surface density; (e, f) connectivity. Points indicate means, whiskers denote pooled standard errors.

Table 2
Total porosity in relation to management type at the core (base resolution 40 µm) and aggregate (base resolution 1.5 µm) scale of the clay soil, expressed as percentage of pores relative to the total volume (mean ± pooled standard error).

Treatment	*n*	Core	Aggregate
Fallow	3	8.07 (± 0.76)	14.3 (± 1.08)
Arable	4	8.29 (± 0.66)	23.4 (± 0.94)
Grassland	4	12.0 (± 0.66)	31.1 (± 0.94)
P_F		0.53	< 0.001

Table 3
Total porosity in relation to management type (expressed as percentage of pores relative to the total volume), and modelled saturated permeability, of the volume of interest used for modelling, for the clay soil at the core and aggregate scale (mean ± pooled standard error).

Treatment	*n*	Core		Aggregate	
		Porosity (%)	Permeability (mm^2)	Porosity (%)	Permeability (mm^2)
Fallow	3	9.3 (± 2.32)	387 (± 202)	14.8 (± 1.78)	0.55 (± 0.09)
Arable	4	12.0 (± 2.01)	702 (± 175)	23.0 (± 1.54)	0.62 (± 0.08)
Grassland	4	16.3 (± 2.01)	827 (± 175)	31.0 (± 1.54)	1.13 (± 0.08)
P_F		0.54	0.38	0.002	0.003

permeability in all cases which was weakest for fallow (Fig. A3b), but as for the core-scale, there was no significant difference between the regression coefficients across all treatments (Table 4; overall mean 1.0 ± 0.23).

Table 4
Linear regression coefficients (mean ± standard error) in relation to management type of log porosity vs. log modelled saturated permeability for the clay soil, at the core (Fig. A3a) and aggregate scale (Fig. A3b).

Treatment	*n*	Core		Aggregate	
		Coefficient[a]	P_{uncorr}	Coefficient[a]	P_{uncorr}
Fallow	9	0.27 (± 0.13)	0.08	0.71 (± 0.24)	0.02
Arable	12	0.79 (± 0.07)	< 0.001	0.93 (± 0.14)	< 0.001
Grassland	12	1.00 (± 0.15)	< 0.001	1.38 (± 0.32)	0.002
Coefficients P_F		0.01		0.30	

[a] Coefficient of x value in fitted equation.

3.2. Effect of management on sandy soil

3.2.1. 3D image assessment

Fig. 3 and A4 illustrate the visualisation of the ROI of the four treatments. In the cores, the presence of stones decreased from bare fallow > arable inorganic equivalent to arable manure > grass (Fig. 3b, A4). The bare fallow and arable inorganic cores appeared relatively porous and contained some large pores (Fig. 3a, c). The arable manure and grassland showed similar types of pores: mostly medium (0.25–1.0 mm) and small pores (< 0.25 mm; respectively Fig. 3b, d). There was no significant difference in the proportion of stone for all treatments (Table A2). However, no noticeable difference was observed between the aggregates (Fig. 3, A4).

3.2.2. 3D characteristic analysis

Total porosity at the core scale was significantly greater under fallow and inorganically fertilised arable than grassland and manured arable with no significant difference between fallow and inorganically fertilised arable, nor between grassland and manured arable (Table 5). In contrast, at the aggregate scale, there were no significant differences in total porosity between any of the four treatments (Table 5).

At the core scale, the pore size distribution normalised to the total ROI volume displayed contrasting behaviours for the pore sizes < 0.25 mm: inorganically fertiliser arable and fallow treatment contained the greatest proportion of the pore sizes compared to grassland and manured arable treatments (Fig. A5a). There was no difference beyond 0.25 mm, except for pores larger than 1.12 mm, here with a greater proportion for fallow and manured arable compared to grassland and inorganically fertiliser arable (Fig. A5a). The nature of the cumulative pore size distributions was different than the pore size distribution normalised to the total ROI and could be classified into three categories: fallow and inorganically fertilised arable, where 70% of the pore sizes were < 0.16 mm; manured arable where 70% of the pore sizes were < 0.32 mm and had a greater proportion of larger pores; and grassland where 70% of the pore sizes were < 0.32 mm and had a larger proportion of pores < 0.64 mm compared to manured arable. The proportion of pores > 1.1 mm ranged from 14.9% (manured arable) to 5.1% (inorganically fertilised arable), with the extremes being significantly different (Fig. 4a). The cumulative pore sizes were characterised by two distinct patterns in relation to treatment associated with fallow, inorganically fertilised arable treatments; and grassland and manured arable. For pore sizes < 0.64 mm, Fig. 4a showed a greater proportion of pore sizes smaller than 0.16 mm under fallow and inorganically fertilised arable than grassland and manured arable (respectively around 70% and 50%). The fallow and inorganically fertilised arable treatments showed a similar cumulative pore size distribution across the size range measured, with very few medium sized pores. The distribution of pore sizes under grassland and manured arable were more diverse, as indicated by greater linearity in abundance across the pore size range. Pore size distributions of grassland and manured arable soils were similar up to a pore size of 0.56 mm and beyond this point, the distribution diverged and there was a significantly greater proportion of the largest pore size under manured arable than grassland (Fig. 4a).

At the aggregate scale, a similar separation of the treatments was observed as for the core scale, although the cumulative pore size distributions were more linear, with only a significant difference for the largest pores under fallow and inorganically fertilised arable, than for grassland and manured arable (Figs. 4b, A5b).

At the core scale, pore surface density profiles also divided into two distinct groupings: fallow and inorganically fertilised arable, and grassland and manured arable. These were significantly greater under fallow and inorganically fertilised arable than grassland and manured arable, both of which were congruent across the size range measured (Fig. 4c). The surface density at the smallest pore size (0.05 mm) was greater under fallow than inorganically fertilised arable, however for the larger pore size (> 0.05 mm) the surface density was not significantly different and decreased similarly under both treatments. For pore sizes > 0.25 mm, there were no significant differences between any of four treatments (Fig. 4c). At the aggregate scale, pore surface profiles were similar in their non-linear nature, with inorganically fertilised arable being significantly lower than other treatments over the range < 3.56 µm (treatment x size class $P < 0.001$; Fig. 4d).

At the core scale, the connectivity was significantly greater for fallow and inorganically fertiliser arable compared to grassland and manured arable, with respect to pores < 0.09 mm, and there was no significant differences beyond this ($P > 0.05$; Fig. 4e). At the aggregate scale, the values of pore connectivity for all treatments were relatively small, suggesting that the overall pore system for the sandy soil was poorly connected. The connectivity of pores at 1.86 µm was significantly different under all four treatments with a ranking of fallow > grassland > manured arable > inorganically fertilised arable (Fig. 4f). There was then a general trend of decreasing connectivity above this size, with convergence of all treatments for pores > 5.97 µm.

At the core scale, the soil porosity within the VOI (which was used for permeability calculations) was linearly related to the porosity of the entire core ($P < 0.001$), but was on average 22% less (data not shown), again revealing that centimetre-scale pore geometry was highly heterogeneous. Within the VOI, the porosity of manured arable and grassland treatments was similar (mean 17%), as was the case for inorganically fertilised arable and fallow (mean 21%), with the later pair being significantly greater than the former ($P < 0.01$; Table 5). At this scale, modelled permeability was not significantly different between all treatments (Table 6). There was a significant positive power-law relationship between mean porosity and permeability for fallow, arable manure and grassland treatments, which was marginally significant for arable inorganic (Fig. A6a). There was no significant difference between the regression coefficients of the power-law relationship between all treatments (Table 7; overall mean 0.68 ± 0.22). At the aggregate scale, there was a direct linear relationship between the porosity for whole aggregates and the VOI used for modelling (data not shown). There was no difference in total porosity between treatments within the VOI used for modelling permeability (Table 6; overall mean 2.22 ± 0.44). Permeability was not significantly different all treatments (Table 6). At this scale, there was a highly significant positive power-law relationship between porosity and permeability for all treatments (Fig. A6b). The regression coefficient was significantly greater in the case of fallow than all other treatments, and significantly smaller in the case of grassland than all other treatments, with arable and arable manure essentially the same, and median to fallow and grassland (Table 7).

4. Discussion

3D quantification of the soil architecture in terms of pore connectivity, pore surface density and pore size distribution, is essential for linking soil structure to fluid flow, gaseous diffusion and soil functions

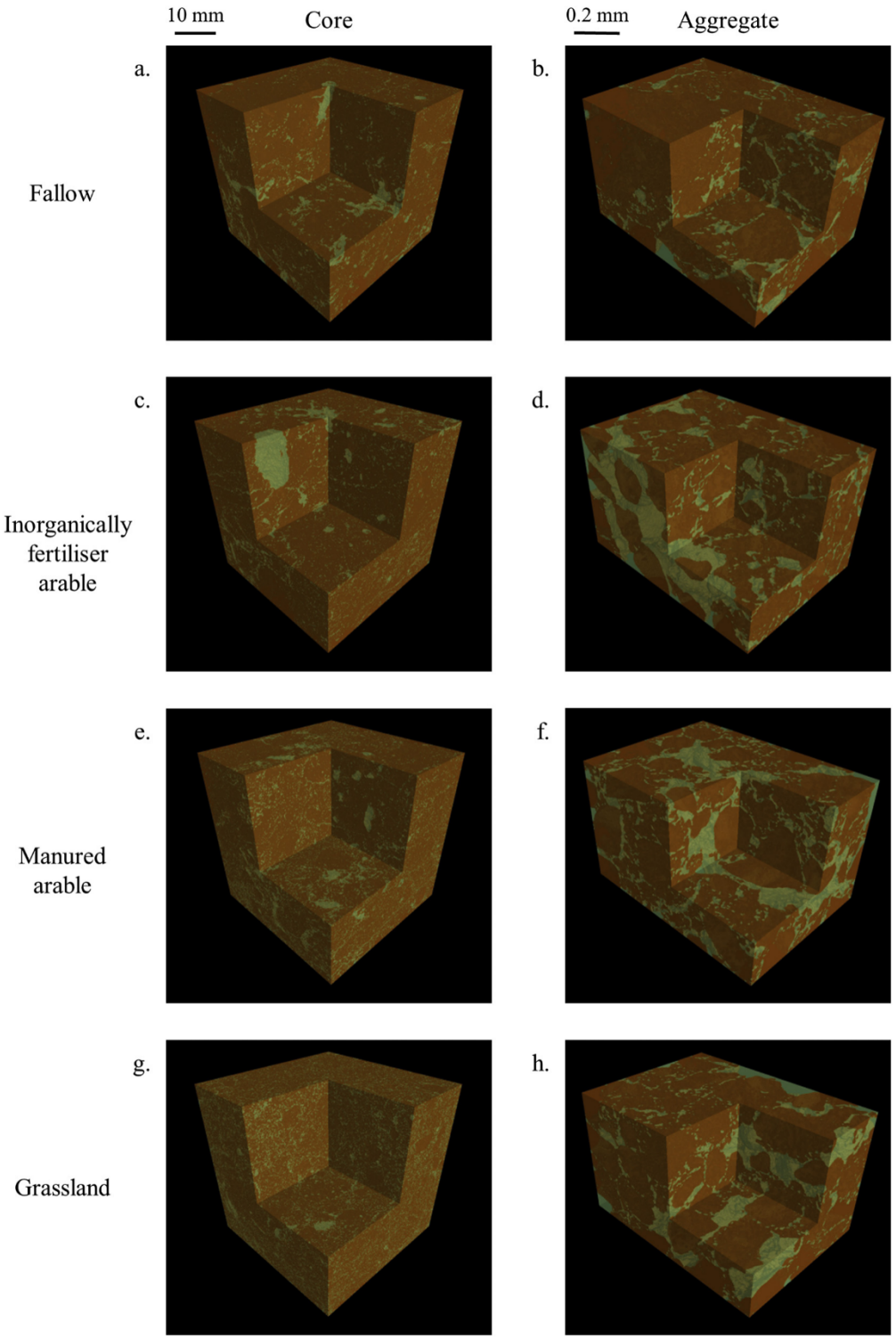


Fig. 3. 3D representation of sandy soils under different cropping systems visualised at core (40 µm resolution; a, c, e, g) and aggregate (1.5 µm resolution; b, d, f, h) scales, displayed as thresholded images denoting pore (green) or solid (brown) phases. (a, b) bare fallow; (c. d) inorganically fertiliser arable; (e, f) manured arable (g, h) grassland. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 5
Total porosity in relation to management type at the core (base resolution 40 µm) and aggregate (base resolution 1.5 µm) scale of the sandy soil, expressed as percentage of pores relative to the total volume (mean ± pooled standard error).

Treatment	*n*	Core	Aggregate
Fallow	4	21.1 (± 0.98)	23.7 (± 0.68)
Inorganically fertilised arable	5	19.6 (± 0.87)	24.4 (± 0.61)
Manured arable	4	14.6 (± 0.98)	24.8 (± 0.68)
Grassland	4	13.3 (± 0.98)	25.4 (± 0.68)
P_F		0.002	< 0.001

(Rabot et al., 2018) including metabolism. Our data revealed substantial effects of agricultural practice upon both pore architecture and function from these perspectives.

4.1. Effect of management on clay soil

The presence of plants increased the soil porosity, as also shown by Helliwell et al. (2017) albeit in the rhizosphere soil images acquired at 12 µm resolution. Where present, perennial vegetation increased soil porosity at both core and aggregate scales to a greater extent than either annual plants interspersed by tilling or bare fallow. Grassland soils had not been ploughed for at least 150 years and the sampled soil contained a very low number of stones (Fig. 1c, Table A1). The greater proportion of larger pores is most likely to have been induced by the diversity of plant roots and their inputs and the presence of associated soil biota (Pires et al., 2017). The decreased occurrence of stones in the surface 10 cm of grassland soil is likely to be due in part to bioturbation, specifically via the surface-deposition of soil by anecic earthworms, which are known to effectively bury objects to lower soil horizons over time (Table A1; Canti, 2003; Hanson et al., 2009). Regular tillage of the

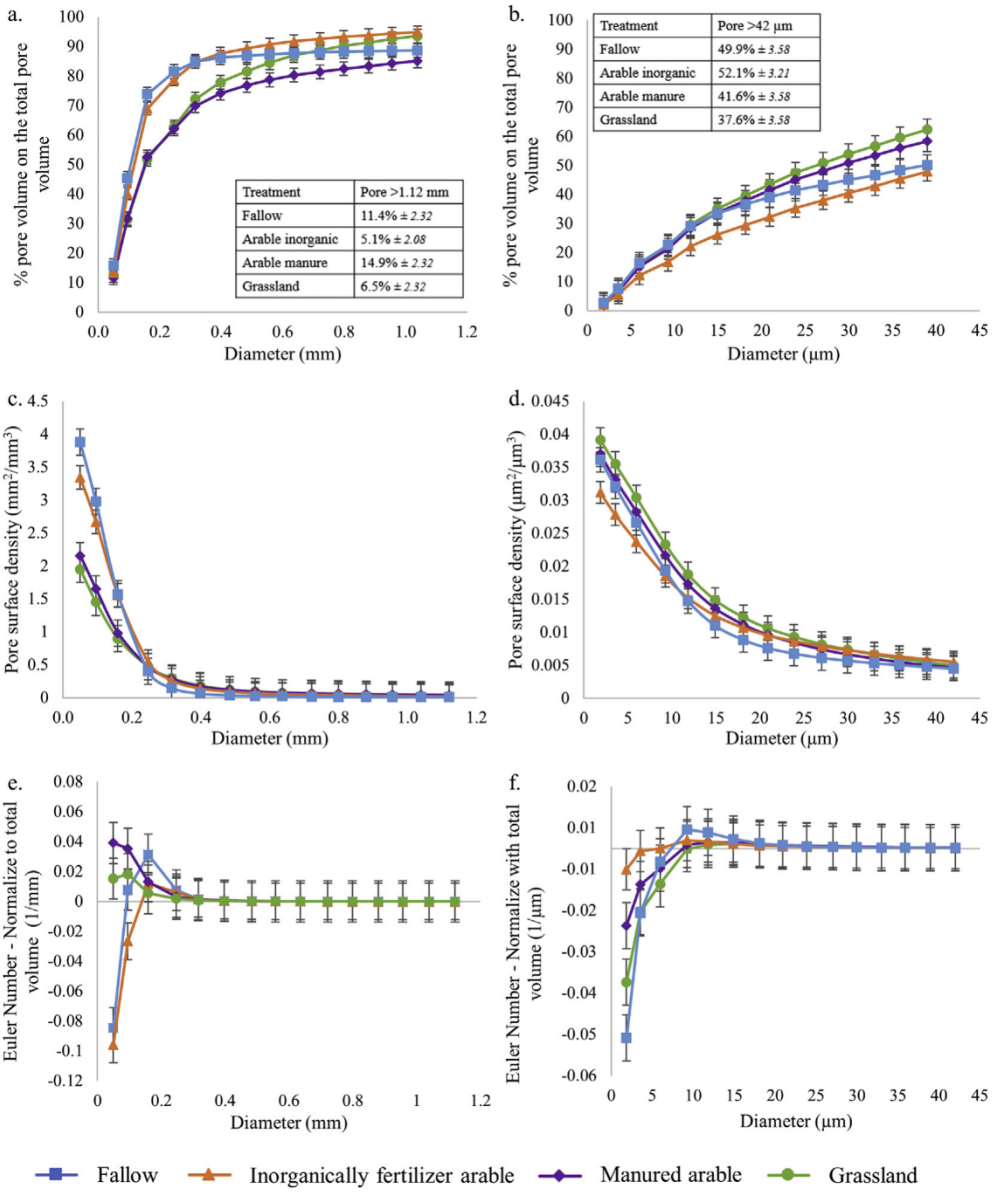


Fig. 4. Soil pore characteristics of sandy soils under different cropping systems at core (40 µm resolution; a, c) and aggregate (1.5 µm resolution; b, d, e) scales: (a, b) cumulative pore distribution of cores; (c, d) surface density; (e, f) connectivity. Points indicate means, whiskers denote pooled standard errors.

Table 6
Total porosity in relation to management type (expressed as percentage of pores relative to the total volume), and modelled saturated permeability, of the volume of interest used for modelling, for the sandy soil at the core and aggregate scale (mean ± pooled standard error).

Treatment	*n*	Core		Aggregate	
		Porosity (%)	Permeability (mm^2)	Porosity (%)	Permeability (mm^2)
Fallow	4	20.9 (± 1.55)	266 (± 59.0)	25.0 (± 1.71)	2.44 (± 0.45)
Inorganically fertilised arable	5	21.2 (± 1.38)	406 (± 52.8)	26.0 (± 1.71)	2.84 (± 0.40)
Manured arable	4	16.4 (± 1.55)	501 (± 59.0)	24.3 (± 1.52)	1.88 (± 0.45)
Grassland	4	17.0 (± 1.55)	464 (± 59.0)	24.6 (± 1.71)	1.72 (± 0.45)
P_F		0.61	0.37	0.98	0.54

Table 7
Linear regression coefficients (mean ± standard error) in relation to management type of log porosity vs. log modelled saturated permeability for the sandy soil, at the core (Fig. A6a) and aggregate scale (Fig. A6b).

Treatment	*n*	Core		Aggregate	
		Coefficient[a]	P_{uncorr}	Coefficient[a]	P_{uncorr}
Fallow	12	0.58 (± 0.25)	0.04	2.69 (± 0.51)	< 0.001
Inorganically fertilised arable	15	0.37 (± 0.18)	0.06	2.08 (± 0.21)	< 0.001
Manured arable	12	1.08 (± 0.25)	0.002	1.90 (± 0.20)	< 0.001
Grassland	12	0.70 (± 0.22)	0.01	1.37 (± 0.13)	< 0.001
P_F		0.08		0.02	

[a] Coefficient of x value in fitted equation.

fallow and arable soils would however retain stones in the plough layer.

The pore size distributions were expressed in two ways: normalised to the total ROI volume (Fig. A2) and normalised to the total pore volume (Fig. 2a, b), each highlights different information regarding the pore structure. Normalising to the total ROI volume expressed the absolute proportion of the different pore sizes, whereas normalising to the pore volume shows the relative proportion of each pore sizes to the total porosity. At the core scale, both normalisations showed the same trend: a smaller proportion of pore sizes < 0.25 mm for the arable compared to grassland and fallow and greater proportion of pores > 1.12 mm for grassland and arable treatment compared to the fallow treatment (Fig. 2a, A2a). However, at the aggregate scale, the absolute proportion of the pores < 25 µm was greater for grassland and arable than fallow, while the relative abundance showed a greater proportion for arable and fallow compared to grassland. This difference between absolute and relative abundances might be due to the fact that the porosity observed in the grassland was twofold greater than fallow, so the relative proportion accounted for the difference in porosity. At both scales, grassland soil showed a greater range of pore sizes compared to arable or fallow (Fig. 2a, b; Fig. A2) which could be emphasised by a greater presence of organic carbon, fungi and a greater abundance of bacteria in grassland treatment as shown by Hirsch et al. (2009) in a previous study working on the same site. Under the arable and fallow treatments, the frequency of stones was greater than the grassland system (Table A1). The presence of stones could be accounted for as a result of the regular ploughing of the soil over the past 60 years which brought them to the soil surface (Rossi et al., 2013). Recent data from another long-term experiment has shown that no-till arable soils have a significantly lower percent of macro-pores than soils subjected to tilling with a chisel plough (Ambert-Sanchez et al., 2016), which corroborates our observation of the high proportion of large pores under the arable at the core scale. The arable treatments showed fewer larger pores at the aggregate scale which emphasizes that ploughing apparently introduced a greater macro-porosity at the core scale.

At the core scale, grassland systems had a greater surface density for the smallest pores (Fig. 2c), in this circumstance the pore-solid interface was expected to be more accessible to micro-organisms and roots, which would be beneficial for water and nutrient uptake. In contrast, the surface density of all visible pores at the core scale was lowest under arable management which could be due to the mechanical disturbance but also the presence of stones since the morphology of a stone has a smoother edge than a pore. Moreover, the decreased surface density in the arable and fallow treatments can be induced by the loss of the elongated pores due to the impact of the machinery (Pagliaia et al., 2003). At the aggregate scale, treatments involving plants had a greater surface density of all pores than bare fallow soil. However, the surface density values were very low. There were statistically significant differences in surface densities between treatments at the aggregate scale but that the magnitude of the effect was very small.

The volume of interest (VOI) was derived from the region of interest (ROI). VOI was used to model the water saturation of the volume and the ROI was used to calculate the pore characteristics using QuantIm. At the core scale, basic pore characteristics were significantly linearly correlated but absolute values were different. These disparities are likely due to the heterogeneous distribution of the pores within ROI, but given the correlation, it is admissible for comparative purposes to study treatment effects. At the aggregate scale, VOI porosity was congruent with ROI porosity (data not shown).

At both scales, across all clay treatments, permeability generally increased with porosity (Table 3). At the core scale this followed a positive power-law relationship with the exponent varying significantly between treatments (Table 4; Fig. A3a). The increase in permeability, with respect to porosity, increased significantly ranking from fallow < arable < grassland, i.e. there is a substantive effect of the extent of plant presence upon this relationship and the intrinsic ability of the soils to conduct water.

At the aggregate scale, the permeability of the fallow and arable treatments was not significantly different despite the difference in porosity (Table 3). However, under both these treatments, the pore size distribution normalised to the total pore volume was similar for the smaller pores (< 9.26 µm) and in greater proportion than grassland (Fig. 2b). Here, the increased proportion of smaller pores appears to reduce permeability. Surprisingly, the differences in grassland and arable treatment permeability (Table 3) were not matched by differences in pore connectivity (Fig. 2e) This suggests that pore size distribution and porosity are better pore characteristic descriptors in our study, which is consistent with observation of Blackwell et al. (1990). This observation complements other studies showing that permeability is dependent upon macro-porosity (Cercioglu et al., 2018) and pore-connectivity at the macroscale (Ball, 1981).

4.2. Effect of management on sandy soil

For sandy soil, there was similarity between pore structures derived from grassland (i.e. perennial plants) and manured arable (i.e. annual plants with organic inputs); and between the fallow and inorganically fertilised arable (i.e. annual plants with inorganic inputs; Fig. 4a). This observation is supported by another experiment studying C sequestration over 70 years. In this particular soil, addition of organic manure (38 t ha^{-1} every fifth year) was as efficient at C sequestration as growing 3 years' grass and clover in a 5-year arable rotation (Johnston et al., 2017). At the core scale, the systems involving perennial and annual plants with organic inputs (manure) reduced porosity, surface density and connectivity (Table 5, Fig. 4c, e) but increased the diversity of pore sizes relative to the absence of plants and annual plants with inorganic inputs (Fig. 4a, c). This reduction in porosity was also observed, in the rhizosphere soil, for the growth of tomato plant root

systems in sandy loam soil by Helliwell et al. (2017). Furthermore, the increase of the porosity in inorganically fertilised arable and fallow treatments could be due to the conventional tillage which is applied to loosen the soil particles (Arvidsson, 1998). The soil structural characteristics associated with manured arable were similar to the grassland treatment, which supports the notion that addition of organic C helps support arable soil structure. Organic inputs decreased the soil porosity and the connectivity, which could decrease permeability of water and nutrients. Both normalisations of pore size distributions showed the same trend: a greater proportion of pores < 0.25 mm under inorganically fertiliser arable and fallow treatments compared to grassland and manured arable (Fig. 4a, A5a).

At the core scale, VOI porosity was linearly correlated with ROI porosity, but the absolute values were different (data not shown). These differences might be induced by the heterogeneous distribution of the pores within the ROI, however due to the correlation, these values were admissible for the comparison of the treatment effects (Table 7). There were no significant differences amongst treatments for the permeability (Table 7).

At the aggregate scale, the only relative difference was observed for the pore connectivity; however, the Euler numbers were less negative, suggesting the pore systems of all treatments were poorly connected (Fig. 4e). Sandy soil is predominantly composed of larger grains than clay soils. This implies that the aggregate scale may not be optimal to observe differences between treatments in the sandy soil. However, the long-term organic management had been proven to have a greater variability in intra-aggregate spatial pore structure for a fine-loamy soil (Kravchenko et al., 2014). In this study, organic matter inputs increased the presence of both larger (> 188 μm) and smaller (< 13 μm) pores. The most relevant scale to characterise soil micro-structure is contingent upon the texture (Kravchenko et al., 2014; Peth et al., 2008). This was apparent in our study where management effects were detected at the aggregate scale only in the case of the clay soil.

4.3. Contrasting effect of management on both soils

The two soil textures exhibited striking differences in soil structure following application of various long-term managements. In both soils, the grassland (and manured arable for the sandy soil) appeared to influence the porosity at the resolutions considered here, increasing for clay soil and decreasing for the sandy soil. Perennial plant inputs and the addition of manure for the arable treatment appear to affect system porosity in their vicinity, contingent on soil texture. We propose that plants modify soil porosity in their vicinity, improving hydraulic function – for example water retention and flow. Indeed, for both soils, under grassland and manured arable there was a decrease in the proportion of smaller pores, which may lead to an increase in permeability. We found that at the core scale, pore connectivity of the clay soil was not significantly different (Fig.2e) and was significantly greater for the inorganically fertilised arable and the fallow treatments compared to manured arable and grassland treatments. Therefore, carbon inputs from perennial plants and addition of organic carbon apparently decreased the connectivity of the pore system which may recover from disturbance associated with management. This supports the observations of Gregory et al. (2009) who found a positive relationship between organic matter content and the resilience of soils to withstand physical compression. However, at the aggregate scale, the clay soil treatments were significantly different depending on management whereas the sandy aggregates were not significantly different in regard to pore structures between the different managements.

There was a positive power-law relationship between porosity and permeability in nearly all treatments, consistent with Luijendijk and Gleeson (2015); this suggests that soil structure is not random but structured through self-organization (Crawford, 1994). The type of cropping system did not significantly affect this relationship except at the core scale in clay soils and aggregate scale in the sandy soils. In both cases, permeability was much greater relative to porosity under grassland than in fallow, with arable intermediate regardless of increased organic status.

5. Conclusions

This study revealed profound but contrasting effects of different agricultural management systems, and in particular the role of plants, on soil structure over the long-term and in the context of two soil textures. For both soil textures, perennial and annual plants (associated with organic input for the sandy soil) increased the diversity of pore sizes. In contrast, the effect of plants on porosity and pore connectivity was markedly different between the two soil textures: for clay soil, plants increased the porosity and the connectivity of the pore system, whereas for sandy soil, plants decreased the porosity and the pore connectivity. Hence the hypothesis that the presence of plants increases porosity requires qualification since plants contributed to soil porosity only in the presence of clay: for sandy soil, the presence of plants reduced porosity and connectivity. Our results confirmed the hypothesis that perennial plants invoked greater structural heterogeneity, manifest as a wider range of pore sizes. This study also showed that addition of manure to arable soil had essentially the same effect as continual perennial plants on the maintenance of the soil structure. Incorporation of organic matter (such as root, organic carbon) can be considered as an agent which assists soil structure to reorganise from the tillage by increasing the diversity of the pore sizes and decreasing porosity. Different crop/management systems create different kinds of soil structure, and for each there are a range of consequences depending on the function under consideration.

These data suggest that management systems generate soil structure differently, conferring to soil structure a variety of functions. The contrasting effects of increased plant presence in the two textures bear an intriguing relationship to what may be considered an optimal configuration of pore architecture in the different circumstances. In the context of a cohesive soil, here clay, plant inputs induced greater porosity, pore-connectivity and permeability, arguably advantageous to plants and the soil biome since it increases water availability via diffuse flow paths. For less cohesive soil, here represented by sand, the presence of plants decreased porosity and connectivity, which likewise is beneficial to the plant and soil biota by increasing the propensity for water storage. The inherent cohesion of the soil may alter a plant's response to its environment in terms of optimising water storage and flow at a system level.

Acknowledgements

We thank *Pr*. Hu Zhou for his assistance with the software QuantIm. This work was performed at the University of Nottingham Hounsfield facility. The University of Nottingham Hounsfield Facility receives funding from BBSRC (Swindon, UK), and The Wolfson Foundation (London, UK). Work at Rothamsted is supported by the BBSRC-funded Soil to Nutrition strategic programme (BBS/E/C/000I0310) and jointly by the Natural Environment Research Council and BBSRC as part of the Achieving Sustainable Agricultural Systems research programme (NE/N018125/1 LTS-M). Access to the Highfield and Woburn Ley-Arable experiments is supported by the UK's Long-Term Experiment National Capability funded by the Biotechnology and Biological Sciences Research Council Grant [grant number BBS/E/C/000J0300)]. We also thank Dr. Steffen Schlüter and an anonymous reviewer for their insightful suggestions for improvement of this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.geoderma.2018.07.002.

References

Ambert-Sanchez, M., Mickelson, S.K., Ahmed, S.I., Gray, J.N., Webber, D.F., 2016. Evaluating soil tillage practices using X-ray computed tomography and conventional laboratory methods. Trans. ASABE 59, 455–463. https://doi.org/10.13031/trans.59.11308.

Arvidsson, J., 1998. Effects of Cultivation Depth in Reduced Tillage on Soil Physical Properties, Crop Yield and Plant Pathogens. vol. 9. pp. 79–85.

Ball, B.C., 1981. Pore characteristics of soils from two cultivation experiments. J. Soil 32, 483–498.

Blackwell, P.S., Ringrose-Voase, A.J., Jayawardane, N.S., Olsson, K.A., McKenzie, D.C., Mason, W.K., 1990. The use of air-filled porosity and intrinsic permeability to characterise macropore structure and saturated hydraulic conductivity of clay soils. J. Soil Sci. 41, 215–228.

Canti, M.G., 2003. Earthworm activity and archaeological stratigraphy: a review of products and processes. J. Archaeol. Sci. 30, 135–148. https://doi.org/10.1006/jasc.2001.0770.

Cercioglu, M., Anderson, S.H., Udawatta, R.P., Haruna, S.I., 2018. Effects of cover crop and biofuel crop management on computed tomography-measured pore parameters. Geoderma 319, 80–88. https://doi.org/10.1016/j.geoderma.2018.01.005.

Chenu, C., 1993. Clay- or sand-polysaccharide associations as models for the interface between micro-organisms and soil: water related properties and microstructure. Geoderma 56, 143–156. https://doi.org/10.1016/0016-7061(93)90106-U.

Chenu, C., Cosentino, D., 2011. Microbial regulation of soil structural dynamics. In: Ritz, K., Young, I. (Eds.), The Architecture and Biology of Soils: Life in Inner Space. CABI, Wallingford, Oxfordshire OX10 8DE, UK, pp. 37–70.

Coleman, K., Jenkinson, D.S., Croker, G.J., Grace, P.R., Klír, J., Körschens, M., Poulton, P.R., Richter, D.D., 1997. Simulating trends in soil organic carbon in long-term experiments using RothC-26.3. Geoderma 81, 29–44.

Crawford, J.W., 1994. The relationship between structure and the hydraulic conductivity of soil. Eur. J. Soil Sci. 45, 493–502.

Crawford, J.W., Deacon, L., Grinev, D., Harris, J.A., Ritz, K., Singh, B.K., Young, I., 2012. Microbial diversity affects self-organization of the soil-microbe system with consequences for function. J. R. Soc. Interface 9, 1302–1310. https://doi.org/10.1098/rsif.2011.0679.

Dorioz, J.M., Robert, M., Chenu, C., 1993. The role of roots, fungi and bacteria on clay particle organization. An experimental approach. Geoderma 56, 179–194. https://doi.org/10.1016/0016-7061(93)90109-X.

Feeney, D.S., Crawford, J.W., Daniell, T., Hallett, P.D., Nunan, N., Ritz, K., Rivers, M., Young, I.M., 2006. Three-dimensional microorganization of the soil-root-microbe system. Microb. Ecol. 52, 151–158. https://doi.org/10.1007/s00248-006-9062-8.

Gregory, A.S., Watts, C.W., Griffiths, B.S., Hallett, P.D., Kuan, H.L., Whitmore, A.P., 2009. The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England. Geoderma 153, 172–185. https://doi.org/10.1016/j.geoderma.2009.08.002.

Gregory, A.S., Dungait, J.A.J., Watts, C.W., Bol, R., Dixon, E.R., White, R.P., Whitmore, A.P., 2016. Long-term management changes topsoil and subsoil organic carbon and nitrogen dynamics in a temperate agricultural system. Eur. J. Soil Sci. 67, 421–430. https://doi.org/10.1111/ejss.12359.

Hanson, I., Djohari, J., Orr, J., Furphy, P., Hodgson, C., Cox, G., Boradbridge, G., 2009. New observations on the interactions between evidence and the upper horizons of the soil. In: Ritz, K., Dawson, L., Miller, D. (Eds.), Criminal and Environmental Soil Forensic, pp. 239–251.

Helliwell, J.R., Miller, A.J., Whalley, W.R., Mooney, S.J., Sturrock, C.J., 2014. Quantifying the impact of microbes on soil structural development and behaviour in wet soils. Soil Biol. Biochem. 74, 138–147. https://doi.org/10.1016/j.soilbio.2014.03.009.

Helliwell, J.R., Sturrock, C.J., Mairhofer, S., Craigon, J., Ashton, R.W., Miller, A.J., Whalley, W.R., Mooney, S.J., 2017. The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. Sci. Rep. 7 (14875). https://doi.org/10.1038/s41598-017-14904-w.

Hirsch, P.R., Gilliam, L.M., Sohi, S.P., Williams, J.K., Clark, I.M., Murray, P.J., 2009. Starving the soil of plant inputs for 50 years reduces abundance but not diversity of soil bacterial communities. Soil Biol. Biochem. 41, 2021–2024. https://doi.org/10.1016/j.soilbio.20.

Hodge, C.A.H., Burton, R.G.O., Corbett, W.M., Evans, R., Seale, R.S., 1984. Soils and Their Use in Eastern England. Bull. 13. Soil Surv. Engl, Wales, Harpenden, United Kingdom.

Johnston, A.E., Poulton, P.R., Coleman, K., Macdonald, A.J., White, R.P., 2017. Changes in soil organic matter over 70 years in continuous arable and ley-arable rotations on a sandy loam soil in England. Eur. J. Soil Sci. 68, 305–316. https://doi.org/10.1111/ejss.12415.

Kravchenko, A.N., Negassa, W.C., Guber, A.K., Hildebrandt, B., Marsh, T.L., Rivers, M.L., 2014. Intra-aggregate pore structure influences phylogenetic composition of bacterial community in macroaggregates. Soil Sci. Soc. Am. J. 78, 1924. https://doi.org/10.2136/sssaj2014.07.0308.

Luijendijk, E., Gleeson, T., 2015. How well can we predict permeability in sedimentary basins? Deriving and evaluating porosity-permeability equations for noncemented sand and clay mixtures. Geofluids 15, 67–83. https://doi.org/10.1111/gfl.12115.

Naveed, M., Moldrup, P., Schaap, M.G., Tuller, M., Kulkarni, R., Vogel, H.J., De Jonge, L.W., 2016. Prediction of biopore- and matrix-dominated flow from X-ray CT-derived macropore network characteristics. Hydrol. Earth Syst. Sci. 20, 4017–4030. https://doi.org/10.5194/hess-20-4017-2016.

Neal, A.L., Rossman, M., Brearley, C., Akkari, E., Guyomar, C., Clark, I.M., Hirsch, P.R., 2017. Land-use influences phosphatase gene microdiversity in soils. Environ. Microbiol. 19, 2740–2753.

Oades, J.M., 1993. The role of biology in the formation, stabilization and degradation of soil structure. Geoderma 56, 377–400. https://doi.org/10.1016/0016-7061(93)90123-3.

Pagliaia, M., Marsilib, A., Servadiob, P., Vignozzi, N., Pellegrini, S., 2003. Changes of some physical properties of a clay soil following the passage of rubber tracked and wheeled tractors of medium power. Soil Tillage Res. 73, 119–129.

Peth, S., Horn, R., Beckmann, F., Donath, T., Fischer, J., Smucker, A.J.M., 2008. Three-dimensional quantification of intra-aggregate pore-space features using synchrotron-radiation-based microtomography. Soil Sci. Soc. Am. J. 72, 897. https://doi.org/10.2136/sssaj2007.0130.

Pires, L.F., Borges, J.A.R., Rosa, J.A., Cooper, M., Heck, R.J., Passoni, S., Roque, W.L., 2017. Soil structure changes induced by tillage systems. Soil Tillage Res. 165, 66–79. https://doi.org/10.1016/j.still.2016.07.010.

Rabot, E., Wiesmeier, M., Schlüter, S., Vogel, H.J., 2018. Soil structure as an indicator of soil functions: a review. Geoderma 314, 122–137. https://doi.org/10.1016/j.geoderma.2017.11.009.

Rossi, R., Amato, M., Pollice, A., Bitella, G., Gomes, J.J., Bochicchio, R., Baronti, S., 2013. Electrical resistivity tomography to detect the effects of tillage in a soil with a variable rock fragment content. Eur. J. Soil Sci. 64, 239–248. https://doi.org/10.1111/ejss.12024.

Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.

Tisdall, J.M., Oades, J.M., 1982. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–165.

Vogel, H.J., Kretzschmar, A., 1996. Topological characterization of pore space in soil - sample preparation and digital image-processing. Geoderma 73, 23–38. https://doi.org/10.1016/0016-7061(96)00043-2.

Vogel, H.J., Weller, U., Schlüter, S., 2010. Quantification of soil structure based on Minkowski functions. Comput. Geosci. 36, 1236–1245. https://doi.org/10.1016/j.cageo.2010.03.007.

Watts, C.W., Whalley, W.R., Longstaff, D., White, R.P., Brooke, P.C., Whitmore, a P., 2001. Aggregation of a soil with different cropping histories following the addition of organic materials. Soil Use Manag. 17, 263–268. https://doi.org/10.1079/Sum200189.

Zhang, X., Lv, M., 2007. Persistence of anomalous dispersion in uniform porous media demonstrated by pore-scale simulations. Water Resour. Res. 43, 1–11. https://doi.org/10.1029/2006WR005557.

Zhang, X., Crawford, J.W., Deeks, L.K., Stutter, M.I., Bengough, A.G., Young, I.M., 2005. A mass balance based numerical method for the fractional advection-dispersion equation: theory and application. Water Resour. Res. 41https://doi.org/10.1029/2004wr003818. (n/a-n/a).

Zhang, X., Crawford, J.W., Flavel, R.J., Young, I.M., 2016. A multi-scale Lattice Boltzmann model for simulating solute transport in 3D X-ray micro-tomography images of aggregated porous materials. J. Hydrol. 541, 1020–1029. https://doi.org/10.1016/j.jhydrol.2016.08.013.

Supplementary data – as published

Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture

Aurelie Bacq-Labreuil, John Crawford, Sacha J. Mooney, Andrew L. Neal, Elsy Akkari, Cormac McAuliffe, Xiaoxian Zhang, Marc Redmile-Gordon, Karl Ritz

Supplementary Materials and Methods A.1. Modelling the saturated hydraulic conductivity

Fig. A1. 3D representation of clay soils under different cropping systems visualised at core (40 μm resolution; a, c, e) and aggregate (1.5 μm resolution; b, d, f) scales, displayed as greyscale images denoting Hounsfield attenuation (darker shades relate to lower attenuation: (a, b) fallow; (c.d) arable; (e, f) grassland.

Fig. A2. Volume of pores normalised to the total ROI volume (mm^3) (a) at the core scale (40 μm resolution) and (b) at the aggregate (1.5 μm resolution) scale. Points indicate means, whiskers denote pooled standard errors; ● grassland ▲Arable ■ Fallow.

Fig. A3. Regression analysis in relation to management type of log porosity vs. log modelled saturated permeability for the clay soil: (a) at the core scale and (b) at the aggregate scale. Doted-line are the linear regression on the log-log values; ● grassland ▲arable ■ fallow.

Fig. A4. 3D representation of sandy soils under different cropping systems visualised at core (40 μm resolution; a, c, e, g) and aggregate (1.5 μm resolution; b, d, f, h) scales, displayed as greyscale images denoting Hounsfield attenuation (darker shades relate to lower attenuation: (a, b) fallow; (c. d) inorganically fertiliser arable; (e, f) manured arable (g, h) grassland.

Fig, A5. Volume of pores normalised to the total ROI volume (mm^3) (a) at the core scale (40 μm resolution) and (b) at the aggregate (1.5 μm resolution) scale. Points indicate means,

whiskers denote pooled standard errors; ● grassland ♦ manured arable ▲ inorganically fertilizer Arable ■ Fallow.

Fig. A6. Regression analysis in relation to management type of log porosity vs. log modelled saturated permeability for the clay soil: (a) at the core scale and (b) at the aggregate scale. Doted-line are the linear regression on the log-log values; ● grassland ♦ manured arable ▲inorganically fertiliser arable ■ fallow.

Supplementary Materials and Methods A.1. Modelling the saturated hydraulic conductivity The hydraulic conductivity of both core and aggregate samples was calculated numerically by simulating water flow through the pore geometry derived by imagery for each sample as driven by gravity. The flow process was simulated with the lattice Boltzmann model by tracking the movement and collisions of a number of fictitious particles under rules that the collisions conserve mass and momentum. We use the multiple-relaxation time (MRT) (d'Humieres *et al.* 2002) to describe the propagations of all particle distribution functions as follows:

$$f_i\left(\boldsymbol{x}+\delta t\boldsymbol{e}_i, t+\delta t\right)=f_i\left(\boldsymbol{x},t\right)+M^{-1}SM\left[f_i^{eq}\left(\boldsymbol{x},t\right)-f_i\left(\boldsymbol{x},t\right)\right], \quad (1)$$

where $f_i\left(\boldsymbol{x},t\right)$is the particle distribution function at location $\boldsymbol{x}$ and time t moving with velocity of $\boldsymbol{e}_i$, δx is the size of the voxels in the image, δt is time step, $f_i^{eq}\left(\boldsymbol{x},t\right)$ is equilibrium distribution function - the value of $f_i\left(\boldsymbol{x},t\right)$at equilibrium, M is a transform matrix and S is the collision matrix. In Eq. (1), the product Mf transforms the particle distribution functions to a moment space and the operation $m=SM\left[f_i^{eq}\left(\boldsymbol{x},t\right)-f_i\left(\boldsymbol{x},t\right)\right]$ performs the collision in the moment space. The post-collision results in the moment space are transformed back to particle distribution functions by $M^{-1}m$. In this paper, we used the D3Q19 lattice in which the particle distribution functions move in 19 directions with 19 velocities (Qian *et al.* 1992). The

collision matrix is diagonal, the terms in which used in this paper are

$$
\begin{aligned}
&S=\left(s_0,s_1,s_2,s_3,s_4,s_5,s_6,s_7,s_8,s_9,s_{10},s_{11},s_{12},s_{13},s_{14},s_{15},s_{16},s_{17},s_{18}\right)^T,\\
&s_0=s_3=s_5=s_7=0,\\
&s_1=s_2=s_{9-15}=1/\tau,\\
&s_4=s_6=s_8=s_{16-18}=8(2-\tau^{-1})/(8-\tau^{-1}),
\end{aligned}
\qquad (2)
$$

The water simulated by the above model has a kinematic viscosity of $\mu=\delta x^2(\tau-0.5)/6\delta t$ and pressure of $p=\rho\delta x^2/3\delta t^2$. The equilibrium moments $m^{eq}=Mf^{eq}$ are defined as follows

$$
\begin{aligned}
&m_0^{eq}=\rho,\\
&m_1^{eq}=-11\rho+19\left(j_x^2+j_y^2+j_z^2\right)/\rho_0,\\
&m_2^{eq}=3\rho-5.5\left(j_x^2+j_y^2+j_z^2\right)/\rho_0,\\
&m_3^{eq}=j_x,\ m_5^{eq}=j_y,\ m_7^{eq}=j_z,\\
&m_4^{eq}=-2j_x/3,\ m_6^{eq}=-2j_y/3,\ m_8^{eq}=-2j_z/3,\\
&m_9^{eq}=\left(2j_x^2-j_y^2-j_z^2\right)/\rho_0,\ \ m_{10}^{eq}=\left(2j_x^2-j_y^2-j_z^2\right)/2\rho_0,\\
&m_{11}^{eq}=\left(j_y^2-j_z^2\right)/\rho_0,\ m_{12}^{eq}=\left(j_y^2-j_z^2\right)/2\rho_0,\\
&m_{13}^{eq}=j_xj_y/\rho_0,\ \ m_{14}^{eq}=j_yj_z/\rho_0,\ \ m_{15}^{eq}=j_xj_z/\rho_0,\\
&m_{16}^{eq}=m_{17}^{eq}=m_{18}^{eq}=0.
\end{aligned}
\qquad (3)
$$

The density ρ and moment $\boldsymbol{j}$ of the water are calculated from

$$
\begin{aligned}
&\rho=\sum_{i=0}^{18}f_i,\\
&\boldsymbol{j}=\rho_0\boldsymbol{u}=\sum_{i=1}^{18}f_i\boldsymbol{e}_i,
\end{aligned}
\qquad (4)
$$

where ρ_0 is a reference density to ensure that the water simulated by the above model is incompressible when the flow reaches steady state.

Numerical implementation of the above model on the soil image involves two steps. The first one is to calculate the collision in moment space and then transform the results back to particle distribution function, i.e. to calculate $f_i^*=f_i(\boldsymbol{x},t)+M^{-1}SM\left[f_i^{eq}(\boldsymbol{x},t)-f_i(\boldsymbol{x},t)\right]$. The second step is to stream the post-collision result f_i^* to a new location at $\boldsymbol{x}+\delta t\boldsymbol{e}_i$ over a time period of δt. Whenever f_i^* hits a solid voxel during the streaming step, we used the bounce-

back method to solve it by sending f_i^* back to where it was before the streaming. Such a treatment results in a non-slip boundary at which the water velocity is zero.

For each VOI, we calculating its permeability by maintaining a thin water film on its top and then simulating its flow in the pore geometry driven by gravity. The four sides of the VOI were treated as periodic boundary in which a particle distribution function coming out of the image from one side was sent back into the image through its opposite side by keeping its mass and momentum unchanged. The initial water velocity in the simulations was zero everywhere and once the flow was deemed to have reached steady state, water velocity in all voxels were sampled. The permeability of the VOI was calculated as follows assuming that the average water flow rate across the VOI was proportional to the gravity applied to drive the water flow

$$Q = -\frac{k}{u} g, \qquad \text{(S5)}$$

where Q is the average flow rate across the image and g is the gravitational acceleration. The permeability k is calculated from

$$k = \frac{\mu}{Ng} \sum_{i=1}^{N} u_z(x_i), \qquad \text{(S6)}$$

where N is the number of voxels including the solid voxels and $u_z(x_i)$ is the vertical velocity component in the voxel centred at x_i.

References

d'Humieres, D., Ginzburg, I., Krafczyk, M., Lallemand, P. & Luo, L.-S. 2002. Multiple-relaxation-time lattice Boltzmann models in three dimensions. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences, 360, 437–451, (At: http://rsta.royalsocietypublishing.org/cgi/doi/10.1098/rsta.2001.0955.).

Qian, Y.H., Dhumieres, D. & Lallemand, P. 1992. Lattice BGK models for Navier-Stokes equation. Europhysics Letters, 17, 479–484.

Table A.1. Quantification of the stones contained in ROI volume (expressed as a percentage of stones relative to ROI volume) for the clay soil at the core scale (mean ± pooled standard error).

Treatment	n	Proportion of stone (%)
Fallow	3	15.01 (± 4.89)
Arable	4	11.23 (± 4.24)
Grassland	4	1.55 (± 4.24)
P_F		0.039

Table A.2. Quantification of the stones contained in ROI volume (expressed as a percentage of stones relative to ROI volume) for the sandy soil at the core scale (mean ± pooled standard error).

Treatment	n	Proportion of stone (%)
Fallow	4	3.89 (± 0.94)
Inorganically fertiliser arable	5	5.21 (± 0.85)
Manured arable	4	5.96 (± 0.94)
Grassland	4	3.48 (± 0.94)
P_F		0.315

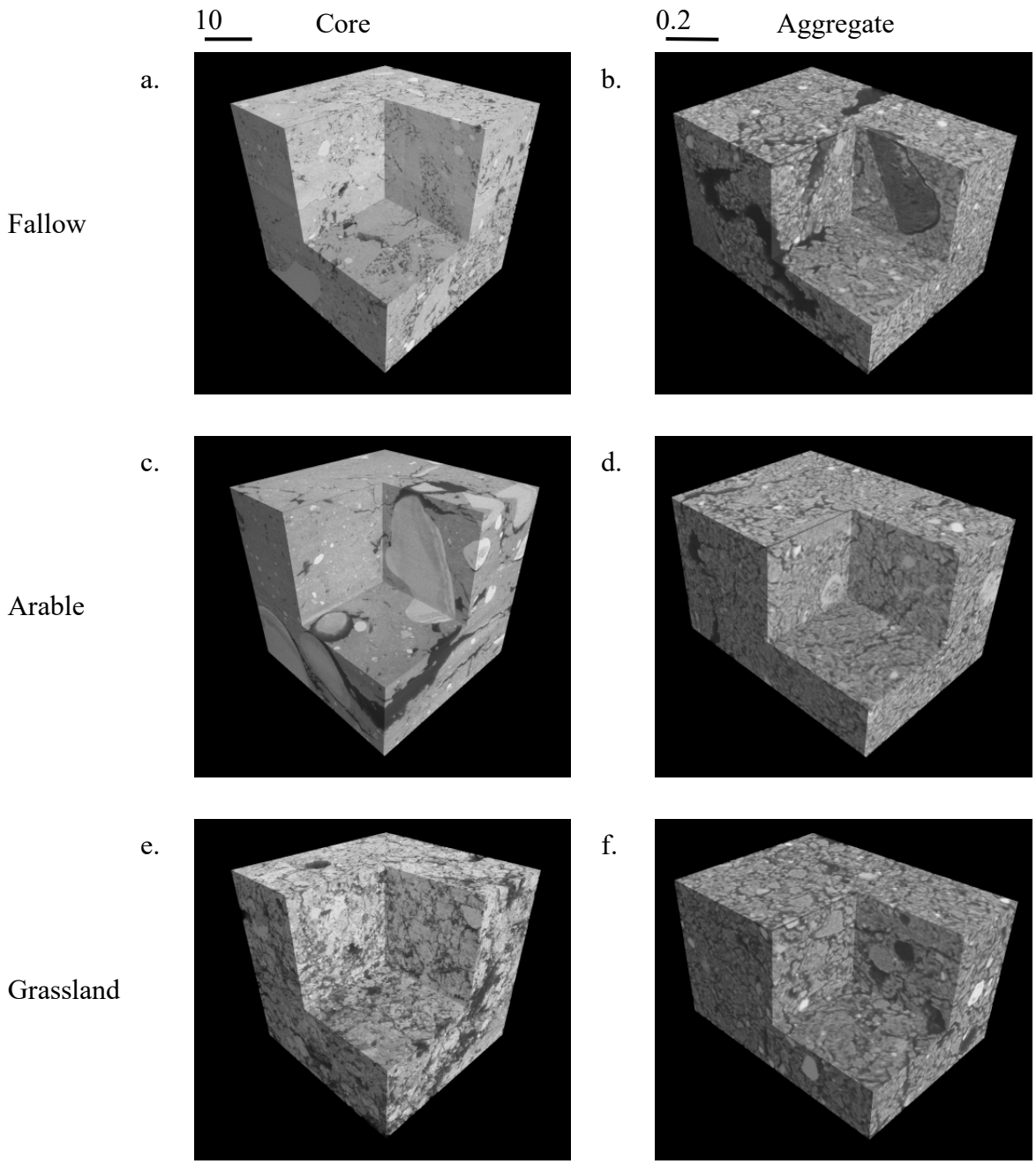


Fig. A1. 3D representation of clay soils under different cropping systems visualised at core (40 μm resolution; a, c, e) and aggregate (1.5 μm resolution; b, d, f) scales, displayed as greyscale images denoting Hounsfield attenuation (darker shades relate to lower attenuation: (a, b) fallow; (c.d) arable; (e, f) grassland.

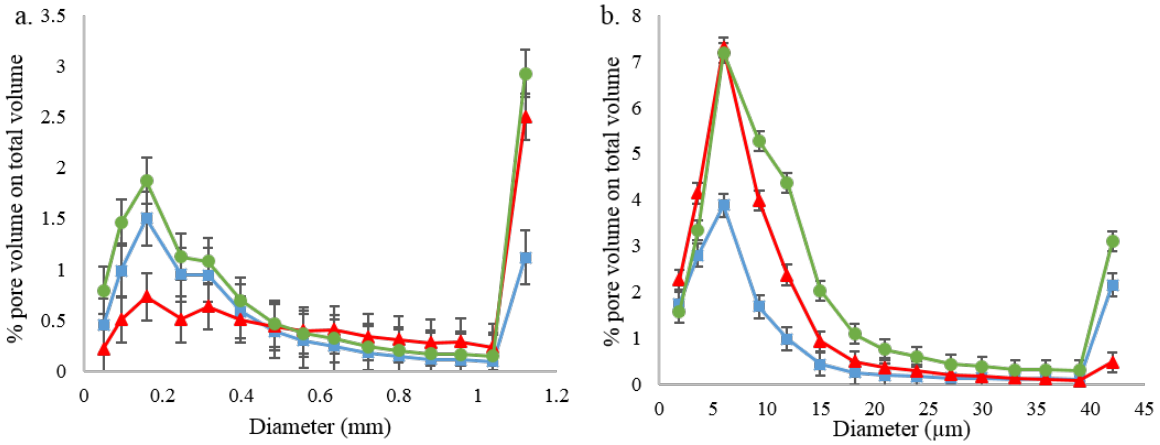


Fig. A2. Volume of pores normalised to the total ROI volume (mm³) (a) at the core scale (40 µm resolution) and (b) at the aggregate (1.5 µm resolution) scale. Points indicate means, whiskers denote pooled standard errors; ● grassland ▲Arable ■ Fallow.

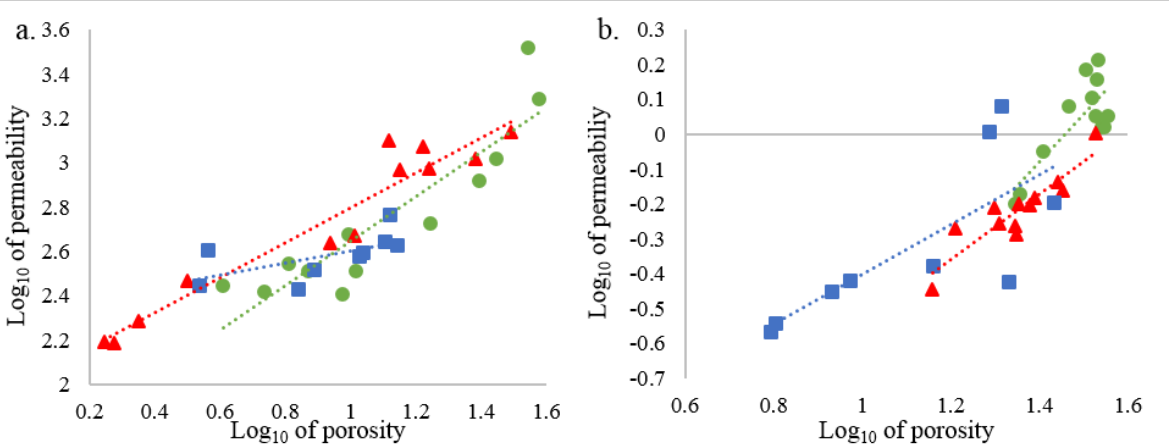


Fig. A3. Regression analysis in relation to management type of log porosity vs. log modelled saturated permeability for the clay soil: (a) at the core scale and (b) at the aggregate scale. Doted-line are the linear regression on the log-log values; ● grassland ▲arable ■ fallow.

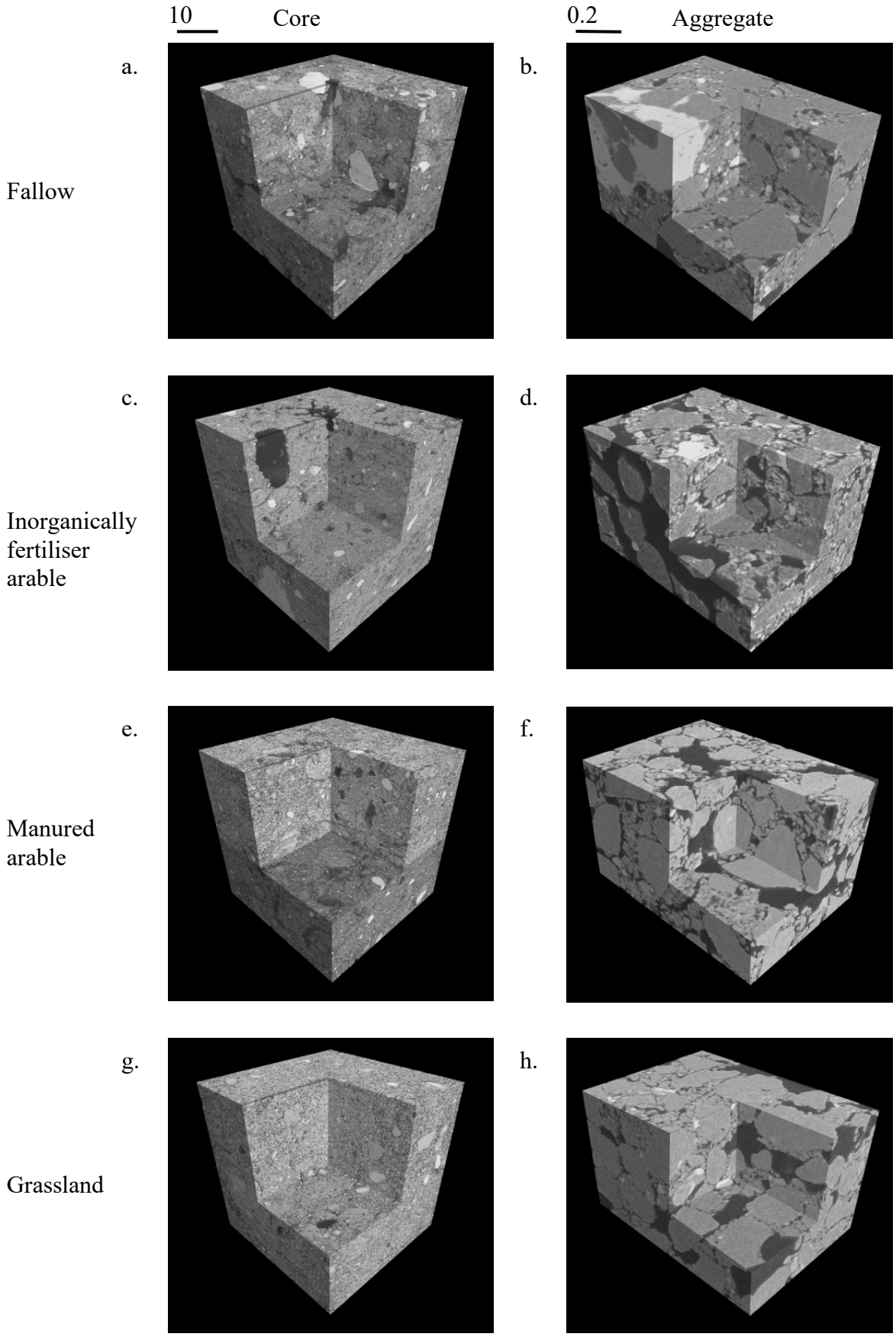
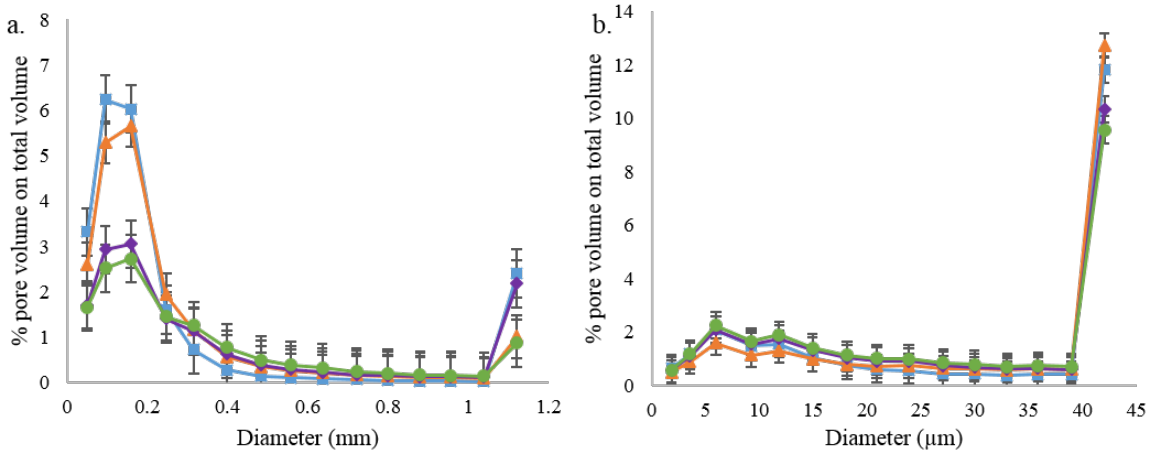

10
Core
0.2
Aggregate
a.
b.
Fallow
c.
d.
Inorganically fertiliser arable
e.
f.
Manured arable
g.
h.
Grassland

Fig. A4. 3D representation of sandy soils under different cropping systems visualised at core (40 µm resolution; a, c, e, g) and aggregate (1.5 µm resolution; b, d, f, h) scales, displayed as greyscale images denoting Hounsfield attenuation (darker shades relate to lower attenuation: (a, b) fallow; (c. d) inorganically fertiliser arable; (e, f) manured arable (g, h) grassland.



Fig, A5. Volume of pores normalised to the total ROI volume (mm³) (a) at the core scale (40 µm resolution) and (b) at the aggregate (1.5 µm resolution) scale. Points indicate means, whiskers denote pooled standard errors; ● grassland ♦ manured arable ▲ inorganically fertilizer arable ■ fallow.

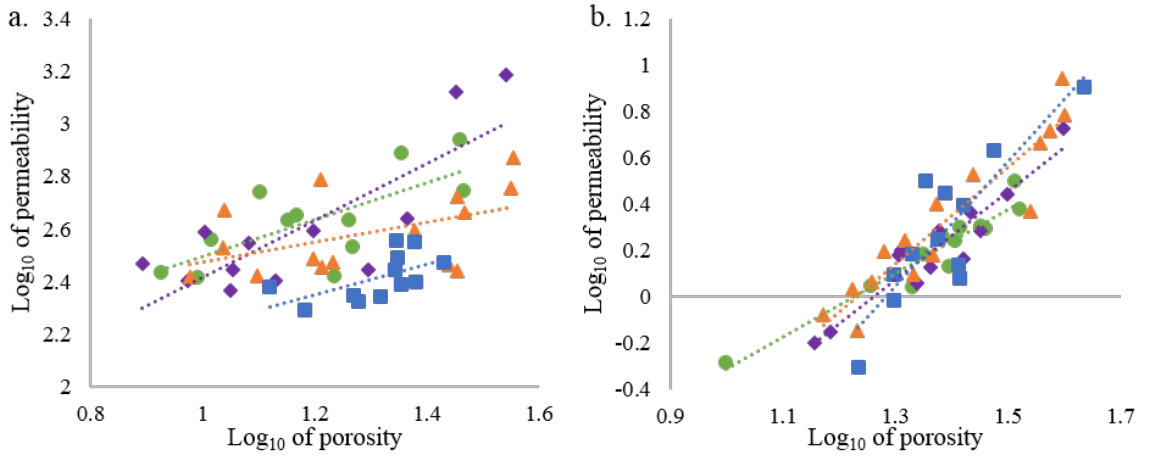


Fig. A6. Regression analysis in relation to management type of log porosity vs. log modelled saturated permeability for the clay soil: (a) at the core scale and (b) at the aggregate scale.

Doted-line are the linear regression on the log-log values; ● grassland ♦ manured arable ▲ inorganically fertiliser arable ■ fallow.

This chapter is structured and formatted in accordance with specification for the European Journal of Soil Science.

Current status is: to be submitted.

Chapter 3: Structural recovery of a long-term fallow soil in response to annual or perennial cropping requires at least 10 years after conversion

Aurelie Bacq-Labreuil[a], John Crawford[b], Sacha J. Mooney[a], Andrew L. Neal[b], Elsy Akkari[b], Karl Ritz[a*]

[a] *Division of Agriculture & Environmental Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK*

[b] *Sustainable Agriculture Science, Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK*

*Author contributions: A.B.L. led and conducted the experimental work, analysed the experimental results, drafted the manuscript and coordinated the revisions. K.R., S.J.M., and A.N. contributed advice on the analyses. All authors contributed to the revision of the manuscript. K.R. and S.J.M. supervised the overall project. All authors give final approval for publication.

3.1. Abstract

Agricultural practices can have significant effects on soil physical and biological properties. Crop rotation, modification of the cropping system, can lead to marked impacts on these properties and enhance plant growth, sequestration of carbon and soil structure. The aim of this study was to understand how the structure of a compromised soil, arising from a long-term bare fallow period, was modified by changing the field management. Three conversion plots were studied using aggregates collected from the converted field of a long-term experiment: bare fallow, bare fallow converted to arable, and bare fallow converted to grassland. We hypothesised that: plant inputs impacts on the modification of pore structure via an increase of porosity, diversity of pore sizes and pore connectivity; and the effect of plants exerts a rapid recovery of soil structure after conversion. Soil structure was assessed by X-ray Computed Tomography at a 1.5 μm resolution (i.e. the aggregate scale). The greatest presence of plants, here represented as the grassland system, increased porosity, diversity of pore sizes, pore-connectivity and pore-surface density. However, soil structure recovery, at this scale, required a long time after conversion to show any effect of plant presence (approximately 10 years) except for the pore size distribution which was modified by plants only 2 years after conversion. Moreover, the magnitude of the plant effect was low, thus the full recovery of the soil structure characteristics might require a longer time.

3.2. Keywords

Soil structure, soil recovery, 3D pore characteristics, cropping systems, X-ray computed tomography, porosity

3.3. Introduction

Agricultural practices applied for decades to soil can have beneficial or detrimental effects on soil physical and biological properties, depending on the nature of such practices (Pagliai *et al.* 2004; Bronick and Lal 2005; Denef *et al.* 2009; Ashworth *et al.* 2017). Agricultural management generally aims to increase, or at least stabilise the yield every year, but intensive farming can lead to soil erosion, compaction and pollution (Bronick and Lal 2005). Moreover, conventional tillage can lead to decline of soil aggregation and soil structure (Watts *et al.* 2001), and a depletion of nutrients and organic carbon within soil (Coleman *et al.* 1997). The addition of organic matter or crop rotations can prevent soil disruption from tillage via improving soil porosity and aggregation (Pagliai *et al.* 2004; Abdollahi *et al.* 2014). Modification of the cropping management can have beneficial impacts on soil properties. For example, after 50 years of continuous cultivation, a desert aeolian sandy soil was turned into a sustainable agricultural soil via the increase of soil organic matter and the content of silt and clay (caused by the irrigation) leading to an increase of aggregation (Su *et al.* 2010).

Furthermore, microbial biomass – a fundamental component of soil fertility - is highly sensitive to tillage (Ashworth *et al.* 2017). The composition of the bacterial communities are more closely related to soil characteristics (such as pH and soil texture) than cropping management, and fungal community is more associated with the nutrients within the soil than the cropping management (Lauber *et al.* 2008). By contrast another recent study showed that crop

management did not have any impact on the microbial community structure, but had an effect on the distribution of genes coding for different functions (Neal *et al.* 2017). This study focused on phosphatase gene coding, and showed that bare-fallow soil contained more genes coding for extracellular and outer-membrane enzymes compared to the grassland and arable treatments, leading to a community with greater foraging functions to utilised nutrients from a higher distance under the bare-fallow (Neal *et al.* 2017). Moreover, different management practices can have a substantial impact on soil structural dynamics (Bacq-Labreuil *et al.* 2018). After 50 years under a bare fallow treatment, soil structure was compromised (Bacq-Labreuil *et al.* 2018) with carbon and nitrogen markedly decreased, as well as the population of the biota (Hirsch *et al.* 2009). The conversion from bare fallow to arable and grassland increased the organic carbon, soil nitrogen and the population of meso-fauna and fungi within 2 to 4 years after conversion (Hirsch *et al.* 2017). However, soil structure modification was not assessed in this experiment.

The aim of the study we report here was to understand how the structure of a compromised soil was modified by changing the field management. Three conversion plots were studied from the converted field of the long-term Highfield experiment, based at Rothamsted Research: bare fallow, bare fallow converted to arable, and bare fallow converted to grassland. We hypothesised that: (1) plant inputs are an active factor in the modification of pore structure via the increase of porosity, diversity of pore sizes and pore connectivity, and (2) plants exerts a rapid change, on soil structural properties.

3.4. Materials and Methods

3.4.1. Aggregates samples

Sample were obtained from the conversion plots from the long-term experiment Highfield Ley-Arable experiment (LATLONG 51.8103N, -0.3748E) on a silty-clay loam textured soil developed on clay-with-flints over Eocene London Clay (Batcome series) and classified as a Chromic Luvisol by FAO criteria. In 2008, each long-term treatment (bare fallow, arable and grassland) managed for more than 40 years were converted into the two other managements and every two or three years, all the plots were sampled, aggregates were air-dried and sieved to 2 mm before storage in the archive. Aggregates from bare fallow (Bf), bare fallow converted into arable (Bf-A) and bare fallow converted to grassland (Bf-G) were selected from the years; 2008, 2010, 2012, 2015 and 2018. The replication of treatments was: 3 plots per treatment and 3 aggregates per plot were randomly selected to be scanned, therefore a total of 9 scanned aggregates per year and per treatment.

3.4.2. X-ray Computed Tomography

Aggregates were scanned using a Phoenix Nanotom® (GE Measurement and Control solution, Wunstorf, Germany) set at a voltage of 90 kV, a current of 65 µA and at a resolution of 1.50 µm. A total of 1,440 projection images were taken at 500 ms period using an averaging of 3 images and skip of 2. The total scan time per sample was 60 minutes. Scanned images were reconstructed using Phoenix datos | x2 rec reconstruction software. They were optimised to correct for any movement of the sample during the scan and reduced noise using the beam hardening correction algorithm, set at 8.

3.4.3. Image analysis

Image analysis was performed using two software packages, ImageJ (Schneider *et al.* 2012) and QuantIm (Vogel *et al.* 2010) following the method from Bacq-Labreuil *et al.* (2018). QuantIm was used to output the 3D characteristics of the pore network calculated from the Minkowski functions where porosity is the percentage of pores >1.5 μm; pore size distribution is the proportion of each size class in the volume normalised to the total pore volume, expressed here as a cumulative value; pore connectivity calculated from the Euler number was normalised to the total volume (the more negative the Euler number is, the greater the pore-connectivity is); the pore surface density represents the roughness of the surface of pores: a lower surface density means a lower roughness, i.e. less surface to be colonised by living organisms (Vogel *et al.* 2010). The Gini-coefficient is applied in economics research to measure the statistical dispersion representing the income or wealth distribution of a population, commonly used as a measurement of inequality (Bellù and Liberati 2006). Here, the Gini-coefficient was applied to measure the distribution of pore size classes as a pointer of the equality of pore size distribution: a Gini-coefficient close to 0 represents an equal distribution of the pore sizes amongst all pore sizes and a Gini-coefficient close to 1 represents an unequal distribution of pore sizes.

3.4.4. Statistical analysis

A standard analysis of variance (ANOVA) was performed using Genstat v 17.1 (VSN International Ltd 2014) on the porosity. A two-way ANOVA was

conducted on all the Minkowski characteristics divided by year using a split plot design with the treatment and the diameter of pores as factors. An analysis of co-variance (ANCOVA), for the porosity and the Gini-coefficient, was also calculated between the Bf-A and Bf-G with time as a co-variate using Past (Hammer *et al.* 2001).

3.5. Results

3.5.1. Visual appearance of soil structures

Representative 2D images showed that in 2008 and 2010, all three treatments had a similar pore architecture (Fig. 3.1.a-f). In 2012, Bf-A and Bf-G started to display different pore architecture manifest by a greater proportion of large pore (>40 μm; Fig. 3.1.g-i). The evolution of the pore characteristics over time was apparent, in 2015 and 2018 for the Bf-A and Bf-G treatments (Fig. 3.1.j-o).

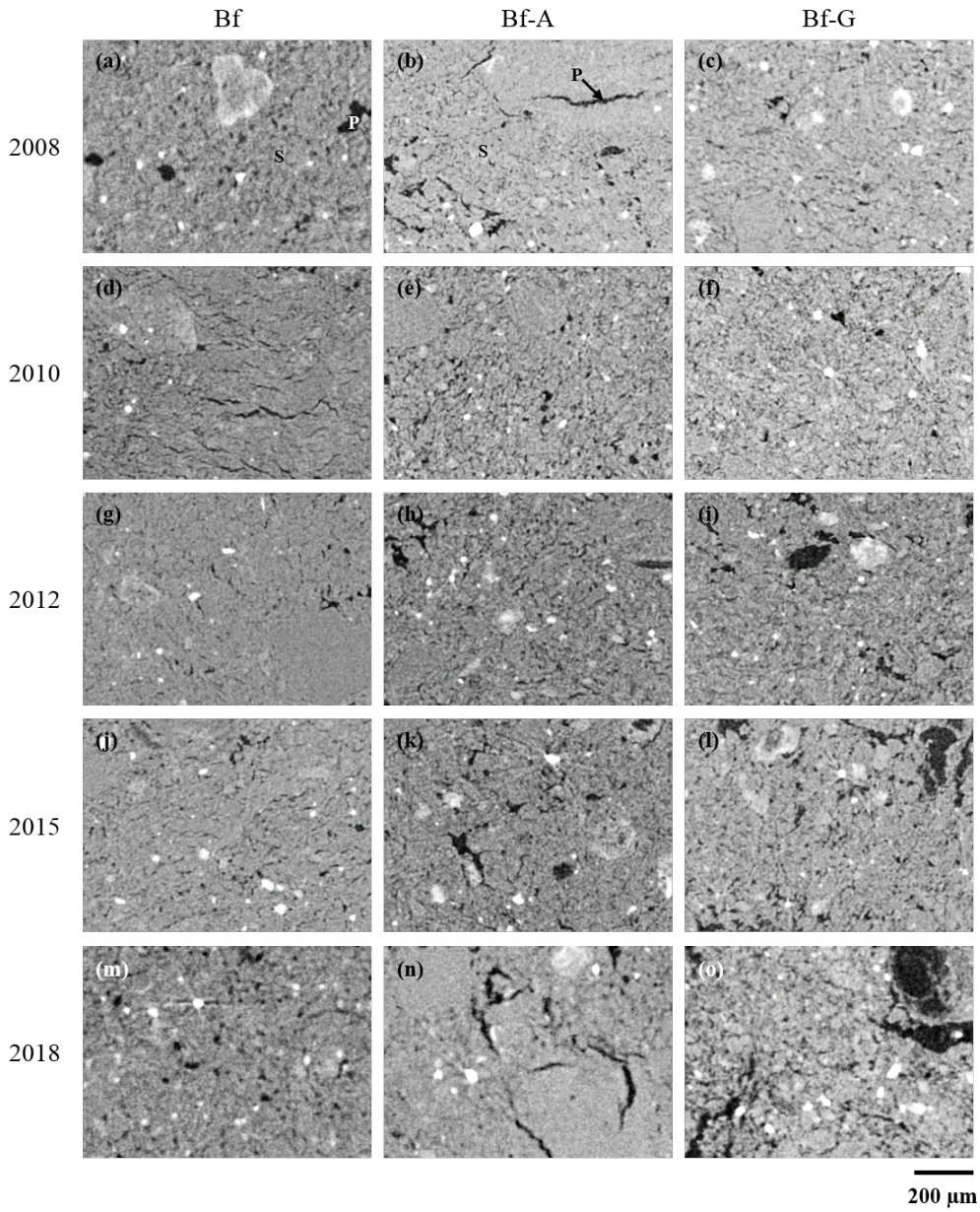


Figure 3.1.: Representative 2D X-ray attenuation images of soils subjected to different forms of management over a ten year period after conversion to these treatments. Base resolution is 40 µm; (P) pores are the darker shades and (S) soil matrix are the lighter shades which relates to the attenuation of the X-ray (a sharpening algorithm has been passed over these images to increase contrast of features); (a, d, g, j, m) bare fallow; (b, e, h, k, n) bare fallow to arable; and (c, f, i, l, o) bare fallow to grassland.

3.5.2. Porosity

In 2008, 2010 and 2012, there was no significant treatment effect with regards to the porosity (*P>0.05*; Fig. 3.2.a-c) compared to 2015 and 2018 (respectively *P=0.029* and *P=0.002*; Fig. 3.2.d, e). In 2015, the porosity of Bf-G was greater than the porosity Bf and Bf-A which was congruent (Fig. 3.2.d). However, in 2018, porosity increased in the treatment in the presence of plants ranking from Bf < Bf-A < Bf-G (Fig. 3.2.e). ANCOVA analysis revealed that porosity under grassland was significantly greater than arable (*P<0.001*), however, the rate of the increase was not significantly different between both treatments (*P>0.05*, Supplementary Fig. 3.1.).

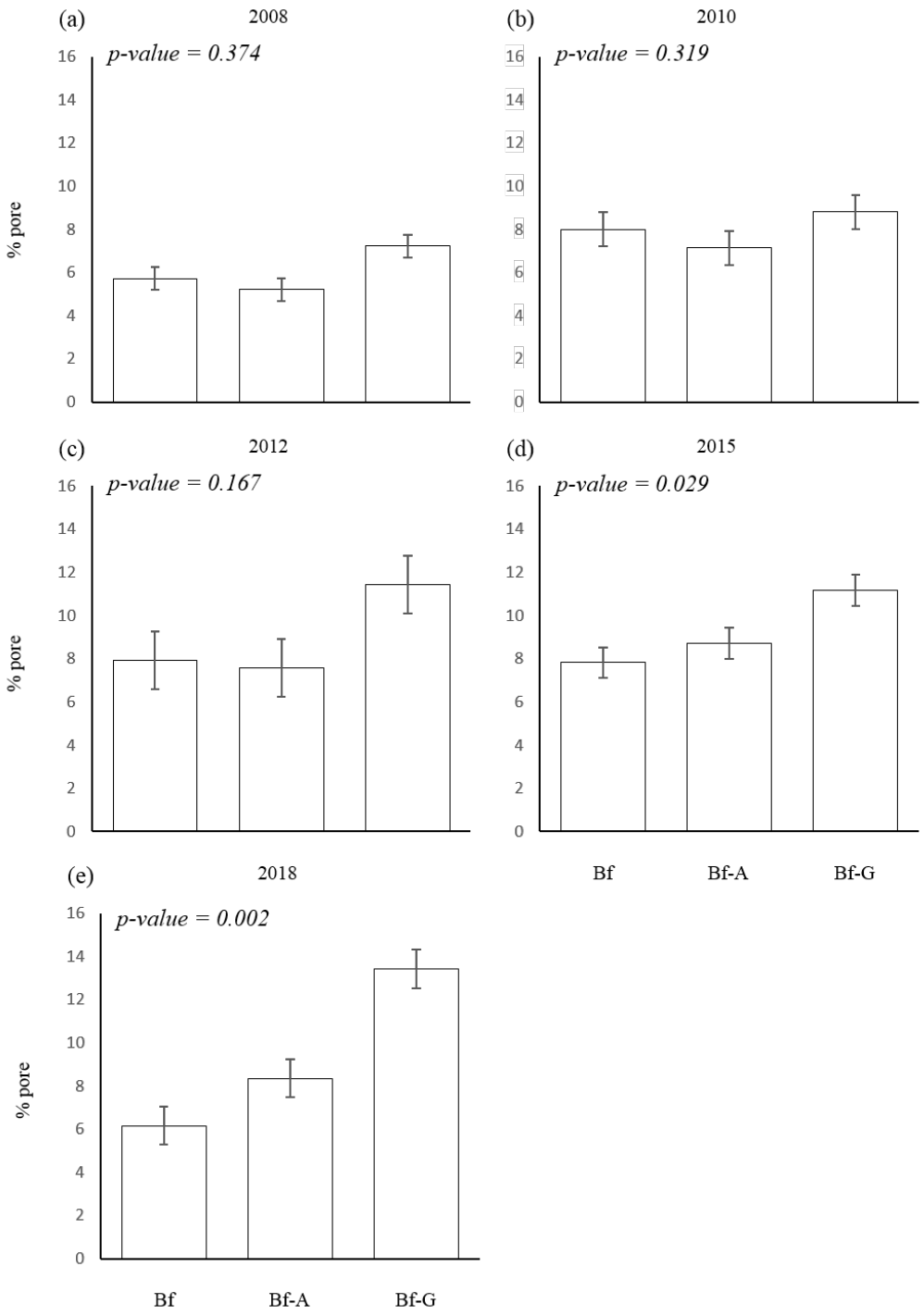


Figure 3.2.: Porosity (based on resolution of 1.5 µm) in relation to bare fallow (Bf), bare fallow converted to arable (Bf-A) and bare fallow converted to grassland (Bf-G) in regards to the years: (a) 2008; (b) 2010; (c) 2012; (d) 2015; (e) 2018. Bar charts were means (n=9) expressed as the percentage of

pores relative to the total volume, whiskers denote pooled standard errors p-values from one-way ANOVA.

3.5.3. Pore size distribution

In 2008, there was no significant treatment effect on the cumulative pore size distribution (*P>0.05*; Fig. 3.3.a). Between 2010-2018, there was a significant diameter x treatment interaction with respect to the cumulative pore size distribution (2010 and 2015: *P<0.001*; 2012 and 2018: *P<0.05*; Fig. 3.3.b-e). In 2010, there was a greater proportion of smaller pores under Bf and Bf-A treatment than Bf-G: for Bf and Bf-A, approximately 50% of pores were <3.56 µm and 70% of pores <5.97 µm compared to Bf-G where 50% of pores were <5.97 µm and 70% <14.9 µm. Moreover, the proportion of pores >42 µm was greater under Bf-G (13% of pores) than Bf and Bf-A (respectively 1% and 2% of pores; Fig. 3.3.b). In 2012, this trend was not apparent: the difference between Bf-G compared to Bf and Bf-A was less significant than in 2010. The proportion of pores <9.26 µm was greater under Bf and Bf-A compared to Bf-G but the proportion of pore >42 µm was not significant between all treatments (Fig. 3.3.c). In 2015, the trend observed in 2010 was more apparent: the proportion of pore sizes <14.9 µm was greater ranking from Bf > Bf-A > Bf-G and the proportion of pore sizes >42 µm was greater under Bf-A and Bf-G (respectively 7% and 10% of pores) than Bf (2% of pores; Fig. 3.3.d). In 2018, this trend was also observed, but only for the pore sizes <9.26 µm, where the proportion of pores was ranking from Bf > Bf-A > Bf-G (Fig. 3.3.e). Beyond this pore size, the proportion of pore sizes was not significantly different between Bf and Bf-A. The proportion of pore sizes >42 µm was greater under

Bf-G (15% of pores) than Bf and Bf-A (respectively 4% and 2% of pores; Fig. 3.3.e).

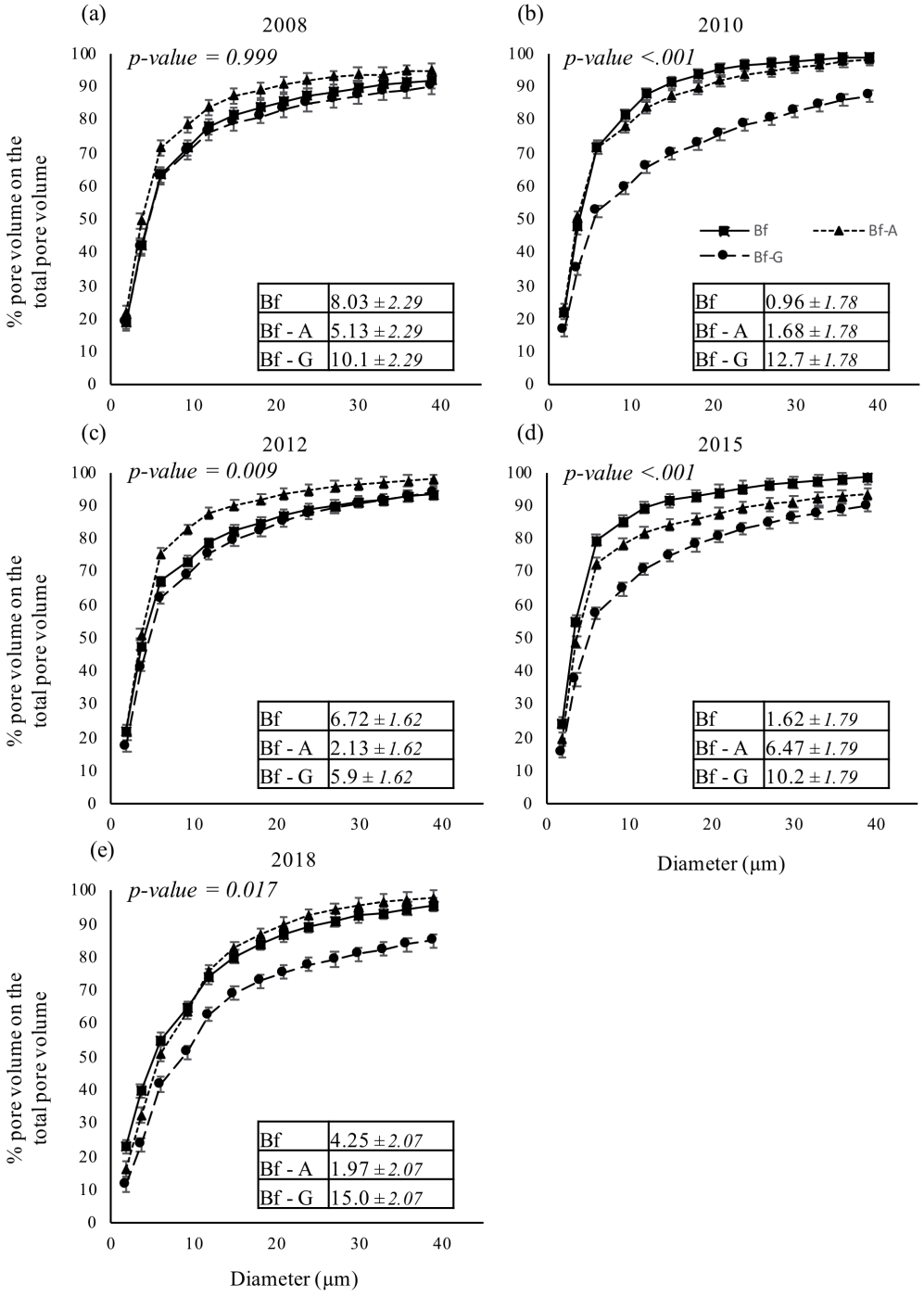


Figure 3.3.: Cumulative pore size normalized to the total pore volume in relation to Bf, Bf-A and Bf-G in regards to the years: (a) 2008; (b) 2010; (c) 2012; (d) 2015; (e) 2018. Points indicate means (n=9), whiskers denote pooled

standard errors, p-values from two-way ANOVA describe treatment and diameter interaction.

The Gini-coefficient was significantly lower for Bf-G compared to Bf-A (*P<0.001*), but the rate of the decrease between Bf-G and Bf-A was not significantly different (*P>0.05*; Supplementary Fig. 3.2.).

3.5.4 Pore connectivity

In 2008 and 2015, there was no significant diameter x treatment interaction with regards to pore connectivity (*P 0.05*; Fig. 3.4.a, d). However, there was a significant diameter x treatment interaction for the year 2010, 2012 and 2018 (with 2010 and 2018: *P<0.001*; 2012: *P<0.05*; Fig. 3.4.b, c, e). In 2010 and 2012, the difference was significant only for the pore sizes <3.56 µm. In 2010, pore connectivity was greater ranking from Bf > Bf-G > Bf-A (Fig. 3.4.b) and in 2012, pore connectivity was greater under Bf-A and Bf-G than Bf (Fig. 3.4.c). In 2018, the same trend as 2012 was shown with a greater difference in the values (Fig. 3.4.e).

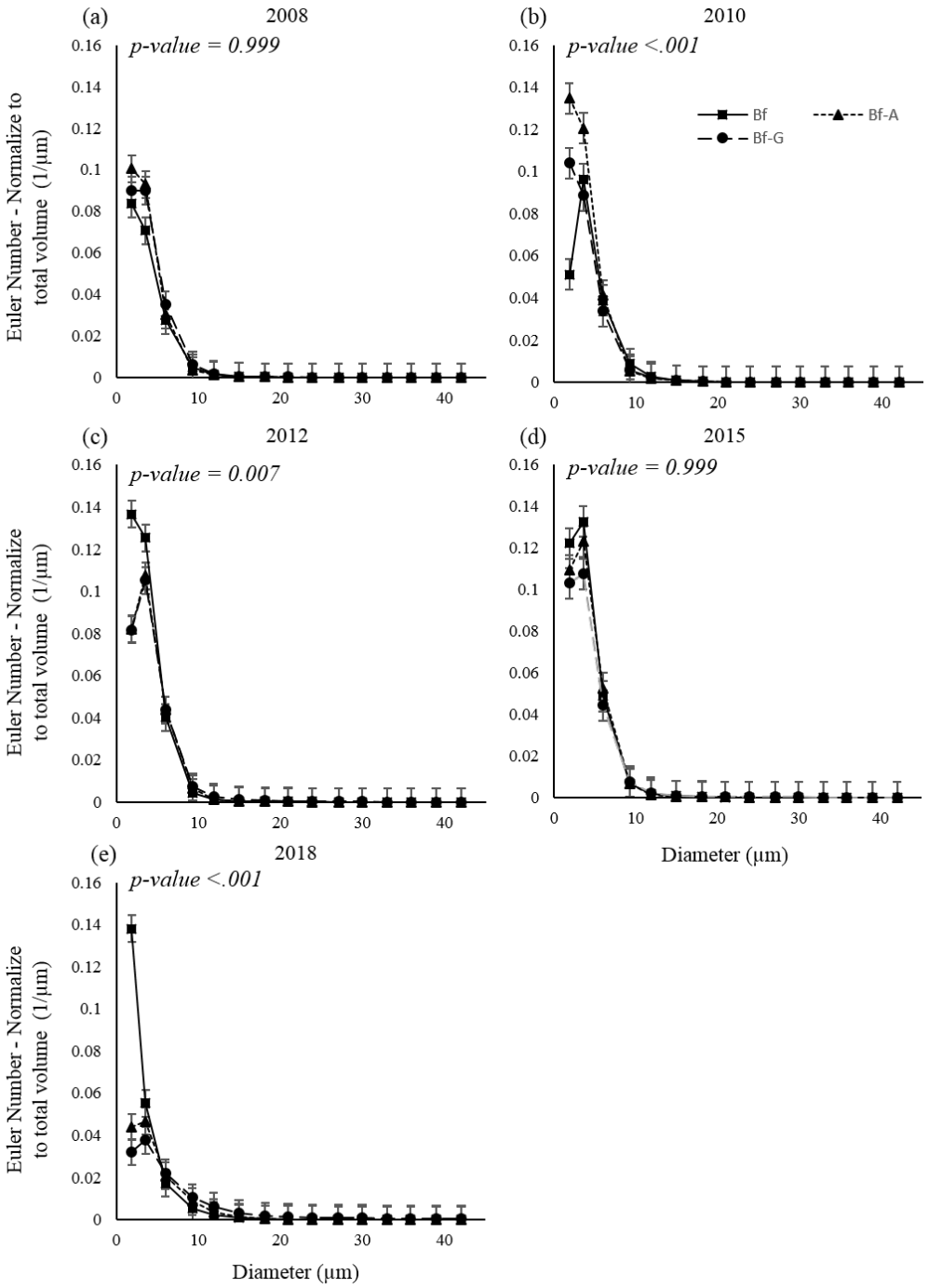


Figure 3.4.: Pore connectivity normalize to total volume in relation to Bf, Bf-A and Bf-G in regards to the years: (a) 2008; (b) 2010; (c) 2012; (d) 2015; (e) 2018. Points indicate means (n=9), whiskers denote pooled standard errors, p-values from two-way ANOVA describe treatment and diameter interaction.

3.5.5. Pore surface density

For the year 2008, 2012 and 2015, there was no significant diameter x treatment interaction with respect to pore surface density (*P>0.05*; Fig. 3.5.a, c, d). There was a significant diameter x treatment interaction for the year 2010 and 2018 (respectively *P<0.05* and *P<0.001*; Fig. 3.5.b, e). In 2010, the difference in pore surface density was greater ranking from Bf > Bf-A > Bf-G for the pore sizes equal to 1.86 µm, and the difference between Bf-A and Bf-G was not significant for the pore sizes equal to 3.56 µm. Beyond this pore size, there was no significant difference between treatments (Fig. 3.5.b). In 2018, pore surface density was greater ranking from Bf-G > Bf-A > Bf, for all pore sizes <14.9 µm, there was no significant difference beyond this pore size (Fig. 3.5.e).

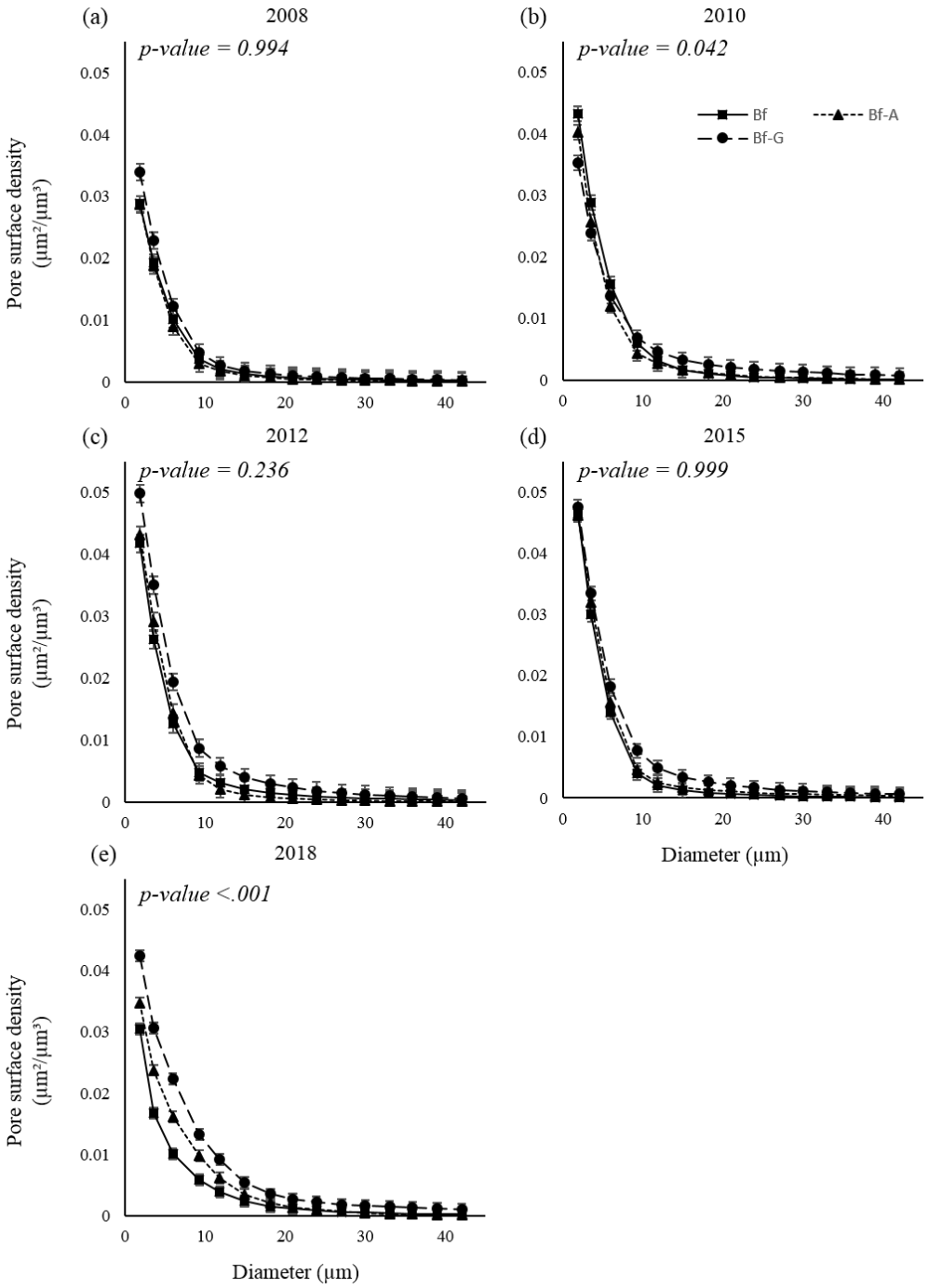


Figure 3.5.: Surface density in relation to Bf, Bf-A and Bf-G in regards to the years: (a) 2008; (b) 2010; (c) 2012; (d) 2015; (e) 2018. Points indicate means (n=9), whiskers denote pooled standard errors, p-values from two-way ANOVA describe treatment and diameter interaction.

3.6. Discussion

The conversion plots were derived from long term bare-fallow management and converted to arable and grassland. The non-significance of treatment effect on the porosity until 2015 showed that the modification of the micro-porosity took a long time to recover, at this scale (Fig. 3.2.). Despite the re-population of the meso-fauna after 2 years of conversion and the increase of soil organic matter and microbial community after 4 and 2 years respectively (Hirsch *et al.* 2017), the recovery of the porosity at the micrometre was longer. This might be related to the carbon cycling processes which is modified by the microbial communities and plants (via decomposition of organic matter and rhizodeposition). In turn, this would likely have affected the soil structure at the microscale but the effect was not instantaneous. The greater recovery of the porosity under grassland compared to arable was consistent with a previous study, which showed the greater resistance and recovery of soil structure from the arable treatment to a physical stress (Gregory *et al.* 2009). They posited that the greater proportion of organic matter enhanced the elastic recovery of the soil structure from a physical stress (Gregory *et al.* 2009).

In comparison, the pore size distribution had a more rapid change than the porosity, after only 2 years of conversion (in 2010), a greater diversity of pore sizes was observed under the grassland treatment, and this trend was also recorded in the 2015 and 2018 data (Fig. 3.3.). Here, the Gini-coefficient showed that the grassland had a more homogeneous distribution of pore sizes than the other treatments, thus the grassland treatment had a greater diversity of pores for 2010, 2015 and 2018 (Supplementary Fig. 3.2.). This increase in diversity of pore sizes might be due to the increase of presence of plants, living organisms and organic matter (Hirsch *et al.* 2017) and the cessation of tillage.

Moreover, plants increase aggregation (Chan and Heenan 1996; Haynes and Beare 1997). Plants can also break down large aggregates (Materechera *et al.* 1994; Chan and Heenan 1996) by growing through existing pores in the large aggregates which disrupt large aggregates. However, plants enmesh soil particles from the large aggregates (Tisdall and Oades 1982), and release mucilage that can bind to the soil particles as the organic matter which in turn stabilise new aggregates and pores (Chenu *et al.* 2000; Bronick and Lal 2005). Furthermore, addition of organic matter can increase the transmission (50 – 500 μm) and the storage (0.5 – 50 μm) pores and decrease the macro-pores (> 500 μm), thus the action of plants and the increase of organic matter increases the proportion of pores between 0.5 to 500 μm (Metzger and Yaron 1987; Watts and Dexter 1997) leading to a more homogeneous distribution of pore sizes, i.e. a greater diversity of pore sizes. Thus, the grassland treatment decreased the relative proportion of storage pore and increase the relative proportion of transmission pores (Fig. 3.3.) which could be beneficial for the transport of water and nutrient. Furthermore, the greater diversity of pore sizes under the grassland was consistent with a previous study looking at the long-term effect of grassland on the same field experiment (Bacq-Labreuil *et al.* 2018). In 2012, the pore size distribution did not follow this trend (Fig. 3.3.c), which could be due to the weather conditions prior to sampling in that specific year. Indeed, 2012 and 2008 were the wettest years during the experimental period (Supplementary Fig. 3.3.). In presence of water, clay particles can swell, and the compression of entrapped air in capillary pores can disturb the pore architecture, in turn disrupting the aggregation (Grant and Dexter 1990; Denef *et al.* 2001). Therefore, the pore network can be re-structured upon re-wetting

due to the nature of soil particles. Changes in pore size between 2010, 2012 and 2015 raised the question of the dynamics of this mechanism. The pore size distribution appears to have a non-linear response over time due to the impact of the wet year in 2012, which shows the rapid recovery of the pore size distribution after a sustained wet period compared to the impact of plant growth.

For the pore connectivity, the magnitude of the plant effect in 2010 and 2012 was very small compared to 2018 (Fig. 3.4.b, c, e). However, in 2018, for the arable and grassland, the pore connectivity was still low (Fig. 3.4.e), suggested the pore network was less connected compared to a previous study (Bacq-Labreuil *et al.* 2018), meaning that pore connectivity may require more than 10 years to be recovered. A greater connection of pores allows the water, gas and nutrient flows within the pore structure (Tisdall and Oades 1982; Dexter 1988). Therefore, the subtle increase in the pore connectivity might increase the water, gas and nutrient movement within the soil. As well as the pore connectivity, the pore surface density was significantly impacted by the presence of plants after 10 years since conversion (Fig. 3.5.e). Our results are congruent with the previous study (Bacq-Labreuil *et al.* 2018), showing that plant increased pore surface density, i.e. plants increased the pore-solid interface which led to a greater surface of the pore where micro-organisms and plant roots can colonise and water films can develop. This can lead to the formation of new habitat and niches which can be beneficial for microbial community diversity (Holden 2011). Moreover, the greater surface of pores might increase the water and nutrient uptake by the microbial community and plants.

This study has shown that the overall impact of the presence of plants required at least 10 years after conversion before being effective in terms of the recovery of soil structure. In general, the recovery the biological components and organic matter (Griffiths *et al.* 2000; Hirsch *et al.* 2017) were more rapid than the recovery of soil structure (Gregory *et al.* 2009). The pore size distribution was the only characteristic which was more sensitive to changes such as wet and dry cycles, living organisms.

3.7. Conclusions

Soil structural recovery of a compromised soil requires at least 10 years of a new management before showing any effects of plant presence. The first hypothesis was validated as porosity, diversity of pore sizes, pore connectivity, and pore surface density were enhanced by the presence of plants. The presence of plants increased the diversity of pore sizes after only 2 years since conversion compared to all the other Minkowski functions which only showed recovery after 7 to 10 years after conversion, which validated the second hypothesis for the pore size distribution and invalidated it for all the other Minkowski functions. The mechanisms behind the recovery of pore sizes appeared to be dynamic over the years and dependent of the weather condition before sampling. Apart from the pore size distribution, the magnitude of the plant effects on all the other Minkowski functions was lower than the difference observed after 50 years of management (Bacq-Labreuil *et al.* 2018). In this study, the effect of grassland upon porosity and pore connectivity were twice greater than the bare fallow treatment. Here, the difference was significant but not as major, which means that soil structure requires more time to fully recover its micro-structure after being converted to grassland and

arable. Therefore, the use of bare fallow for this extreme period (> 50 years) is detrimental for the physical and biological characteristics and the recovery of the soil structure requires more than 10 years. This observation raises the question about the application of certain managements in agricultural practices. For example, instead of applying a bare fallow treatment in a crop rotation, it would be beneficial for the soil characteristics to apply a vegetation cover which increases the organic matter inputs and impact on soil structure, leading to a 'conditioning' of soil physical and biological characteristics for the next crop. This would prevent the further degradation of the soil and also help for its recovery if the soil characteristics were compromised. Moreover, the recovery of the soil is a long process, thus a modification of cropping managements might require some time before the observation of beneficial impacts on soil structural dynamics. Therefore, this should be accounted for the future research and conclusions.

3.8. Acknowledgements

This work was performed at the University of Nottingham Hounsfield facility. The University of Nottingham Hounsfield Facility receives funding from BBSRC (Swindon, UK), and The Wolfson Foundation (London, UK). Work at Rothamsted is supported by the BBSRC-funded Soil to Nutrition strategic programme (BBS/E/C/000I0310) and jointly by the Natural Environment Research Council and BBSRC as part of the Achieving Sustainable Agricultural Systems research programme (NE/N018125/1 LTS-M). Access to the Highfield Ley-Arable experiments is supported by the UK's Long-Term

Experiment National Capability funded by the Biotechnology and Biological Sciences Research Council Grant [grant number BBS/E/C/000J0300)].

3.9. References

Abdollahi, L., Munkholm, L. J., and Garbout, A. (2014). Tillage System and Cover Crop Effects on Soil Quality: II. Pore Characteristics. Soil Sci. Soc. Am. J. 78, 271. doi:10.2136/sssaj2013.07.0302.

Ashworth, A. J., DeBruyn, J. M., Allen, F. L., Radosevich, M., and Owens, P. R. (2017). Microbial community structure is affected by cropping sequences and poultry litter under long-term no-tillage. Soil Biol. Biochem. 114, 210–219. doi:10.1016/j.soilbio.2017.07.019.

Bacq-Labreuil, A., Crawford, J., Mooney, S. J., Neal, A. L., Akkari, E., McAuliffe, C., *et al.* (2018). Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture. Geoderma 332, 73–83. doi:10.1016/J.GEODERMA.2018.07.002.

Bellù, L. G., and Liberati, P. (2006). Inequality Analysis: The Gini Index. doi:10.1016/0306-4573(92)90089-I.

Bronick, C. J., and Lal, R. (2005). Soil structure and management: A review. Geoderma 124, 3–22. doi:10.1016/j.geoderma.2004.03.005.

Chan, K. Y., and Heenan, D. P. (1996). The influence of crop rotation on soil structure and soil physical properties under conventional tillage. Soil Tillage Res. 37, 113–125. doi:http://dx.doi.org/10.1016/0167-1987(96)01008-2.

Chenu, C., Le Bissonnais, Y., and Arrouays, D. (2000). Organic Matter Influence on Clay Wettability and Soil Aggregate Stability. Soil Sci. Soc. Am. J. 64, 1479. doi:10.2136/sssaj2000.6441479x.

Coleman, K., Jenkinson, D. S., Croker, G. J., Grace, P. R., Klír, J., Körschens, M., *et al.* (1997). Simulating trends in soil organic carbon in long-term experiments using RothC-26.3. Geoderma 81, 29–44.

Denef, K., Roobroeck, D., Manimel Wadu, M. C. W., Lootens, P., and Boeckx, P. (2009). Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biol. Biochem. 41, 144–153. doi:10.1016/j.soilbio.2008.10.008.

Denef, K., Six, J., Bossuyt, H., Frey, S. D., Elliott, E. T., Merckx, R., *et al.* (2001). Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol. Biochem. 33, 1599–1611. doi:10.1016/S0038-0717(01)00076-1.

Dexter, A. R. (1988). Advances in characterization of soil structure. Soil tillage Res. 11, 199–238.

Grant, C. D., and Dexter, A. R. (1990). Air entrapment and differential swelling as factors in the mellowing of moulded soil during rapid wetting. Aust. J. Soil Res. 28, 361–369. doi:10.1071/SR9900361.

Gregory, A. S., Watts, C. W., Griffiths, B. S., Hallett, P. D., Kuan, H. L., and Whitmore, A. P. (2009). The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England. Geoderma 153, 172–185. doi:10.1016/j.geoderma.2009.08.002.

Griffiths, B. S., Ritz, K., Bardgett, R. D., Cook, R., Christensen, S., Ekelund, F., *et al.* (2000). Ecosystem Response of Pasture Soil Communities to Fumigation-Indicuded Microbial Diversity Reudctions: An Examination of the Biodiversity - Ecosystem Function Relationship. Oikos 90, 279–294. doi:10.2307/3547138.

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. Palaeontol. Electron. 4.

Haynes, R. J., and Beare, M. H. (1997). Influence of six crop species on aggregate stability and some labile organic matter fractions. Soil Biol. Biochem. 29, 1647–1653. doi:10.1016/S0038-0717(97)00078-3.

Hirsch, P. R., Jhurreea, D., Williams, J. K., Murray, P. J., Scott, T., Misselbrook, T. H., *et al.* (2017). Soil resilience and recovery: rapid community responses to management changes. Plant Soil 412, 283–297. doi:10.1007/s11104-016-3068-x.

Holden, P. A. (2011). How do the microhabitats framed by soil structure impact soil bacteria and the processes that they regulate? Eds K. Ritz I. Young. Archit. Biol. Soils Life Inn. Sp. Chapter 7, 118–148.

Materechera, S. A., Kirby, J. M., Alston, A. M., and Dexter, A. R. (1994). Modification of soil aggregation by watering regime and roots growing through beds of large aggregates. Plant Soil 160, 57–66.

Metzger, L., and Yaron, B. (1987). “Influence of sludge organic matter on soil physical properties,” in Advances in Soil Science Vol 7, ed. B. A. Stewart (Springer-Verlag, New York Inc.), 141–163.

Neal, A. L., Rossmann, M., Brearley, C., Akkari, E., Guyomar, C., Clark, I. M., *et al.* (2017). Land-use influences phosphatase gene microdiversity in soils. Environ. Microbiol. 19, 2740–2753. doi:10.1111/1462-2920.13778.

Pagliai, M., Vignozzi, N., and Pellegrini, S. (2004). Soil structure and the effect of management practices. Soil Tillage Res. 79, 131–143. doi:10.1016/j.still.2004.07.002.

Schneider, C. A., Rasband, W. S., and Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.

Su, Y. Z., Yang, R., Liu, W. J., and Wang, X. F. (2010). Evolution of soil structure and fertility after conversion of native sandy desert soil to irrigated cropland in arid region, China. Soil Sci. 175, 246–254. doi:10.1097/SS.0b013e3181e04a2d.

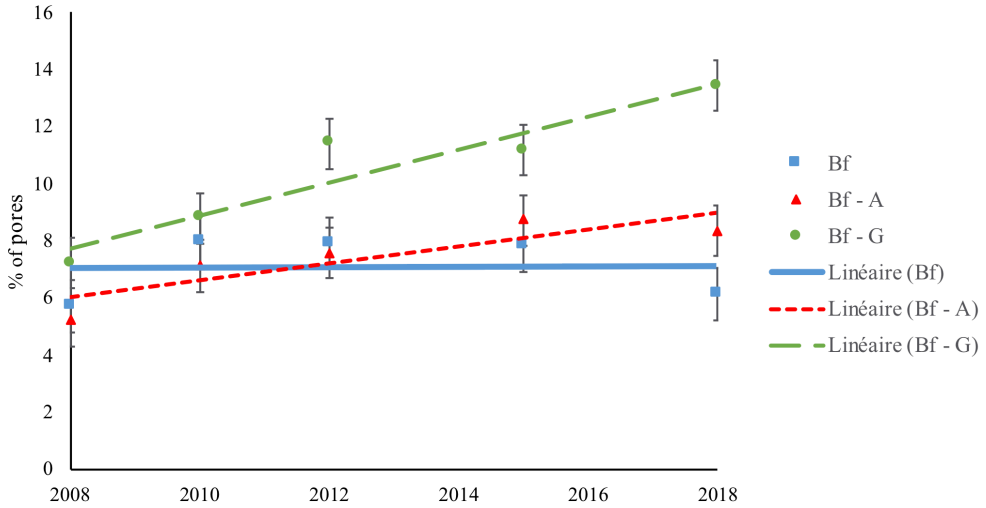
Tisdall, J. M., and Oades, J. M. (1982). Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–165.

Vogel, H. J., Weller, U., and Schlüter, S. (2010). Quantification of soil structure based on Minkowski functions. Comput. Geosci. 36, 1236–1245. doi:10.1016/j.cageo.2010.03.007.

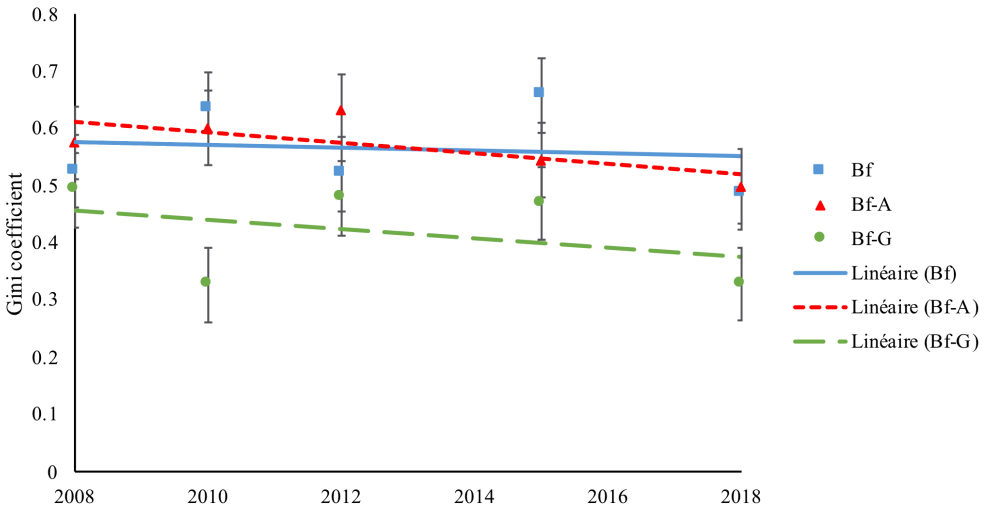
Watts, C. W., and Dexter, A. R. (1997). The influence of organic matter in reducing the destabilization of soil by simulated tillage. Soil Tillage Res. 42, 253–275. doi:10.1016/S0167-1987(97)00009-3.

Watts, C. W., Whalley, W. R., Longstaff, D., White, R. P., Brooke, P. C., and Whitmore, a P. (2001). Aggregation of a soil with different cropping histories following the addition of organic materials. Soil Use Manag. 17, 263–268. doi:Doi 10.1079/Sum200189.

3.10. Supplementary data

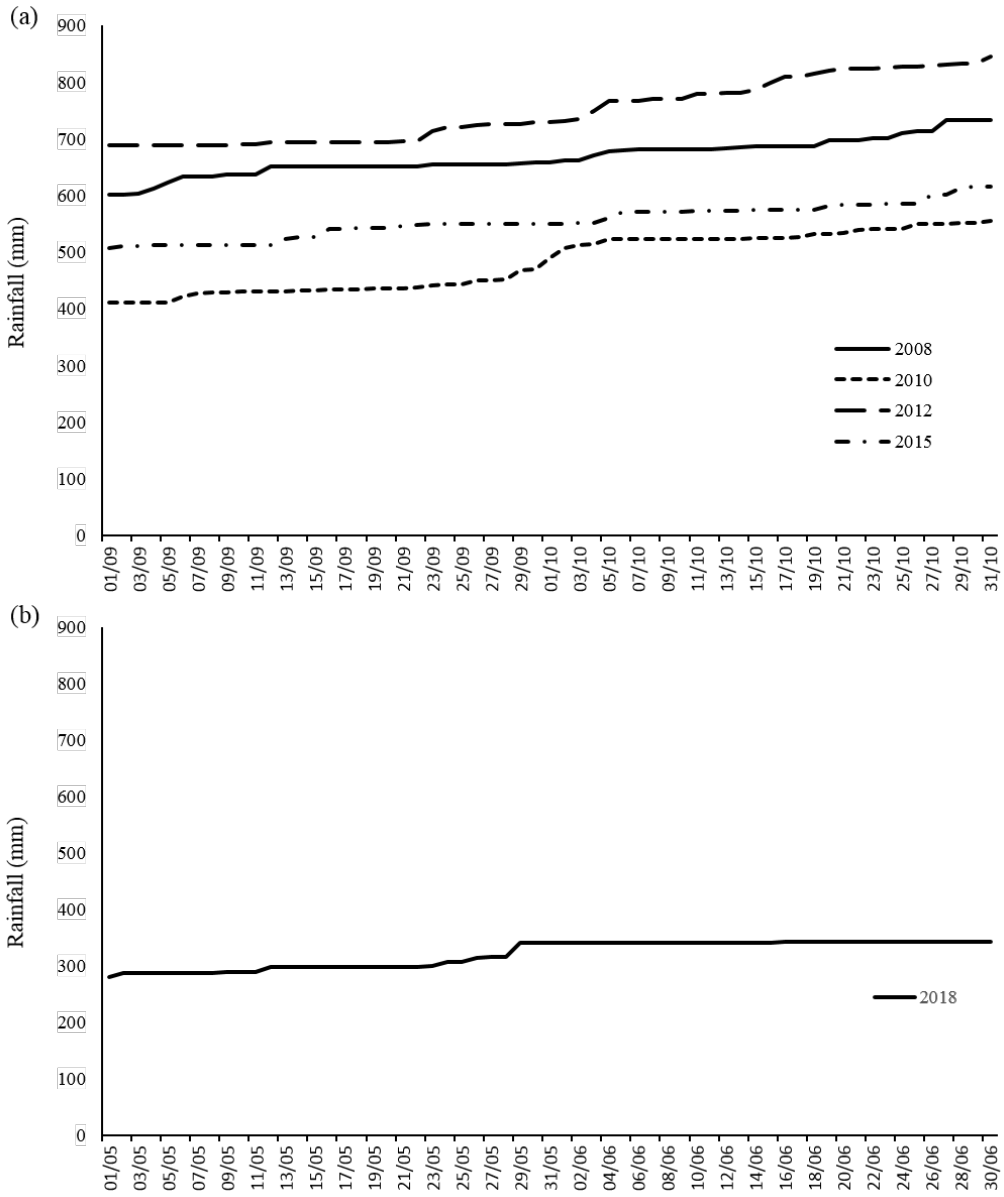


Supplementary figure 3.1.: Porosity analysis comparing the bare fallow (■), the bare fallow converted to arable (▲) and the bare fallow converted to grassland (●). The points represents the replicates and the lines are a linear regression for each treatment.



Supplementary figure 3.2.: Gini coefficient comparing the bare fallow (■), the bare fallow converted to arable (▲) and the bare fallow converted to

grassland (●). The points represents the replicates and the lines are a linear regression for each treatment.



Supplementary figure 3.3.: Cumulative rainfall (mm) on the Highfield from (a) September to October for 2008 to 2015; and (b) May to June for 2018, as the sampling time were different.

This chapter is structured and formatted in accordance with specification for the journal Plant and Soil.

Current status is: submitted.

Chapter 4: *Phacelia tanacetifolia* affects soil structure differently depending on soil texture

Aurelie Bacq-Labreuil[a], John Crawford[b], Sacha J. Mooney[a], Andrew L. Neal[b], Karl Ritz[a*]

[a] *Division of Agriculture & Environmental Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK*

[b] *Sustainable Agriculture Science, Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK*

*Author contributions: A.B.L. led and conducted the experimental work, analysed the experimental results, drafted the manuscript and coordinated the revisions. K.R., S.J.M., and A.N. contributed advice on the analyses. All authors contributed to the revision of the manuscript. K.R. and S.J.M. supervised the overall project. All authors give final approval for publication.

4.1. Abstract

The physical structure of soils is largely influenced by the textural class of the soil, but can also be affected by other factors including the presence of plants, which can directly and indirectly affect soil structural genesis. We studied the effects of *Phacelia tanacetifolia*, often used as a cover-crop species in arable agricultural systems, upon soil structural properties in the context of two contrasting soil textures. A sandy loam and a clay soil were destructured at a scale of 2 mm, and planted with *Phacelia* in a replicated pot experiment, with associated unplanted controls. X-ray Computed Tomography was used to visualise and quantify the soil pore networks in 3D. We hypothesised there would be differential effects of the plants upon soil structure contingent on the texture. The presence of plants did not affect the aggregate size distribution for any of the textures during the time frame of the experiment (6 weeks). However, the inherent 3D porous architecture of the soils were significantly affected differently depending on the soil texture. For the sandy loam soil, the porosity, pore connectivity, and pore surface density decreased in the presence of plants, whereas for the clay, the porosity was constant, the pore-connectivity decreased, and surface density increased in the presence of plants. Therefore, plants impact the structural genesis of soil depending on its inherent textural characteristics, leading to a differential development of pore architecture in different contexts. These results have implications from an ecological perspective, and in terms of the prescription of plants to remediate or condition soil structure in managed systems.

4.2. Keywords: X-ray computed tomography, Soil texture, 3D image analysis, porosity, connectivity, cover crop

4.3. Introduction

Soil structure is classically defined as the arrangement of soil particles and organic materials (Tisdall and Oades 1982), typically creating a dynamic and heterogeneous pore network within the soil matrix (Dexter 1988). The nature of this pore network is to a large extent underpinned by soil texture, but it can also be affected by other factors such as the actions of living organisms, wet:dry cycles, etc. (Ritz & Young, 2011). In terrestrial systems, soil is the fundamental base which supports vegetation growth (van Breemen 1993), but plants also affect the nature of their belowground habitat both directly and indirectly. Plant roots modify the aggregation of soil particles, generally acting to generate and stabilise the aggregate structure (Tisdall and Oades 1982). This occurs by processes of enmeshment of soil particles and excretion of mucilage and other extra-cellular polymeric substances which adhere constituents together (Bronick and Lal 2005). Indirect mechanisms that are mediated by interactions with soil biota, serve to drive aggregation processes (Haynes and Beare 1997; Rillig *et al.* 2002; Ritz and Young 2011). Root mucilage stabilises aggregates by increasing cohesion and decreasing wetting rates of aggregates (Czarnes *et al.* 2000). The inherent diversity of plant species means that the soil is frequently exposed to an increase in the diversity of root architecture within the matrix (e.g. tap, fibrous, fine roots), a increase in the quality and quantity of carbon inputs, and considerable differentiation in the microbial communities associated with the root systems (Chan and Heenan 1999; Rillig *et al.* 2002). For example, Chan and Heenan (1996) demonstrated that extensive fibrous roots enhanced aggregation, and Haynes and Beare (1997) reported a greater microbial biomass and an increase of aggregation associated with leguminous plants.

A recent study revealed tomato root architecture was markedly different for plants after 8 days of growth dependant on soil texture: plants developed a thick tap root in sandy loam soil but grew thinner roots with more laterals in clay soil (Helliwell *et al.* 2017). Furthermore, the porosity of the rhizosphere of the sandy loam soil was decreased whereas for the clay loam soil it was increased. Thus, the root growth strategies of plants is influenced by the surrounding environment. In non-cohesive and coarser soil, root systems generally develop to greater depth and are thicker than roots growing in a cohesive, finer textured soil (Hacke *et al.* 2000; Jackson *et al.* 2000; Li *et al.* 2005). Non-cohesive and coarser soil dries at greater rates in the upper layer, therefore the root systems need to grow deeper to access water (Jackson *et al.* 2000). The influence of plants on soil structural dynamics is also dependant on soil texture: in a silty-clay soil the presence of plant can increase the porosity and pore connectivity compared to a sandy soil where the presence of plants can decrease the porosity and pore-connectivity (Bacq-Labreuil *et al.* 2018). The soil hydraulic properties in finer textured soils are considerably different due to the enhanced water holding in finer pores (Saxton *et al.* 1986). However, the equilibrium between water dynamics and gas exchange is crucial for the development of plant and associated microbial communities.

The aim of this study was to establish the effect of soil texture and plant growth on early stage soil structural genesis. We grew *Phacelia tanacetifolia*, an herbaceous plant commonly used as a cover crop in arable rotations and apocryphally thought to be effective in conditioning soil structure, in a sandy loam and clay soil, along with unplanted control treatments. We hypothesised that (i) plant roots have a contrasting effect on soil structure depending on the

soil texture; and (ii) the presence of a plant increases the porosity, pore-connectivity, and diversity of pore sizes.

4.4. Materials and methods

4.4.1. Preparation of soil cores

Soil from the Newport series, a sandy loam (clay: 9.5%, silt: 26.1%, sand: 65.3%; FAO Brown Soil) and soil from the Worcester series, a clay (clay: 43.3%, silt: 28.4%, sand: 28.2%; FAO Argillic Pelosol) soils were collected from the top 50 cm of arable fields situated in Bunny, Nottinghamshire, UK (52.52 °N, 1.07 °W). After collection, the soil was spread and left to dry over two days before being thoroughly mixed and broken down by passing through a 2-mm mesh sieve. Columns comprised of polypropylene tubes (170 mm height x 68 mm diameter) with a 0.1 mm mesh affixed to the base were packed with soil to a bulk density of 1.2 mg m^{-3}. Columns were placed on a tension table for saturation for 24 h and then equilibration for 3 days at -3 kPa prior to seed sowing. Pre-germinated seeds of *Phacelia tanacetifolia* Benth. *cv. "Angelia"* were planted in the soil surface and adjusted to provide one emergent plant per column. Four planted and four unplanted replicates of each soil type were established and arranged in a randomised block design in a growth chamber providing 16:8 h light:dark cycle at 21°C:50% humidity and 15°C:75% humidity respectively. Plants were grown for 6 weeks since at this age they were fully pot-bound.

4.4.2. X-ray Computed Tomography (CT)

All columns were X-ray CT scanned prior to sowing seeds, and at 2, 4 and 6 weeks thereafter, using a Phoenix v|tome|x M scanner (GE Measurement

and Control solution, Wunstorf, Germany) set at a voxel resolution of 40 μm, 180 kV with a current of 180 μA. A total of 2,160 projection images were collected for each scan at a 250 ms period using an averaging of 3 images and skip of 1, resulting in a total scan time of 90 min. The scanning time was chose to optimise the image processing with greater quality of image. Scans occurred over 4 days with treatments randomly allocated over this period but consistent between the three occasions.

All scanned images were reconstructed using Phoenix datos | x2 rec reconstruction software. The scanned images were optimised to correct any sample movement during the scan and reduce noise using the beam hardening correction algorithm, set at 8.

As a multi-scan routine was performed on the core samples, VG StudioMax® 2.2 was used to merge the top, middle and bottom scans to obtain a single 3D volume for each complete core. Image sequences of 40 x 40 x 120 mm were extracted for image analysis.

4.4.3. Image analysis

Pre-processing of the image sequences was performed using Image J (Schneider *et al.* 2012) and the threshold and the 3D calculation was implemented in QuantIm (Vogel *et al.* 2010). The preparation of the images and the quantification of the 3D pore characteristic were processed using the method described in detail in Bacq-Labreuil *et al.* (2018).

In summary, the following Minkowski function which characterised 3D pore network, collected using QuantIm; the porosity of the selected volume (percentage of the pores greater than 40 μm); the pore size distribution,

expressed here as a cumulative value (proportion of each size class in the volume); the pore connectivity expressed by the Euler number (negative Euler number are associated with greater pore connectivity); and the pore surface density which is the pore-solid interface (a greater surface density suggests a larger roughness of the pore edges, Vogel *et al.* 2010).

4.4.4. Sampling and measurements

After 6 weeks, the columns were destructively harvested, and the soil air-dried. Aggregate size distribution was determined by passing 250 g of air-dried soil through a sieve series of 2000, 1000, 710, 500, 425, 300, 212 and 53 µm, via horizontal shaking for 3 minutes at 300 rotations min^{-1}. The mass of aggregates retained on each sieve was determined and normalized to the total mass (Kézdi 1974).

4.4.5. Statistical analysis

All statistical analyses were conducted using Genstat version 17.1 (VSN International Ltd., 2014). For aggregate size distribution, at Week 0, a one-factor analysis of variance (ANOVA) was performed to assess the difference in soil mass between size classes, at Week 6 and for total porosity a two-factor ANOVA was used to assess the effects of plant status and either size class or time. A three-way ANOVA was performed on all primary variables using a split-plot design with soil type, plant status and size classes of pores as factors.

4.5. Results

Both soil textures resulted in two different pore architecture (Fig. 4.1.a, c). The growth of *Phacelia* after 6 weeks induced cracks in the surrounding of the

primary root but more apparent in the clay soil (Fig. 4.1.b, d, e). Cracks could have resulted from the growth of the primary root through the soil profile (Fig. 4.1.b, d) or from the lateral root growing through aggregates in clay soil and inducing the formation of cracks (Fig. 4.1.e).

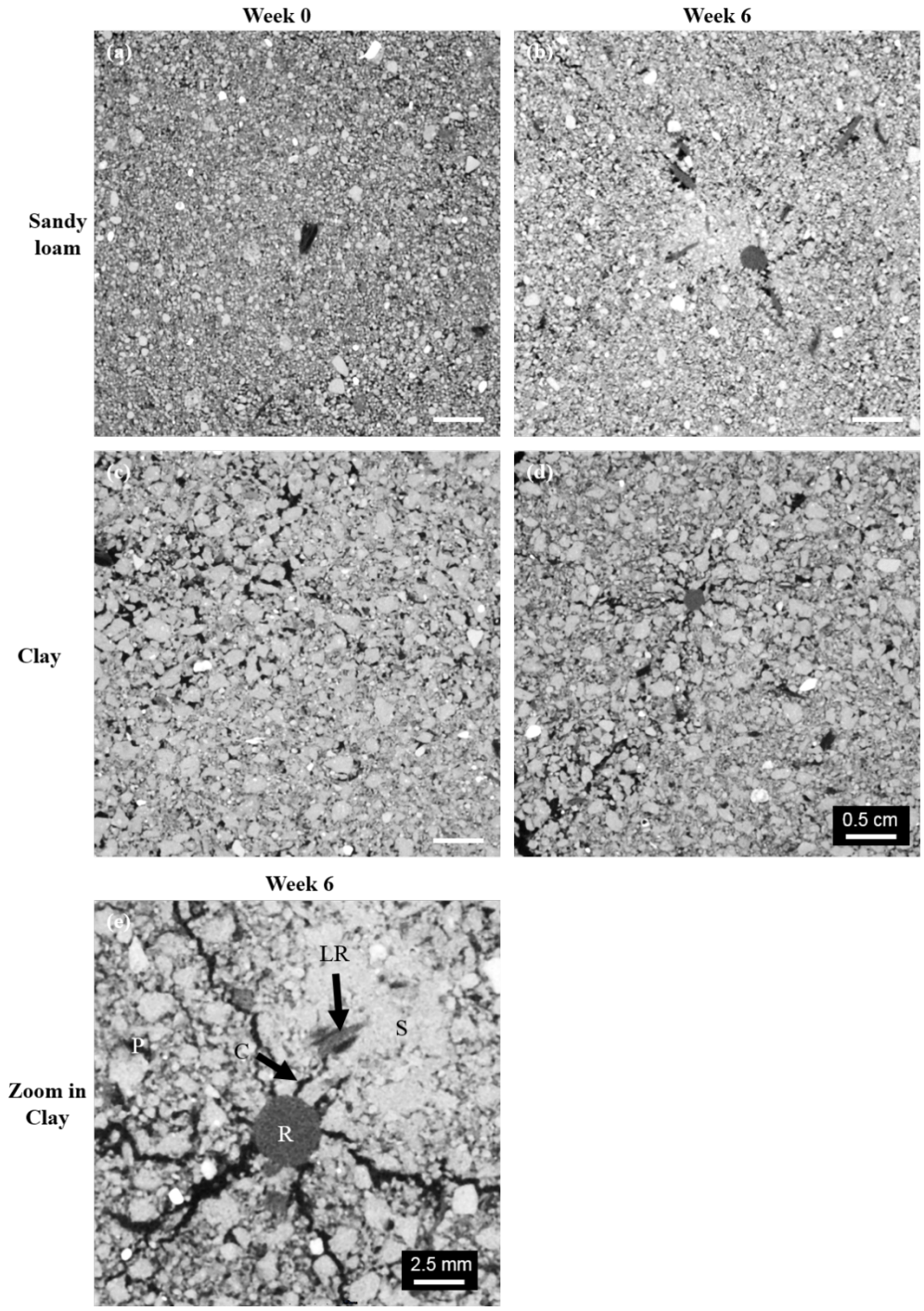


Figure 4.1.: 2D X-ray attenuation images of soils (40 µm resolution; darker shades relate to lower attenuation; a sharpening algorithm has been passed over these images to increase contrast of features) from (a, c) unplanted at Week 0 and (b, d, e) soil planted with phacelia after 6. (a, b) sandy clay soils; (c, d) clay soils. (e) Example of effect of lateral root (LR) growing from a

primary root (R) through aggregate in the clay soil and resulting in crack (C), growing through the soil matrix (S). P represents isolated pores.

4.5.1. Pore characteristics

Pore architectural changes observed visually over time (Fig. 4.1.a-d), resulted in a significant time x treatment interaction term with respect to total porosity of sandy loam as determined by X ray CT measurements (P<0.001; Fig. 4.2.). For the unplanted soil, total porosity decreased between Week 0 and Week 2 but not thereafter, whilst in planted soils there was a consistent decrease in porosity across Weeks 0-6 (Fig. 4.2.a). There was also a significant time x treatment interaction associated with the total porosity of the clay soil (P<0.001). Here, total porosity was less in planted treatments at Week 0, similar at Week 2 and greater in planted soils at Week 6 (Fig. 4.2.b).

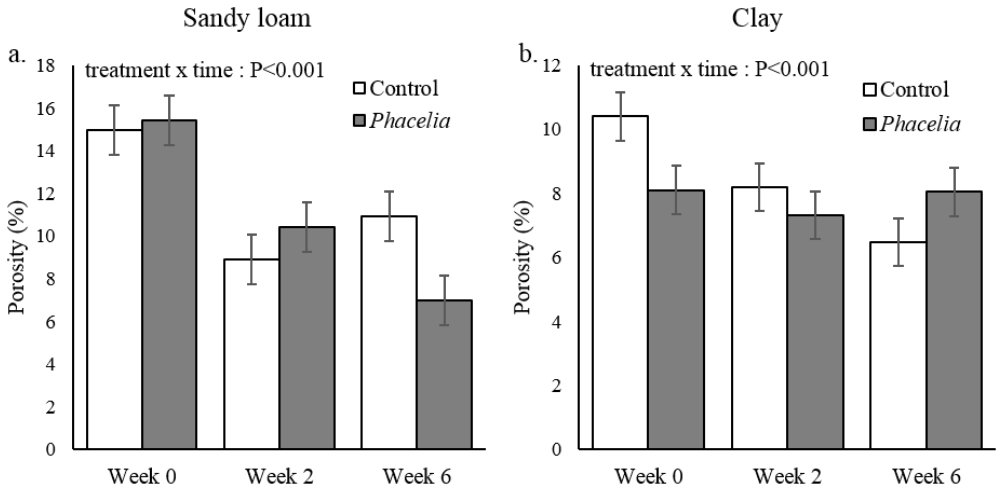


Figure 4.2.: Total soil porosity in unplanted and planted soils (spatial resolution 40 µm). (a) sandy loam soil; (b) clay soil. Bars denote means (n=4) expressed as the percentage of pores relative to the total volume, whiskers denote pooled standard errors.

Minkowski functions only showed significant changes with respect to pore diameters of <0.3 mm for both sandy loam and clay soils (Figs. 4.3. and 4.4.). For sandy loam there was a significant pore size diameter x treatment x time interaction term with respect to all pore size distribution, pore connectivity and pore surface density ($P \leq 0.01$). Whilst this effect was statistically significant with respect to pore size distribution, in numerical terms the effects were minute, and barely discernible when plotted (Fig. 4.3.a-c). Approximately 90% of the pore sizes in all cases were ≤0.16 mm (Fig. 4.3.a-c). The connectivity function of unplanted soils decreased significantly between Weeks 0 and 2, with only a modest increase by Week 6. However, on these occasions, plant effects on connectivity differed depending on pore size. At Week 2, pores <0.1 mm were more connected in planted soils but not above this size. By Week 6 this relationship changed such that pores <0.1 mm were less connected, and those in the range 0.1-0.25 mm were more connected in planted soils. Pore surface density decreased for both unplanted and planted soils between Week 0 and Week 2 but with a greater magnitude for unplanted soils, and with this decline continuing in planted soils to Week 6 (Fig. 4.3.j-l).

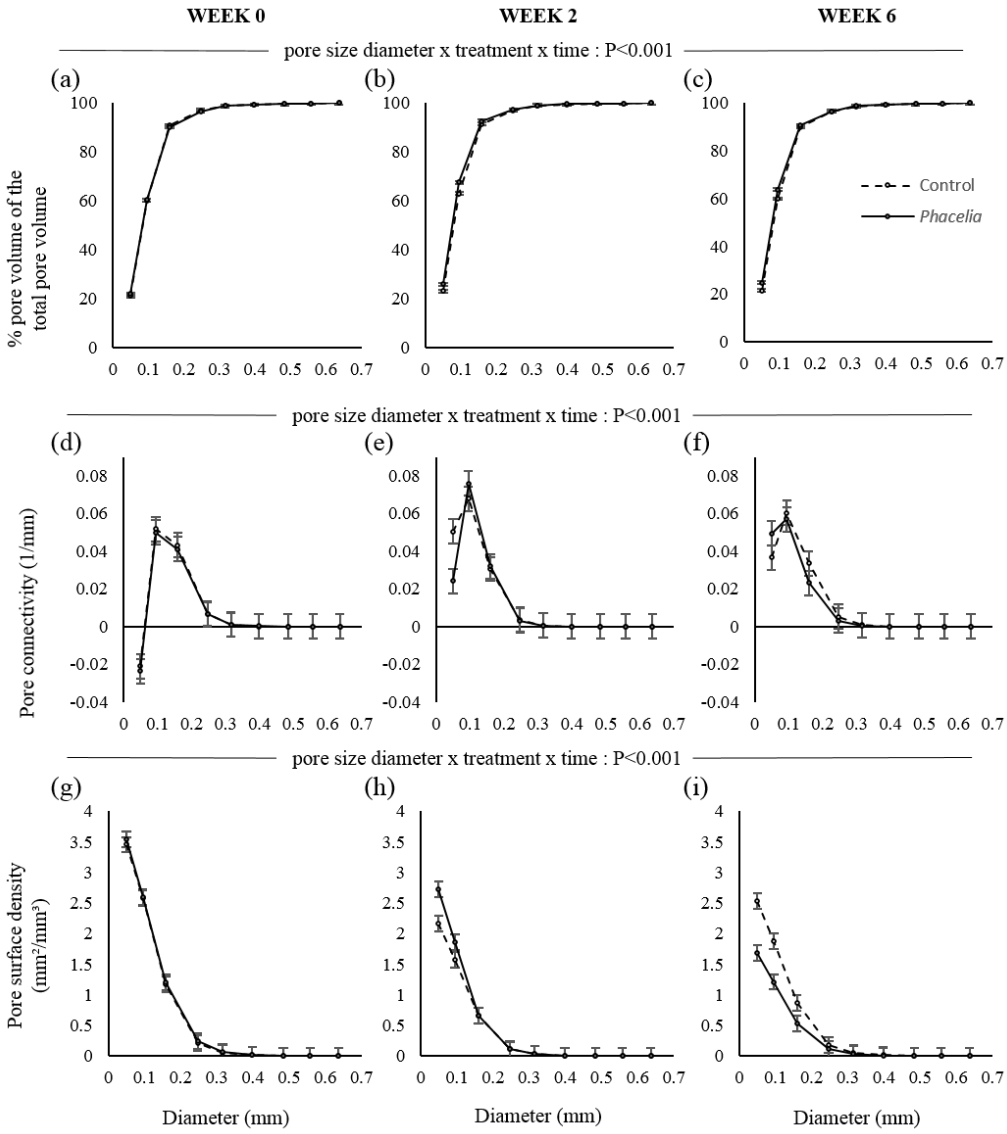


Figure 4.3.: Minkowski functions of sandy loam soils for the unplanted and planted soils at Week 0 (a, d, g), Week 2 (b, e, h) and Week 6 (c, f, i): (a - c) cumulative pore distribution of cores; (d - f) connectivity; (g - i) surface density. Points denote means (n=4), whiskers denote pooled standard errors.

For the clay soil, there was no significant three-way interaction term with respect to pore size distribution ($P>0.05$; Fig. 4.4.a-c), but there was for pore connectivity and pore surface density ($P<0.001$; Fig. 4.4.d-l). Overall, approximately 80% of the pore sizes for both treatments were ≤0.25 mm (Fig.

4.3.a-c). At Week 0, the pore connectivity of the unplanted soils was substantially greater than the planted soils for pores in the 0.05-0.1 mm size range (Fig. 4.4.d). Over the subsequent 6 weeks, pore connectivity in planted and unplanted soils converged to parity (approximately 0.23 mm^{-1}; Fig. 4.4.d-f), leading to a significant interaction. Pore surface density of unplanted soils was greater than planted soils by up to 0.3 mm at Week 0. By Week 2, pore surface density functions had decreased and converged for both treatments, and by Week 6 was significantly smaller for pores <0.2 mm in unplanted soils (Fig. 4.4.j-l).

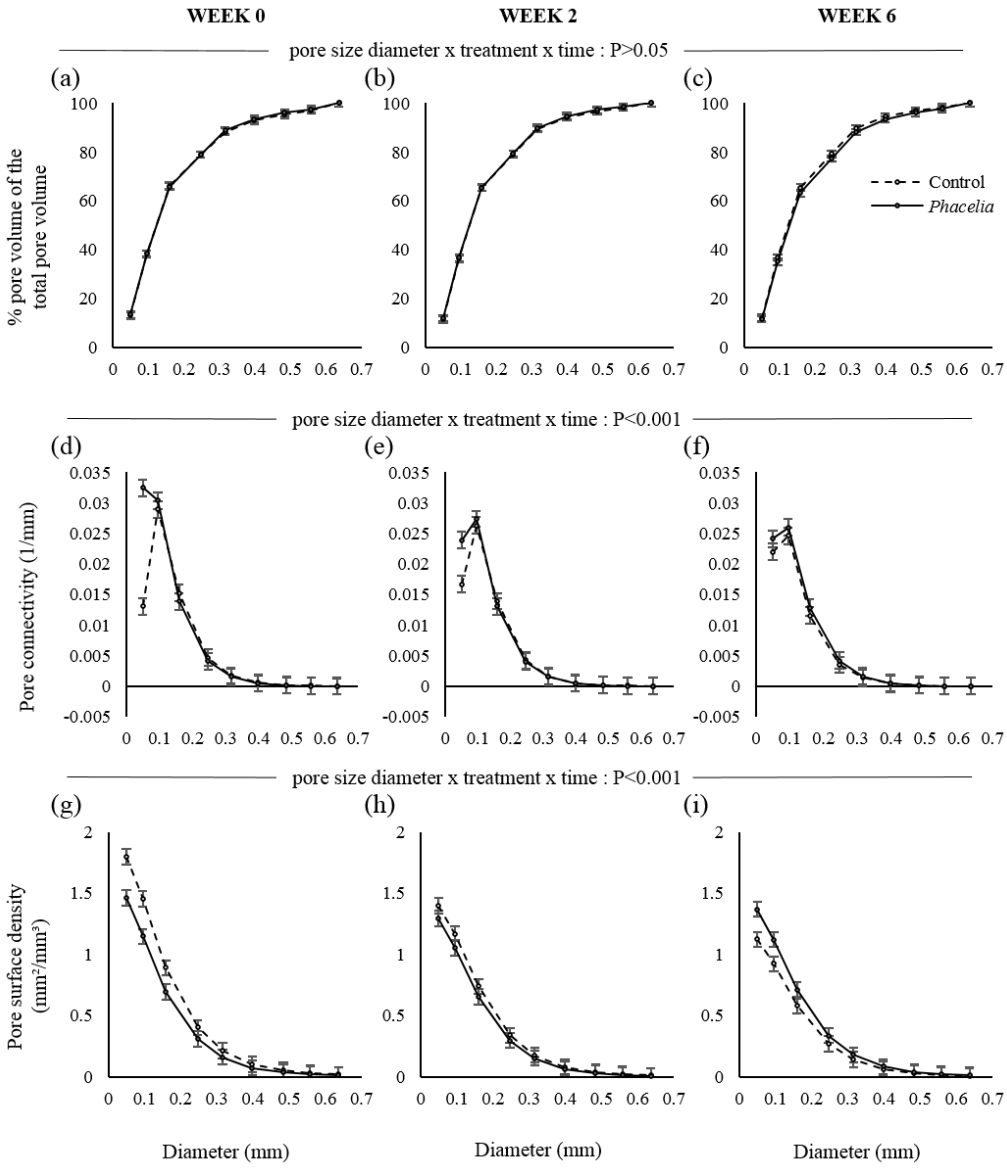


Figure 4.4.: Minkowski functions of clay soils for the unplanted and planted soils at Week 0 (a, d, g), Week 2 (b, e, h) and Week 6 (c, f, i): (a - c) cumulative pore distribution of cores; (d - f) connectivity; (g - i) surface density. Points denote means (n=4), whiskers denote pooled standard errors.

4.5.2. Aggregate size distribution

At Week 0, the aggregate size distribution of the sandy loam showed an increasing proportion of aggregates in size class 53-500 µm, followed by a reverse of this trend for aggregates >2,000 µm (Fig. 4.5.a). This trend was

interrupted at 425-500 µm, where this size class constituted a significantly smaller proportion than neighbouring classes (Fig. 4.5.a). There was an extremely low proportion of aggregates > 2,000 µm (approximately 0.4%, Fig. 4.5.a). At Week 6, this pattern was still manifest, and there was no significant effect of plants (P>0.05; Fig. 4.5.b). For the clay soil, there was a general trend of an increase in proportion of aggregates with increasing size class, but a substantial increase for pores >1,000 µm, with the greatest proportion >2,000 µm (Fig. 4.5.c).

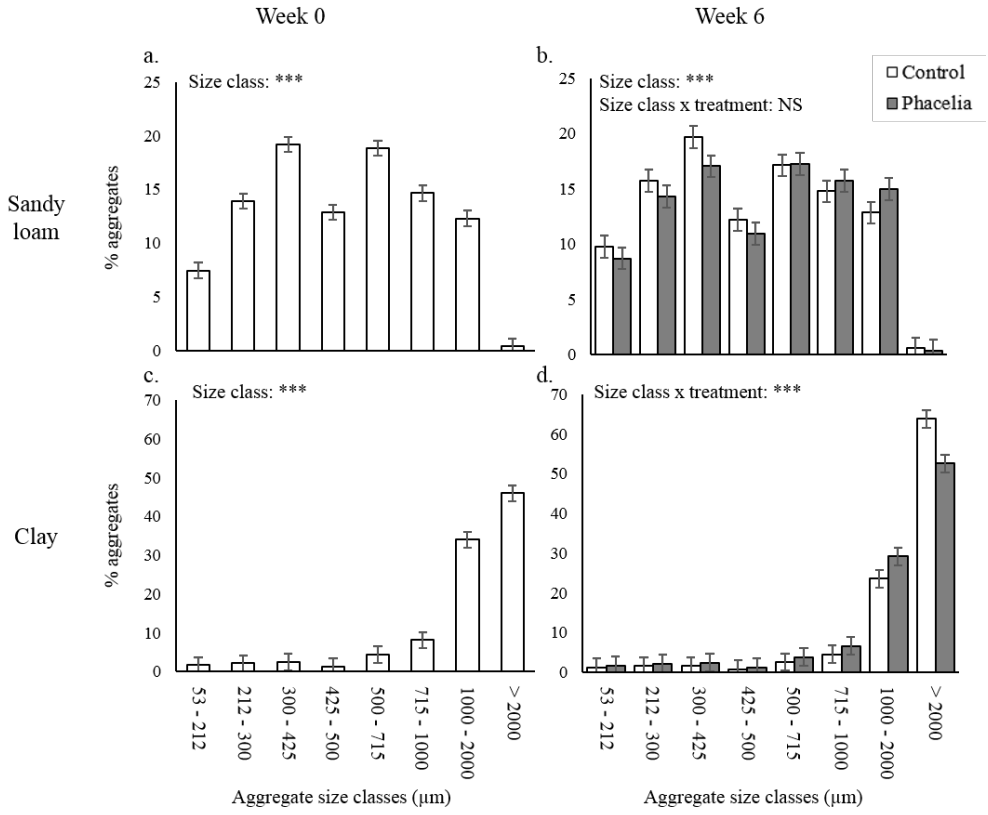


Figure 4.5.: Soil aggregate size distribution showing the starting condition at Week 0 (a, c) and the effect of plants at Week 6 (b, d) for the sandy loam soil (a – b) and the clay soil (c – d). Bars denote means (n=4) expressed as the percentage of aggregates relative to the total volume, whiskers denote pooled standard errors.

This pattern persisted at Week 6, where there was a significant effect of plants with respect to aggregates >1,000 μm; planted soils had a significantly greater proportion of aggregates 1-2 mm than unplanted soils, but this pattern was reversed for aggregates >2,000 μm (P<0.05; Fig. 4.5.d).

4.6. Discussion

As would be expected the nature of the aggregate size distribution was profoundly different between the textures: approximately 80 % of all aggregates were >1,000 μm for the clay, whereas in sandy loam soil the aggregate sizes were more evenly distributed throughout the sizes <2,000 μm with 0.5 % of aggregate sizes >2,000 μm (Fig. 4.5.). For the clay soil, the larger proportion of aggregates >1,000 μm can be attributed to the greater proportion of clay particles due to their capacity to bound together (Tisdall and Oades 1982; Dexter 1988; Blake *et al.* 2003). The presence of plants did not impact on the aggregate size distribution in the sandy loam soil. This may have been due to a lack of any substantial wet:dry cycles imparted, which is known to stabilise aggregation (Bronick and Lal 2005) as the samples were held at a fixed water potential in this experiment. During wetting, water can disperse or swell clay particles which leads to increased contact between clay and other particles, and therefore binding during the drying phase (Singer *et al.* 1992). Furthermore, sandy loam soil contained a low proportion of clay (9.5%), which is representative of a non-cohesive soil. Thus in non-cohesive soil, the binding due to the presence of clay is reduced leading to a reduction of the root action on the aggregation (Degens *et al.* 1994; Six *et al.* 2004). We wished to avoid such effects in this study in order to investigate the inherent effects of the plant on structural genesis. Hence in both soils, the water regime was constant during

the experiment, thus the change in wet and dry cycles were not responsible for the greater proportion of aggregates >2,000 μm observed in the unplanted treatment for the clay soil. Thus, the aggregation in the unplanted treatment might be due to other biotic factors. The planted soils showed a decrease in the percentage of aggregate sizes >2,000 μm and an increase in the percentage of aggregate sizes 1,000-2,000 μm (Fig. 4.5.). The greater proportion of aggregates of the sizes between 1,000-2,000 μm in the planted soil might have resulted from fragmentation of bigger aggregates by root penetration or development via root action (Materechera *et al.* 1994; Chan and Heenan 1996; Jin *et al.* 2013). Therefore, in the more cohesive soil, roots appear to generate fragmented aggregates, which may facilitate water infiltration or drainage within the aggregates (Fig. 1e; Materechera *et al.* 1994). This in turn would have arguably positive effects upon water availability to the plants through the generation of a wider pore sizes from sizes between 0.05 and 0.16 mm, which are associated to the transmission pores (Metzger and Yaron 1987; Watts and Dexter 1997).

For both soil textures, a decrease in porosity was observed in unplanted soil at Week 2 (from 14.9 to 8.9% for the sandy loam soil and from 10.4 to 8.2% for the clay soil) which maintained constant until Week 6 (Fig. 4.2.) which is most likely a consequence of settling of the soil due to gravity. However, soil texture profoundly influenced the soil structural development of planted soil: in sandy loam soil, porosity decreased constantly over the 6 weeks (from 15.4 to 7%) whereas, in clay soil, the porosity stayed constant over the 6 weeks (approximately 7.8%). The results from the sandy loam soil was consistent with a previous study which observed, a decrease of porosity in rhizosphere

soil induced by root growth of tomato plants for the same soil texture (Helliwell *et al.* 2017). However, the results for clay soils are divergent from Helliwell *et al.* (2017) who detected an increase of rhizosphere porosity in this case. The impact of the plants on overall soil porosity, at the scale measured here (40 μm), can be slower compared to that of the rhizosphere porosity (Helliwell *et al.* 2017). This observation was also observed at the field level: the presence of plants decreased the porosity of a sandy soil compared to the increase of the porosity for a clay soil (Bacq-Labreuil *et al.* 2018). Therefore, a plant may modify soil structure differently depending on the soil texture. The results for the sandy loam soil was consistent with another study which showed plants growing at a bulk density of 1.2 g cm^{-3} decreased the soil porosity (Martin *et al.* 2012). However, these results are divergent from Feeney *et al.* (2006) for the soil of the same textural class, at a bulk density of 1.3 g cm^{-3}, where the presence of plants and soil microbiota increased the porosity. Our results suggest that the initial configuration of the pore network, defined by soil texture and bulk density, affects subsequent root growth responses and the associated impacts of roots on soil structural genesis.

Neither soil texture showed a significant plant effect on pore size distribution or pore connectivity after 6 weeks growth. A longer experiment might have revealed a greater influence of plants on soil structural genesis. In the sandy loam soil, the presence of plants decreased the pore surface density, i.e. decreasing pore-solid interfaces (Fig. 4.3.g-i). This meant the presence of plants reduced the irregular shaped-pores or elongated pores within the pore network (Vogel *et al.* 2010; Bacq-Labreuil *et al.* 2018). In clay soil, the pore solid interface increased in the planted soils (Fig. 4.4.g-i), which suggests that

elongated or irregular shaped-pores increased within the pore network. The formation of more irregular-shaped pores would likely influence the microbial community due to the creation of new habitats and a wider range of niches (Holden 2011). A more diverse pore structure and heterogeneity in pore morphology can also affect soil hydrology, via modifying water flow at a local scale and the nature of water film continua. Therefore, the same plant genotype had two distinctive effects upon the modification of pore morphology depending on the inherent soil texture. Therefore, the prescription of crops for a specific characteristics such as root morphology, rhizodeposition, might be better informed by consideration of the soil texture in which they are grown. Especially that the same plant species is affected differently depending on soil textures. This characteristic might be important for breeders and farmers in order to prescribe plant species that are optimal for the needs of the farmers and depending on the soil texture.

4.7. Conclusions

This study revealed a contrasting effect of soil textural characteristics on soil structural genesis. The results confirm our hypothesis that a plant can modify soil aggregation properties and pore networks differently depending on the inherent soil texture, manifest by a very different aggregate size distribution and the contrasting effect of plants on both textural soils. However, the second hypothesis was not completely demonstrated for both soils. For the sandy loam soil, the presence of roots decreased porosity, pore surface density, but had no significant impact on pore size distribution and pore connectivity after 6 weeks of growth. For the clay soil, the presence of roots maintained the porosity constant over the 6 weeks, but had no effect on the pore connectivity,

contradicting the second hypothesis, but increased the pore surface density, which supported it. These results showed that plants can impact soil pore architecture depending on textural characteristics. Sandy soils are usually free drain; thus, the root might have a beneficial impact by reducing the porosity which might enhance water retention. Therefore, cover crops could potentially be used to prime soil structure before sowing the main crop, specifically in sandy soil to enhance the retention of water, and in clay soil to increase water transmission. Therefore, farmers, depending on their requirements (such as water management, compaction) could prescribe different plant species depending on their characteristics, but taking in account the soil texture. Further studies are required to understand whether different plant species affect such soil structural dynamics in different ways (Ehrmann and Ritz 2013). We postulate this is likely given the diversity of root morphologies, rhizodeposition patterns and higher-order interactions between plants and soil biota. These observations also have implications from an ecological perspective, for example in the way vegetation may modulate soil structural dynamics during successional processes, which appears to have been barely considered.

4.8. Acknowledgements

We thank Pr. Hu Zhou for his assistance with the software QuantIm, and Paul Brown for supplying seed of *Phacelia*. This work was performed at the University of Nottingham Hounsfield facility. The University of Nottingham Hounsfield Facility receives funding from BBSRC (Swindon, UK), and The Wolfson Foundation (London, UK).

4.9. References

Bacq-Labreuil A, Crawford J, Mooney SJ, *et al.* (2018) Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture. Geoderma 332:73–83. doi: 10.1016/J.GEODERMA.2018.07.002

Blake JJ, Spink JH, Dyer C (2003) Factors affecting cereal establishment and its prediction. HGCA Res Rev 51:1–53

Bronick CJ, Lal R (2005) Soil structure and management: A review. Geoderma 124:3–22. doi: 10.1016/j.geoderma.2004.03.005

Chan KY, Heenan DP (1999) Microbial-induced soil aggregate stability under different crop rotations. Biol Fertil Soils 30:29–32. doi: 10.1007/s003740050583

Chan KY, Heenan DP (1996) The influence of crop rotation on soil structure and soil physical properties under conventional tillage. Soil Tillage Res 37:113–125. doi: http://dx.doi.org/10.1016/0167-1987(96)01008-2

Czarnes S, Hallett PD, Bengough a. G, Young IM (2000) Root- and microbial-derived mucilages affect soil structure and water transport. Eur J Soil Sci 51:435–443. doi: 10.1046/j.1365-2389.2000.00327.x

Degens BP, Sparling GP, Abbott, K. L (1994) The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long-term clover-based and grass pastures. Eur J Soil Sci 45:459–468. doi: 10.1111/j.1365-2389.1994.tb00531.x

Dexter AR (1988) Advances in characterization of soil structure. Soil tillage Res 11:199–238

Ehrmann J, Ritz K (2013) Plant: Soil interactions in temperate multi-cropping production systems. Plant Soil 376:01-29. doi: 10.1007/s11104-013-1921-8

Feeney DS, Crawford JW, Daniell T, *et al.* (2006) Three-dimensional microorganization of the soil-root-microbe system. Microb Ecol 52:151–158. doi: 10.1007/s00248-006-9062-8

Hacke UG, Sperry JS, Ewers BE, *et al.* (2000) Influence of soil porosity on water use in Pinus taeda. Oecologia 124:495–505

Haynes RJ, Beare MH (1997) Influence of six crop species on aggregate stability and some labile organic matter fractions. Soil Biol Biochem 29:1647–1653. doi: 10.1016/S0038-0717(97)00078-3

Helliwell JR, Sturrock CJ, Mairhofer S, *et al.* (2017) The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. Sci Rep 7:14875. doi: 10.1038/s41598-017-14904-w

Holden PA (2011) How do the microhabitats framed by soil structure impact soil bacteria and the processes that they regulate? Eds K Ritz I Young Archit Biol Soils Life Inn Sp Chapter 7:118–148

Jackson RB, Sperry JS, Dawson TE (2000) Root water uptake and transport : using physiological predictions. Trends Plant Sci 5:

Jin K, Shen J, Ashton RW, *et al.* (2013) How do roots elongate in a structured soil? J Exp Bot 64:4761–4777. doi: 10.1093/jxb/ert286

Kézdi A (1974) Handbook of soil mechanics. Soil Physics, vol 1, Elsevier S

Li Y, Xu H, Cohen S (2005) Long-term hydraulic acclimation to soil texture and radiation load in cotton. Plant, Cell Environ 28:492–499. doi: 10.1111/j.1365-3040.2005.01291.x

Martin SL, Mooney SJ, Dickinson MJ, West HM (2012) Soil structural responses to alterations in soil microbiota induced by the dilution method and mycorrhizal fungal inoculation. Pedobiologia (Jena) 55:271–281. doi: 10.1016/j.pedobi.2012.06.001

Materechera SA, Kirby JM, Alston AM, Dexter AR (1994) Modification of soil aggregation by watering regime and roots growing through beds of large aggregates. Plant Soil 160:57–66

Metzger L, Yaron B (1987) Influence of sludge organic matter on soil physical properties. In: Stewart BA (ed) Advances in Soil Science Vol 7. Springer-Verlag, New York Inc., pp 141–163

Rillig MC, Wright SF, Eviner VT (2002) The role of arbuscular mycorrhizal fungi and glomalin on soil aggregation comparing the effects of five plant species. Plant Soil 238:325–333

Ritz K, Young IM (2011) Architecture and Biology of Soils., CABI, Wall

Saxton KE, Rawls WJ, Romberger JS, Papendick RI (1986) Estimating generalized soil-water characteristics from texture. Soil Sci Soc Am J 50:1031–1036

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675

Singer MJ, Southard RJ, Warrington DN, Janitzky P (1992) Stability of Synthetic Sand-Clay Aggregates after Wetting and Drying Cycles. Soil Sci Soc Am J 56:1843. doi: 10.2136/sssaj1992.03615995005600060032x

Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res 79:7–31. doi: 10.1016/j.still.2004.03.008

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. J Soil Sci 33:141–165

van Breemen N (1993) Soils as biotic constructs favoring net primary productivity. Geoderma 57:183–211. doi: 10.1016/0016-7061(93)90002-3

Vogel HJ, Weller U, Schlüter S (2010) Quantification of soil structure based on Minkowski functions. Comput Geosci 36:1236–1245. doi: 10.1016/j.cageo.2010.03.007

Watts CW, Dexter AR (1997) The influence of organic matter in reducing the destabilization of soil by simulated tillage. Soil Tillage Res 42:253–275. doi: 10.1016/S0167-1987(97)00009-3

This chapter is structured and formatted in accordance with specification for the journal Scientific Reports.

Current status is: to be submitted.

Chapter 5: Four cover crop species have a differential impact upon soil structural genesis and microbial community phenotype

Aurelie Bacq-Labreuil[a], John Crawford[b], Sacha J. Mooney[a], Andrew L. Neal[b], Karl Ritz[a*]

[a] *Division of Agriculture & Environmental Sciences, School of Biosciences, University of Nottingham, Sutton Bonington Campus, Leicestershire LE12 5RD, UK*

[b] *Sustainable Agriculture Science, Rothamsted Research, West Common, Harpenden, AL5 2JQ, UK*

*Author contributions: A.B.L. led and conducted the experimental work, analysed the experimental results, drafted the manuscript and coordinated the revisions. K.R., S.J.M., and A.N. contributed advice on the analyses. All authors contributed to the revision of the manuscript. K.R. and S.J.M. supervised the overall project. All authors give final approval for publication.

5.1. Abstract

Cover crops (plants grown in an agricultural rotation between main crops) can significantly improve soil quality via sequestering carbon, retaining nutrients, decreasing soil erosion, and maintaining belowground biodiversity. However, little is known of the effects of such plants upon soil structure. The aim of the study was to assess the impact of four species typically used as cover crops and which have contrasting root architecture (white clover, black oat, phacelia, tillage radish) on soil structural genesis and the associated modification of microbial community structure, growing in a clay soil. The four plant species were grown in a replicated pot experiment with clay soil destructured at a scale of 2 mm, with unplanted soil as control. X-ray Computed Tomography was used to quantify the formation of pore networks in 3D and phospholipid fatty acid analysis was performed to study the microbial community phenotype. Black oat maintained the porosity and pore-connectivity greater than the other species throughout the 8 weeks of growth, whereas phacelia decreased the porosity and pore-connectivity but increased the proportion of smaller pores. The microbial community phenotype under phacelia was notably different from the other species, with a greater proportion of fungal markers. Thus different plant species have differential effects upon soil structural genesis and microbial community phenotype, which provides evidence that certain species may be more suitable as cover crops in terms of soil structural conditioning.

5.2. Key words:

Cover crops, X-ray Computed Tomography, soil structure, porosity, microbial community phenotype

5.3. Introduction

Soil structure is an important factor affecting crop production primarily due to the characteristics of the soil pore network impacting on root growth, soil fauna, nutrient, water and gas exchanges[1]. Soil quality can be defined as the capacity of soil to maintain or enhance plants and organisms' development, air and water quality, and support human health and habitation[2,3]. Soil structure is considered to be an effective indicator of soil quality[1].

Moreover, plants are known to structure soil via enmeshing and binding soil particles[4] and to break down larger aggregates via root penetration[5]. The genesis of soil structure is dynamic and requires energy which is provided by plant roots and fauna. Furthermore, root architecture plays a critical role in such effects on soil structure formation. Plants producing large quantities of fine roots appear to be more effective in soil aggregation formation compared to fibrous roots which were less effective in fracturing soil aggregates[6]. The presence of roots increases aggregate stability, the permeability of soil[7], soil porosity and connectivity[8], and asserts a great influence on microbial community structure in terms of both richness and diversity[9,10].

In agricultural systems, cover crops are increasingly sown between main crops[11]. Cover crops can sequester carbon[3,12], decrease soil erosion[3], increase soil macro-porosity[13,14], and increase microbial diversity and richness[15,16]. Cover crops enhance also the presence of saprophytic and mycorrhizal fungi in the microbial community structure[10,17,18]. However, the effect of cover crops on soil structural genesis is poorly understood. Most recent studies have focused on the role of cover crops in terms of remediating compacted soils or upon the microbial communities[9,12,14,16,18].

The aim of the study was to assess the impact of four species of plants commonly grown as cover crops (white clover, black oat, phacelia, tillage radish) on soil structural genesis and modification of microbial community structure. These plants were selected for their contrasting root morphologies in terms of tap root formation, vigorous deep-rooting and fibrous multi-branching root systems. X-ray Computed Tomography was used to quantify the formation of pore networks in 3D and phospholipid fatty acid analysis was performed to study the microbial community phenotype. We hypothesised that different root morphology impact soil structural genesis differently: for example, tap root species creates a ring of compaction surrounding the primary root growth which decreases porosity and diversity of pore sizes, compared to fibrous root species which creates a greater diversity of pore sizes and increases porosity and pore-connectivity.

5.4. Methods

5.4.1. Preparation of soil cores

A clay soil (clay: 43.3%, silt: 28.4%, sand: 28.2%) was collected from 0-50 cm depth from an arable field situated at the University of Nottingham experimental farm in Bunny, Nottinghamshire, UK (52.52°N, 1.07°W). The soil was air-dried for 2 days, and passed through a 2 mm mesh sieve, to destructure it at this scale. To re-activate the microbial community, the soil was re-wetted to 15% moisture content and incubated in bulk in black plastic bags slightly opened in a dark room at room temperature and then passed through a 10 mm sieve to ensure effective homogenisation. Soil columns were prepared by packing polypropylene tubes (170 mm height x 68 mm diameter) with a 0.1 mm mesh adhered to the bottom and was packed with the moist soil to a bulk

density of 1 g cm^{-3}. Columns were saturated for 24 h and left to drain for 24 h to reach the field moisture capacity (approximately 20%). Four different cover crop species were selected for their contrasting morphologies: tap root species, tillage radish (*Raphanus sativus* L. c.v. "Mimo"), vigorous deep-rooting species, black oat (*Avena strigosa* L. c.v. "Prate"), and fibrous multi-branching species, white clover (*Trifolium repens* L. c.v. "Galway") and phacelia (*Phacelia tanacetifolia Benth.* c.v. "Angelia"). Pre-germinated seeds of were sown into individual columns and adjusted to contain one emergent plant per column. Twenty replicates of each plant species, and of an unplanted (control) soil, were allocated in a random block design to allow for four replicates of each treatment to be sampled after 0, 2, 4, 6 and 8 weeks. Columns were maintained in a growth chamber set at 16:8 h light:dark cycle, 21:15°C respectively and 70% humidity.

5.4.2. X-ray Computed Tomography (CT) procedures

Homogenisation of column packing was checked by X-ray CT. Planted and unplanted columns were scanned using Phoenix v|tome|x m me scanner (GE Measurement and Control solution, Wunstorf, Germany) set at a voltage of 180 kV with a current of 180 µA and at voxel resolution of 40 µm. A multiple scan was performed for 1 h 29 s, with a total of 2160 projection images taken at a 250 ms period using an averaging of 3 images and skip one. Longer scan was favoured to obtain the best contrast on images which helped the threshold of the soil pores. The cores were destructively harvested after being scanned, and from the air-dried soil, three aggregates were randomly selected per core (Supplementary Materials and Methods 1).

Scanned images were reconstructed using Phoenix datos | x2 rec reconstruction software. The scanned images were optimised to correct any sample movement during the scan and reduce noise using the beam hardening correction algorithm, set at 8. As a multi-scan routine was performed on the core samples, VG StudioMax® 2.2 was used to merge the top, middle and bottom scans to obtain a single 3D volume for the complete core. Image sequences of 40 x 40 x 120 mm were extracted for image analysis for the cores.

5.4.3. Image analysis

Image preparation was performed using Image J[19]. Quantification of 3D pore characteristics was processed using QuantIm[20], both following the method described in[17].

The 3D characteristics of pores quantified were: (i) percentage of pores with a size greater than the scanning resolution (40 μm, hereafter referred as porosity); (ii) pore size distribution, viz. the proportion of each pore size class within the range 0.05 – 1.1 mm (for the cores) normalised by the total pore volume, expressed as a cumulative value; (iii) pore-connectivity, as the Euler number normalized to the total volume20: the more negative the Euler number is, the greater the pore-connectivity.

5.4.4. Sampling and measurement

On each sampling occasion, the allocated columns were scanned as above and then destructively harvested. Subsamples (c. 20 g) of the moist soil were stored at -82°C and then freeze-dried; the rest of the soil was air-dried for further

analysis. The freeze-dried and air-dried soils were stored in the dark at room temperature.

5.4.5. Aggregate size distribution

Aggregate size distributions were determined by passing 250 g of air-dried soil through a sieve series of 2000, 1000, 850, 500, 425, 300, 212 and 53 µm, via horizontal shaking for 3 minutes at 300 rotation.min^{-1} on a horizontal KS 500 shaker (Janke & Kunkel, Staufen, Germany). The mass of soil retained on each sieve was determined and normalized by the total mass of the sieved soil.

5.4.6. Microbial community phenotype profiling

The microbial community phenotypic community structure was profiled using the phospholipid fatty acid (PLFA) technique[21]. PLFA were extracted from 2 g of freeze-dried soil following a method derived from[21,22]. The lipid classes were separated using the solid phase extraction (SPE) column using Hypersep SPE column containing 50 mg of silica per 1 mL column. The extracted lipids were then methyled via a transesterification process to convert them into dried fatty acid methyl ester. The fatty acids were suspended into 75 µL of hexane, for the gas chromatography (GC) analysis. The GC analysis was performed using a GC and a DSQII mass spectroscope from Thermo Electron Corporation®, a Zebron capillary 'ZB-FFAP' column from Phenomex®. The dimension of the column was 30 m length x 0.25 mm inner diameter x 0.25 µm film thickness. The method was 1 µL of the sample was injected in the column maintained at a constant temperature of 250ºC, the carrier gas was helium set at

18psi. For each sample, a chromatogram was obtained with the retention time of each compound and the ion profile provided by the mass spectroscopy. The markers were associated to different microbial groups as follows: Gram +: i-15:00, a-15:00, i-16:00, a-17:00, 10me-16:00, 10me-18:00; Gram -: 16:1n9, 16:1n7, cy17:00, 18:1n7, cy19:00; saprophytic fungi: 18:1n9, 18:2n6,9, 18:3n9; and non-specific: 14:00, i-14:00, 16:00, 18:00, 18:1n16[23–26]. The percentage of the fatty acid indicators was used to analyse the proportion of microbial groups. The relative abundance of the microbial groups were calculated by the sum group marker lipids over the sum of all lipids.

5.4.7. Statistical analysis

For the pore characteristics, two-way analysis of variance (ANOVA) was conducted using Genstat v 17.1 (VSN International Ltd 2014), performed on all primary variables using a split-plot design with the plant treatments and size classes of pores as factors and for the total porosity, time was added to the factor list.

PLFA profiles were analysed by principal component (PC) analysis and resultant PCs analysed by ANOVA.

5.5. Results

5.5.1. Characteristics of the pore architecture

The different species affected soil structure in different ways, which was apparent from visual observation of the X-ray images (Fig. 5.1.). Formal quantification of soil structural parameters confirmed this. There was a significant treatment x time interaction with respect to the porosity ($P<0.001$; Fig. 5.2.). The porosity of unplanted soil was essentially constant over the 8

weeks of the experiment with a slight increase at Weeks 2 and 6. In the presence of black oat, the porosity increased significantly at Weeks 2 and 6, and was similar to control for Weeks 4 and 8. Whilst for planted soils with clover, phacelia and radish, the porosity was essentially constant up to Week 6 and decreased at Week 8 drastically (Fig. 5.2.).

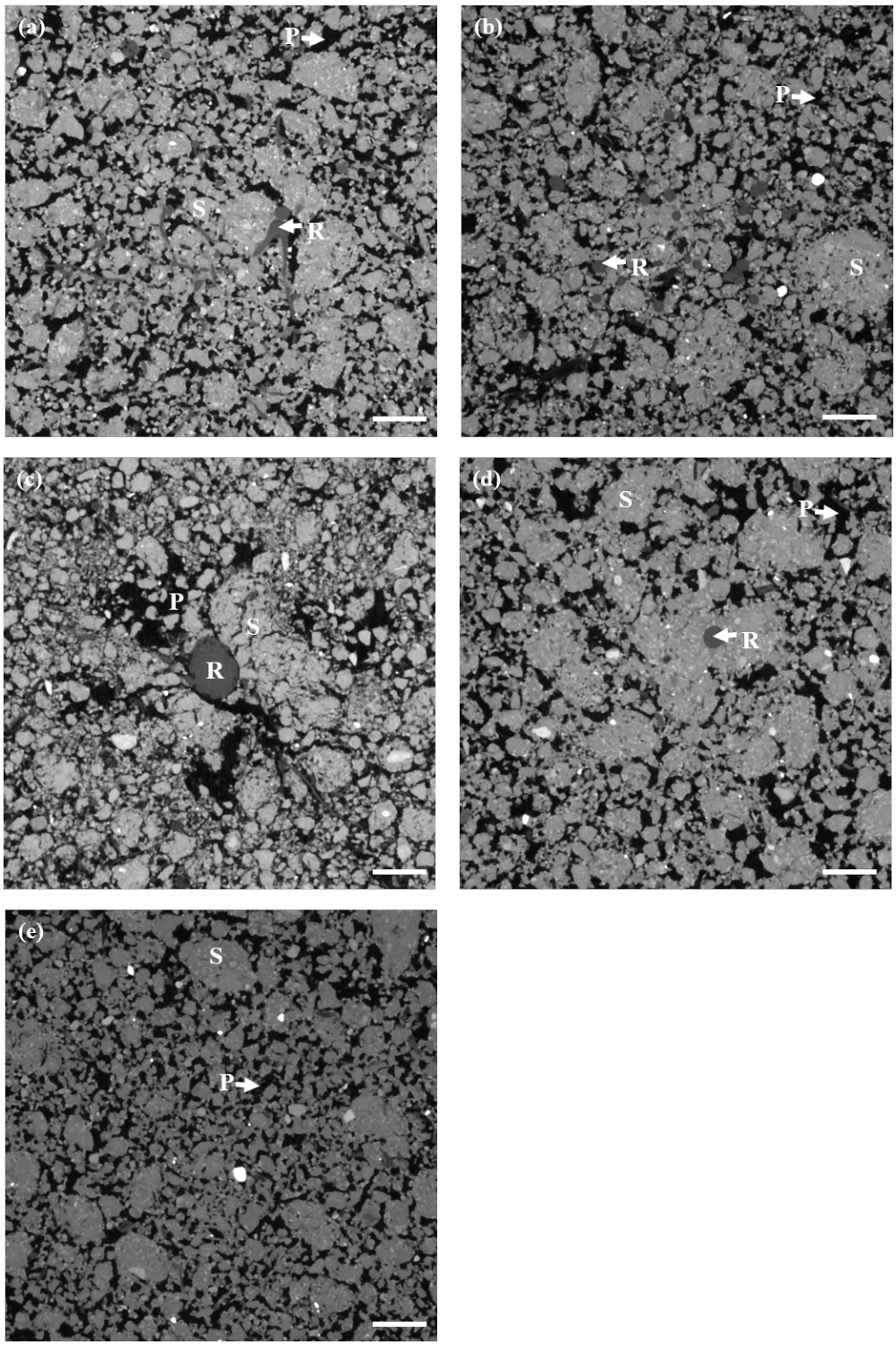


Figure 5.1.: 2D images of cores (40 µm resolution) at Week 8, displayed as greyscale images denoting Hounsfield attenuation (darker shades relate to lower attenuation), region of interest: (a) white clover; (b) black oat; (c) Phacelia; (d) tillage radish; and (e) unplanted soil (S: soil matrix, P: pore, R: root).

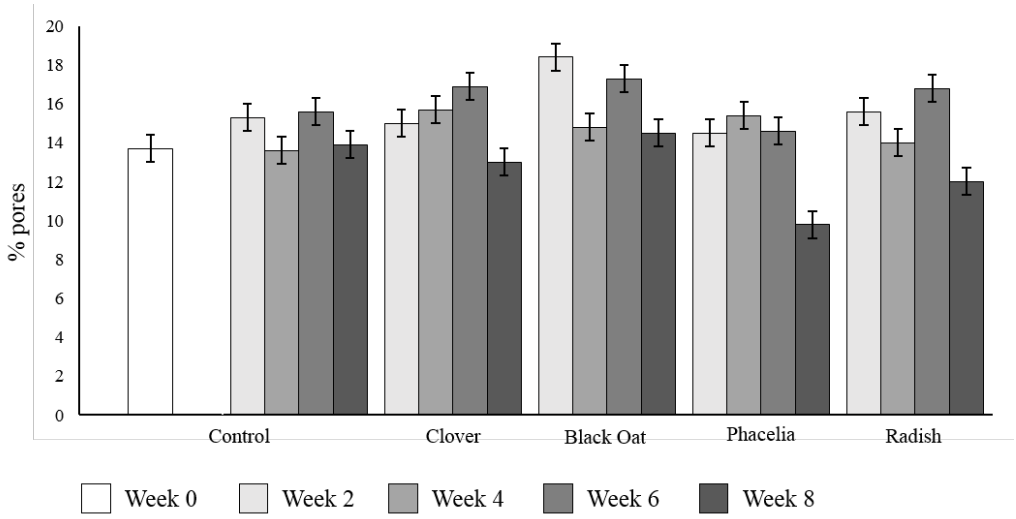


Figure 5.2.: Porosity in relation to the planted treatment over the 8 weeks of growths expressed as percentage of relative pore to the total volume. Bars indicate means and whiskers denote pooled standard errors.

There were significant treatment x time interactions with respect to cumulative pore size distribution at Week 8 (Fig. 5.3.c), and pore-connectivity at Weeks 2 and 8 (Fig. 5.3.e, f; $P<0.001$). At Weeks 0 and 2, the pore size distribution was essentially congruent for all treatments with approximately 50% of pore sizes <0.25 mm and 80% of pore sizes <0.4 mm (Fig. 5.3.a-c). At Week 8, phacelia increased the proportion of the smaller pore sizes with approximately 50% of pore sizes <0.16 mm and 80% of pore sizes <0.31 mm (Fig. 5.3.c).

At Week 0, the control columns showed low pore connection displayed by a small Euler number for the pore sizes <0.09 mm (Fig. 5.3.d). At Week 2, the overall connectivity increased from Week 0. Planted soil with black oat had the greatest pore-connectivity, whilst the soil planted with phacelia was the less connected pore-system (Fig. 5.3.e). At Week 8, the connectivity decreased for all treatments with the same pattern as Week 2: black oat soils had the greatest

pore-connectivity and phacelia the lowest pore-connectivity (Fig. 5.3.f). Pore size distribution and pore-connectivity at Weeks 4 and 6 were intermediate between Week 2 and Week 8, and are shown in Supplementary Fig. 5.1. but omitted from Fig 5.3. for clarity.

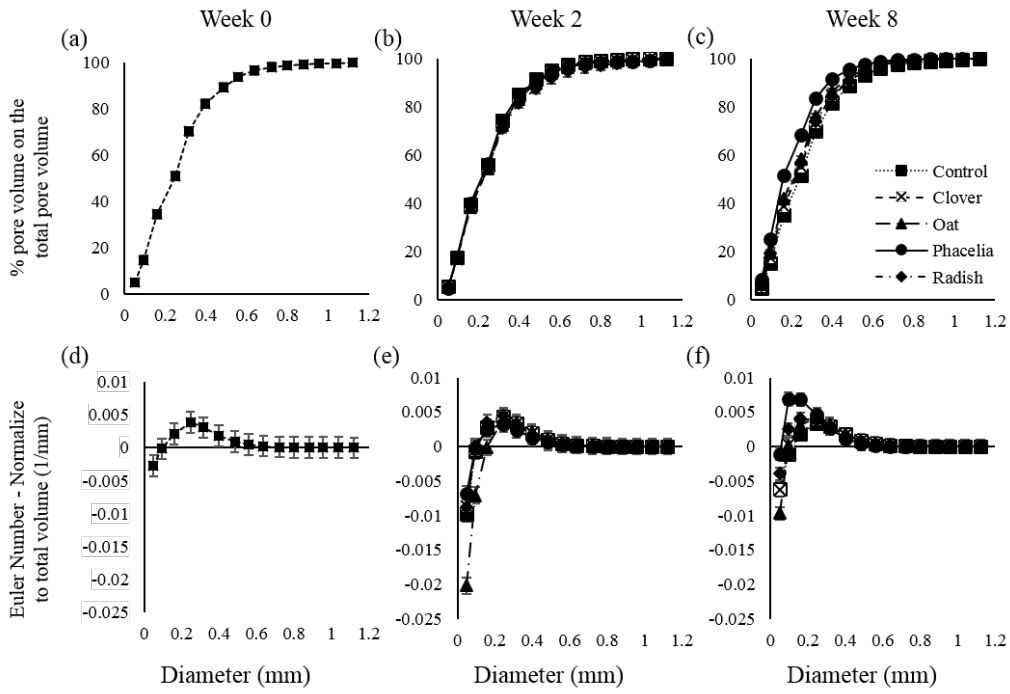


Figure 5.3.: Minkowski functions of treatments at core scale (40 µm resolution) at three time points, week 0 (a, d) week 2 (b, e) and week 8 (c, f): (a - c) cumulative pore size distribution; (d - f) pore-connectivity of cores. Points show means, whiskers denote pooled s.e.

At the aggregate scale, there were no significant differences in porosity, pore size distribution and pore connectivity between any of the treatments (Supplementary Fig. 5.2.).

5.5.2. Aggregate size distribution

At Week 0, the aggregate size distribution of the control showed an increasing proportion of aggregates with increase in size class. This trend was interrupted at 2 size classes (425- 500 and 715-100 µm), where these size contained a significantly smaller proportion than neighbouring ones (Fig. 5.4.a).

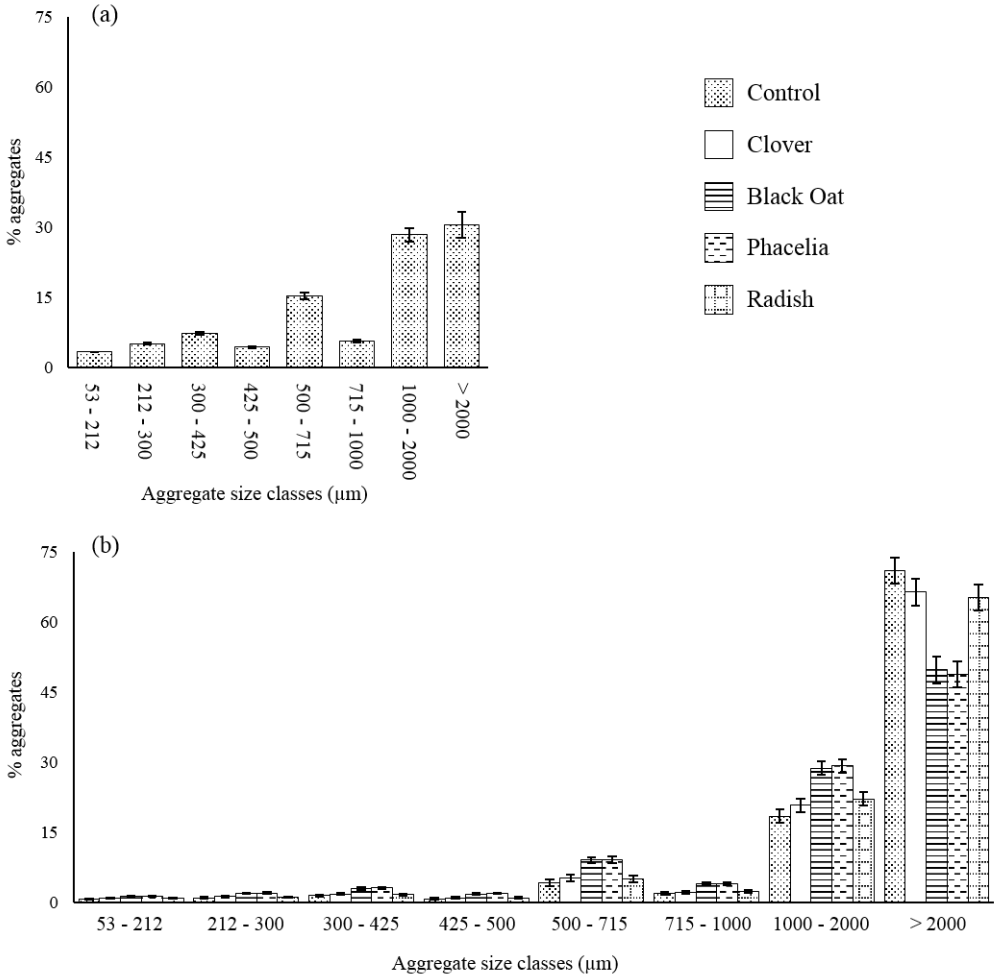


Figure 5.4.: Aggregate size distribution displaying the starting condition at Week 0 (a) and the effect of different plant species at Week 8. Bar charts represent means expressed as percentage of aggregates relative to the total volume, and whiskers are pooled standard errors.

This basic pattern persisted at Week 8 with a substantial increase in proportion of aggregates for the size class >2,000 μm. Planted soils with black oat and phacelia had a significantly greater proportion of aggregate from 2,000-300 μm than the control and the planted soil with clover and radish, but this trend was reversed for aggregates >2,000 μm (Fig. 5.4.b).

5.5.3. Microbial phenotypic profiles

There were significant plant effects upon microbial community phenotypic structure with respect to PC1 and PC3 (both $P<0.001$), which collectively accounted for 61% of the variation (Fig. 5.5.). Microbial community phenotype profiles differed significantly between both planted and unplanted soils, and between plant species. There was a significant effect of the plant species upon the microbial community phenotype apparent via PC1 and PC3 ($P < 0.001$ and $P = 0.012$ respectively) which together accounted for 61% of the variance. Community structure associated with phacelia was notably distinct from the other treatments apparent via PC1, and communities associated with black oat distinct from those of clover, with black oat communities intermediate between these (Fig. 5.5.a). PC3 discriminated communities associated at Week 0 (control) with those present at Week 8 in all cases (Fig. 5.5.a). The loadings associated with PC1 were predominantly two fungal markers (18:2n6,9; 18:3n9) and two Gram negative marker (16:1n9, cy-17:00) and two Gram positive markers (i-16:00; a-15:00; Fig. 5.5.b). Markers associated with Gram positive bacteria contributed large loadings to PC3 (Fig. 5.5.b).

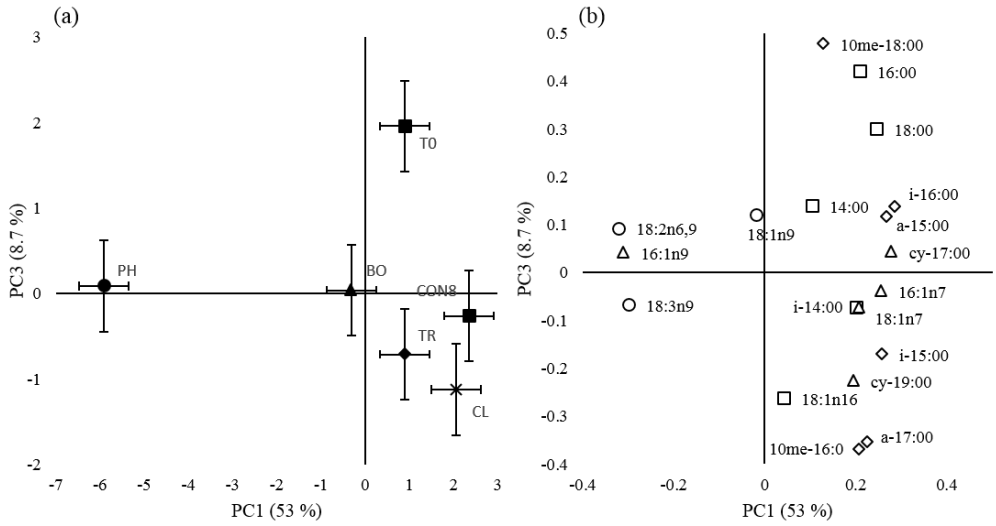


Figure 5.5.: Effects of plant species upon microbial community phenotypes. (a) Principal component (PC) ordination of first and third PCs (T0: control at week 0, CON8: control at week 8, CL: white clover, BO: black oat, PH: Phacelia, TR: tillage radish; points show means, whiskers denote pooled s.e) and (b) associated loading (◊ Gram positive Δ Gram negative ○ fungi □ non-specific markers).

The nature of the effect was due to an increase of the proportion of fungal marker and a decreased of the proportion of Gram + for phacelia compared to the other treatments (Fig. 5.6.).

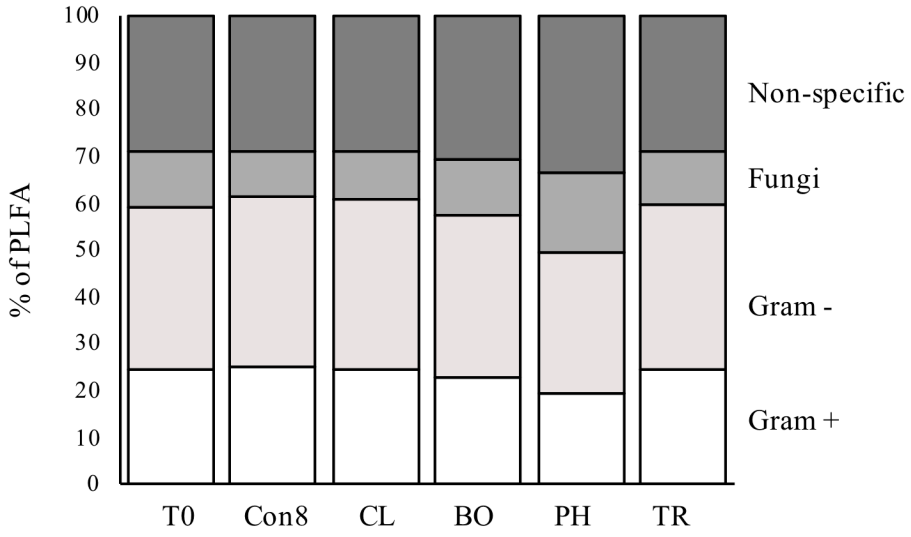


Figure 5.6.: Proportion of PLFA divided per group of community: T0: control at week 0; after 8 weeks of incubation: Con8: control; CL: clover; BO: Black oat; PH: phacelia and TR: Tillage radish

5.6. Discussion

After 8 weeks of growth, black oat and phacelia cores were highly root-bound, suggesting that visualisation of effects beyond this point would be inappropriate, and that any effects of the plants upon soil structure would be amplified by such a high concentration of roots. Whilst this then would be an artificial circumstance in the context of the field, it is appropriate for a comparative study such as this. After 8 weeks, the increase in proportion of the largest aggregates (>2,000 μm) for the control, the white clover and tillage radish might be caused by the high concentration of clay particles[13,27,28]. Limited effects of white clover and tillage radish might be due to a slower development of root systems, as both plant species grew much more slowly than the black oat and phacelia plants. For black oat and phacelia treatments, the presence of plant roots decreased the proportion of the largest aggregates

(>2,000 μm) by maintaining a greater proportion of aggregate sizes of 1,000-2,000 μm compared to the control. Therefore, the high proportion of roots apparently decreased the proportion of larger aggregates (>2,000 μm), and increased the proportion of aggregate sizes from 1,000-2,000 μm (Fig. 5.4.). The greater presence of roots in black oat and phacelia columns could have induced the breakdown of the larger aggregates resulting in an increase in proportion of aggregate sizes from 2,000-1,000 μm[14,15].

Black oat maintained the porosity at a constant level, i.e. at Week 8 the porosity of the columns was not significantly different from all the previous weeks (Fig. 5.2.). Despite the reduction of the connectivity between Week 2 and 8, black oat induced greater pore-connectivity compared to all treatments at both weeks (Fig. 5.3.b, c). Therefore, black oat maintained a greater porosity and pore-connectivity contrary to phacelia which decreased significantly porosity and pore-connectivity at Week 8 (Fig. 5.2. and 5.3.c) and increased the proportion of smaller pores compared to all treatments at Week 8 (Fig. 5.3.f). This formation of pore sizes (between 0.05 to 0.16 mm) facilitates the flow path of water, gas and nutrients[29], however, the decrease of connectivity might counteract this shift in the pore sizes[30,31]. Thus, the formation of new pores smaller than 0.3 mm with a poor-connection might be involved in the creation of water storage pores[32,33]. At Week 8, black oat and phacelia columns were root bound, notwithstanding that the nature of inherent pore network was drastically different for both these treatments. Root biomass could not be determined since aggregate sampling precluded this. Thus, different modifications of pore networks were implemented by the inherent nature of

root systems and not by the extent of rooting. Therefore, after 8 weeks of growth, black oat and phacelia showed evidence of impacting the pore network differently by modifying its characteristics in very different ways.

The non-significant impact of the plant at aggregate scale (Supplementary Fig. 5.2.) might be influenced by the water regime which was kept constant during the experiment. Wet and dry cycles are important for the modification of soil structure[32]. Notably by the disruption of aggregation due to clay particles swelling in the presence of water and the compression of entrapped air in capillary pores which could lead to the creation of new pores[34,35]. The presence of plant applies local wet and dry cycles by drying the immediate root environment leading to a greater cohesion of root exudates and clay particles[36,37]. Hence the non-significant impact of plants on the micro-structure lack of wet and dry cycles.

Principal component analysis discriminated the microbial community at Week 0 compared to all the treatments at Week 8 along the y-axis, meaning microbial community evolved during the incubation period and was not discriminated by PC3 (Fig. 5.5.a). This discrimination was associated with a shift of the bacterial community between both time points (Fig. 5.5.b). Notwithstanding this, PC analysis distinctly discriminated phacelia in relation to PC1, which was associated with the saprophytic fungal marker 18:2n3,6 and 18:3n9 and Gram-negative bacteria 16:1n9 (Fig. 5.5.)[23,24,26,38]. Moreover, phacelia showed a greater proportion of fungal marker compared to all treatments which revealed that phacelia increased the presence of saprophytic fungi (Fig. 5.6.). Such microbes were likely utilising rhizodeposits as Gram-negative bacteria and fungi have been described to be involved in immediate assimilation of

rhizodeposit carbon in grassland soils[38,39]. Another study showed that approximately two months after sowing, fungi were an important factor, especially the non-mycorrhizal fungi, to discriminate microbial community structure between different cover crops[11]. Phacelia has been described as forming mycorrhizal associations[40–42], but there is no record in UK soils of mycorrhizal formation. A quantification of the mycorrhizal infection was performed on the phacelia root (Supplementary material and methods 5.2.), which revealed no colonisation of roots by mycorrhizal hyphae (Supplementary Fig. 5.3.). The discrimination of black oat and tillage radish via PC1 between control and clover treatments (Fig. 5.5.a), showing that both plant species impacted slightly microbial community structure but not to the same extent as phacelia, which could be due to the nature of root characteristics[43].

5.7. Conclusions

These results revealed a contrasting effect of the root morphologies on the soil structure genesis which validated our hypothesis. Vigorous deep-rooting species (represented by the black oat) maintained the porosity and pore connectivity whereas one species of the fibrous multi-branching root species (phacelia) decreased porosity and pore-connectivity and enhanced the proportion of smaller pore (<0.31 mm). The tap root species (represented by tillage radish) and the second species of the fibrous multi-branching root species (white clover) decreased the porosity but had no significant impact on the pore connectivity. Therefore, the nature of the root architecture of these plant species likely modified the soil pore characteristics differently depending on the growth strategy of the plants.

Moreover, the microbial community phenotype was also modified by the presence of plants.

These results confirmed the postulate that the diversity of root morphology and higher-order interactions between plant and soil biota impact soil structural genesis and dynamics (Chapter 4)[44]. This has practical and ecological implications since the nature of root morphology can have different effects upon soil structure. What is unclear is the extent to which such effects occur where plants are growing in combination, which occurs in natural systems, and can be prescribed in cover-crop mixtures. This warrants further investigation.

5.8. References

1. Pagliai, M. & Vignozzi, N. The soil pore system as an indicator of soil quality. Adv. GeoEcology 35, 69–80 (2002).
2. Karlen, D. L. et al. Soil Quality: A Concept, Definition, and Framework for Evaluation. Soil Sci. Soc. Am. J. 61, 4 (1997).
3. Reicosky, D. C. & Forcella, F. Cover crop and soil quality interactions in agroecosystems. J. Soil Water Conserv. 53, 224–229 (1998).
4. Tisdall, J. M. & Oades, J. M. Organic matter and water-stable aggregates in soils. J. Soil Sci. 33, 141–165 (1982).
5. Materechera, S. A., Kirby, J. M., Alston, A. M. & Dexter, A. R. Modification of soil aggregation by watering regime and roots growing through beds of large aggregates. Plant Soil 160, 57–66 (1994).

6. Chan, K. Y. & Heenan, D. P. The influence of crop rotation on soil structure and soil physical properties under conventional tillage. Soil Tillage Res. 37, 113–125 (1996).
7. Vergani, C. & Graf, F. Soil permeability, aggregate stability and root growth: a pot experiment from a soil bioengineering perspective. Ecohydrology 9, 830–842 (2015).
8. Bacq-Labreuil, A. et al. Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture. Geoderma 332, 73–83 (2018).
9. Ashworth, A. J., DeBruyn, J. M., Allen, F. L., Radosevich, M. & Owens, P. R. Microbial community structure is affected by cropping sequences and poultry litter under long-term no-tillage. Soil Biol. Biochem. 114, 210–219 (2017).
10. Duchene, O., Vian, J. F. & Celette, F. Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. Agric. Ecosyst. Environ. 240, 148–161 (2017).
11. Lehman, R. M. et al. Soil biology for resilient, healthy soil. J. Soil Water Conserv. 70, 12A–18A (2015).
12. Scott, D. A., Baer, S. G. & Blair, J. M. Recovery and Relative Influence of Root, Microbial, and Structural Properties of Soil on Physically Sequestered Carbon Stocks in Restored Grassland. Soil Sci. Soc. Am. J. 81, 50 (2017).

13. Abdollahi, L., Munkholm, L. J. & Garbout, A. Tillage System and Cover Crop Effects on Soil Quality: II. Pore Characteristics. Soil Sci. Soc. Am. J. 78, 271 (2014).

14. Burr-Hersey, J. E., Mooney, S. J., Bengough, A. G., Mairhofer, S. & Ritz, K. Developmental morphology of cover crop species exhibit contrasting behaviour to changes in soil bulk density, revealed by X-ray computed tomography. PLoS One 12, 1–18 (2017).

15. Fernandez, A. L. et al. Structure of bacterial communities in soil following cover crop and organic fertilizer incorporation. Appl. Microbiol. Biotechnol. 100, 9331–9341 (2016).

16. Patkowska, E. & Konopiński, M. The role of oat, common vetch and tansy phacelia as cover plants in the formation of microorganisms communities in the soil under the cultivation of root chicory (cichorium intybus var. sativum bisch.) and salsify (tragopogon porrifolius var. sativus (Gat. Acta Sci. Pol., Hortorum cultus 12, 179–191 (2013).

17. Six, J., Frey, S. D., Thiet, R. K. & Batten, K. M. Bacterial and Fungal Contributions to Carbon Sequestration in Agroecosystems. Soil Sci. Soc. Am. J. 70, 555 (2006).

18. Finney, D. M., Buyer, J. S. & Kaye, J. P. Living cover crops have immediate impacts on soil microbial community structure and function. J. Soil Water Conserv. 72, 361–373 (2017).

19. Schneider, C. A., Rasband, W. S. & Eliceiri, K. W. NIH Image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675 (2012).

20. Vogel, H. J., Weller, U. & Schlüter, S. Quantification of soil structure based on Minkowski functions. Comput. Geosci. 36, 1236–1245 (2010).

21. Buyer, J. S. & Sasser, M. High-throughput phospholipid fatty acid analysis of soils . Appl. Soil Ecol. 61, 127–130 (2012).

22. Buyer, J. S., Teasdale, J. R., Roberts, D. P., Zasada, I. A. & Maul, J. E. Factors affecting soil microbial community structure in tomato cropping systems. Soil Biol. Biochem. 42, 831–841 (2010).

23. Stahl, P. D. & Klug, M. J. Characterization and differentiation of filamentous fungi based on Fatty Acid These include : Characterization and Differentiation of Filamentous Fungi Based on Fatty Acid Composition. Appl. Environ. Microbiol. 62, 4136–4146 (1996).

24. Zelles, L. Phospholip fatty acid profiles in selected members of soil microbial communities. Chemosphere 35, 275–294 (1997).

25. Zelles, L. Identification of single culutured micro-organisms based on their whole-community fatty acid profiles, using an extended extraction procedure. Chemosphere 39, 665–682 (1999).

26. Frostegård, A. & Bååth, E. The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fertil. Soils 22, 59–65 (1996).

27. Dexter, A. R. Advances in characterization of soil structure. Soil tillage Res. 11, 199–238 (1988).

28. Oades, J. M. Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76, 319–337 (1984).

29. Rabot, E., Wiesmeier, M., Schlüter, S. & Vogel, H. J. Soil structure as an indicator of soil functions: A review. Geoderma 314, 122–137 (2018).

30. Luo, L., Lin, H. & Schmidt, J. Quantitative Relationships between Soil Macropore Characteristics and Preferential Flow and Transport. Soil Sci. Soc. Am. J. 74, 1929 (2010).

31. Paradelo, M. et al. X-ray CT-Derived Soil Characteristics Explain Varying Air, Water, and Solute Transport Properties across a Loamy Field. Vadose Zo. J. 15, 0 (2016).

32. Bronick, C. J. & Lal, R. Soil structure and management: A review. Geoderma 124, 3–22 (2005).

33. Pires, L. F. et al. Soil structure changes induced by tillage systems. Soil Tillage Res. 165, 66–79 (2017).

34. Denef, K. et al. Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol. Biochem. 33, 1599–1611 (2001).

35. Grant, C. D. & Dexter, A. R. Air entrapment and differential swelling as factors in the mellowing of moulded soil during rapid wetting. Aust. J. Soil Res. 28, 361–369 (1990).

36. Reid, J. B. & Goss, M. J. Interactions Between Soil Drying Due To Plant Water-Use and Decreases in Aggregate Stability Caused By Maize Roots. J. Soil Sci. 33, 47–53 (1982).

37. Six, J., Bossuyt, H., Degryze, S. & Denef, K. A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res. 79, 7–31 (2004).

38. Denef, K., Roobroeck, D., Manimel Wadu, M. C. W., Lootens, P. & Boeckx, P. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biol. Biochem. 41, 144–153 (2009).

39. Treonis, A. M. et al. Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs. Soil Biol. Biochem. 36, 533–537 (2004).

40. Casanova-Katny, M. A., Torres-Mellado, G. A., Palfner, G. & Cavieres, L. A. The best for the guest: High Andean nurse cushions of Azorella madreporica enhance arbuscular mycorrhizal status in associated plant species. Mycorrhiza 21, 613–622 (2011).

41. Neagoe, A., Iordache, V., Bergmann, H. & Kothe, E. Patterns of effects of arbuscular mycorrhizal fungi on plants grown in contaminated soil. J. Plant Nutr. Soil Sci. 176, 273–286 (2013).

42. Cripps, C. L. & Eddington, L. H. Distribution of Mycorrhizal Types among Alpine Vascular Plant Families on the Beartooth Plateau, Rocky Mountains, U.S.A., in Reference to Large-Scale Patterns in Arctic–Alpine Habitats. Arctic, Antarct. Alp. Res. 37, 177–188 (2005).

43. Chan, K. Y. & Heenan, D. P. Microbial-induced soil aggregate stability under different crop rotations. Biol. Fertil. Soils 30, 29–32 (1999).

44. Ehrmann, J. & Ritz, K. Plant: Soil interactions in temperate multi-cropping production systems. Plant Soil 376, 01-29 (2013).

5.9. Supplementary data

5.9.1. Materials and Methods

5.9.1.1. Supplementary Materials and Methods 5.1. Aggregate extraction and image analysis

Aggregates were selected randomly from the sieve 2000-1000 µm and scanned using Phoenix Nanotom® (GE Measurement and Control solution, Wunstorf, Germany) set at a voltage of 90 kV, a current of 65 µA and at a voxel resolution of 1.51 µm. The total scan time was 69 minutes, with a total of 1440 projection images was taken at 500 ms period using an averaging of 3 images and skip of 2. Scanned images were reconstructed using Phoenix datos | x2 rec reconstruction software. The scanned images were optimised to correct any sample movement during the scan and reduce noise using the beam hardening correction algorithm, set at 8. Image sequences of 0.98 x 0.73 x 0.60 mm were extracted for the image analysis for the aggregates. The image analysis was performed exactly as the core images.

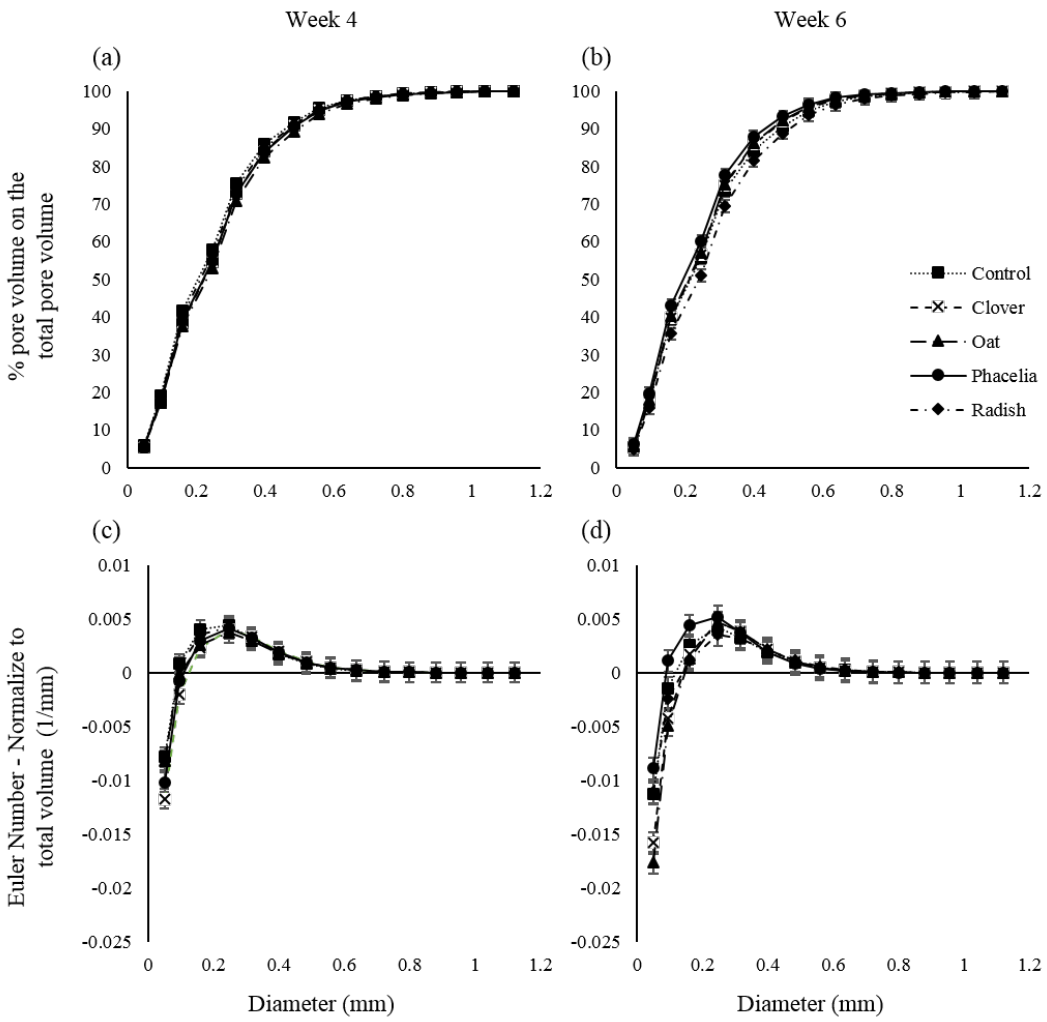
5.9.1.2. Supplementary Materials and Methods 5.2. Clearing, staining and quantifying mycorrhizal roots.

The quantification of mycorrhizal infection of roots was performed on fresh root samples harvested at the end of the experiment. Clearing and staining of roots was proceeded following the method from1. All the baths were kept at 70ºC for the experiment. Roots were cleared in 10% potassium hydroxide solution for 90 mins, then washed for 2 mins in water. Roots were stained in 3% ink in 5% acetic acid solution for 6 mins and washed in 5% acetic acid for 10 mins. Roots were placed in Petri dish with gridlines containing 50% of glycerol, the quantification of the mycorrhizal infection was performed following the method from2 using a binocular microscope (Stemi SV 6, Zeiss, Germany), and an associated camera (Axioma ERc 5S, Zeiss, Germany) using the in-build software Zen® 2.3 lite.

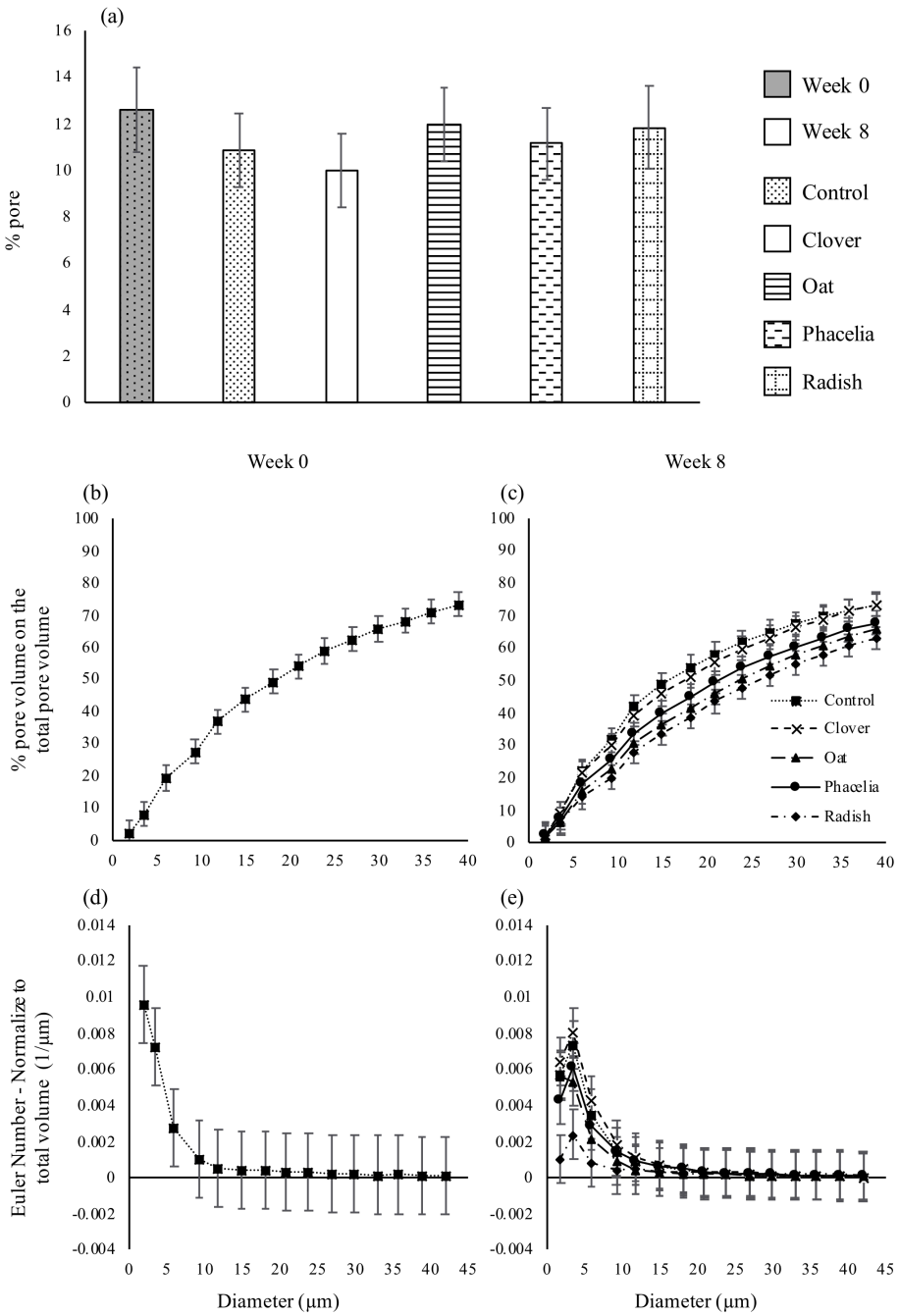
5.9.2. Supplementary references:

1. Vierheilig, H., Coughlan, A. P., Wyss, U. & Piche, Y. Ink and Vinegar, a Simple Staining Technique for Arbuscular-Mycorrhizal Fungi. Appl. Environ. Microbiol. 64, 5004–50070 (1998).
2. Giovannetti, M. & Mosse, B. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist 84, 489–500 (1980).

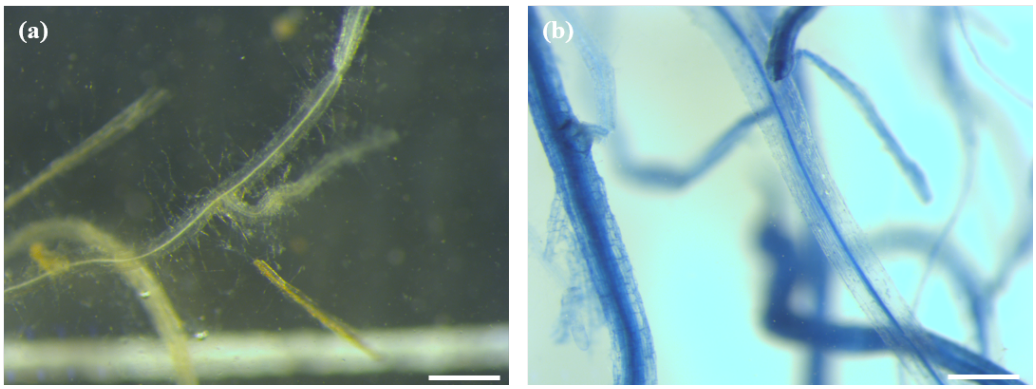
5.9.3. Supplementary figures



Supplementary figure 5.1.: Minkowski functions of treatments at core scale (40 µm resolution) at two time points, Week 4 (a, c) and Week 6 (b, d): (a, b) cumulative pore size distribution; (c, d) pore-connectivity of cores. Points show means, whiskers denote pooled s.e.



Supplementary figure 5.2.: Minkowski functions of treatments at aggregate scale (1.5 µm resolution) at two time points, Week 0 (a, b, d) and Week 8 (a, c, e): (a) porosity (b, c) cumulative pore size distribution; (d, e) pore-connectivity of cores. Bar chart and points show means, whiskers denote pooled s.e.



Supplementary figure 5.3.: An example of images visualised with a binocular microscope from the phacelia (a) unstained roots; (b) stained roots. Scale bare are 500 µm.

Chapter 6: General discussion

6.1. Summary

The main objective of this project was to understand the impacts of plant roots on soil structure. Essentially, two basic processes are considered: *soil structural genesis*, i.e. the creation of new pore networks; and *soil structural dynamics*, which involves the modification of existing pore networks/ characteristics such as porosity, pore size distribution, pore connectivity and pore surface density (which is an indicator of the pore surface roughness).

Soil structure was studied using long-term field experiments at Rothamsted Research, looking at the effect of cropping systems on soil characteristics. Plant effects were shown to be different depending on the soil texture: for a cohesive soil, the presence of plants increased porosity, diversity of pore sizes and pore-connectivity whereas for a non-cohesive soil, the presence of plant decreased both porosity and pore-connectivity (Chapter 2).

Plants had a significant impact on soil structural genesis, the research aimed to address the question concerning the impact of roots on a compromised soil (from a bare-fallow soil, Chapter 2). Archived soil from conversion plots from the long-term management experiment at Rothamsted Research were sampled to study the rate of recovery of micro-structure in a cohesive soil. The presence of plants increased porosity and pore-connectivity after 7 and 10 years respectively since conversion and increased the diversity of pore sizes after 2 years of conversion. However, the magnitude of the effect was lower than in

Chapter 2, which suggested only a partial recovery of the soil structure after 10 years of conversion (Chapter 3).

This led to the hypothesis that soil texture was an important factor in modulating the effects of plants on soil structural dynamics. To test this hypothesis, a controlled pot experiment was designed to specifically look at the role of a single plant species in soil structural genesis on two different soil textures. This study confirmed the hypothesis (Chapter 4). This then led to a further hypothesis that such effects might be contingent upon the plant species, given the variety in plant root traits that exist. Four cover crop species, selected for their contrasting root architures, were studied in a controlled pot experiment to assess their effects upon soil structural genesis and microbial community phenotype. This study validated the hypothesis that four cover crop species have differential effects upon soil structural genesis and microbial community phenotype (Chapter 5).

6.2. Field vs pot experiments

These four case studies were comprised of two experiments based on field trials, aimed to assess the modification of the soil structure, and two pot-experiments conducted in controlled environments, which aimed to assess the genesis of soil structure.

6.2.1. Pot experiments

In a pot experiment, there is a greater control on external factors such as wet/dry cycles, humidity, temperature, light/dark cycles, as the pots can be placed in a growth chamber (precision control of environmental factors), a

glasshouse (controlled environment) or outside (similar environmental conditions as in the field). The boundaries of pots are confining, which generally leads to a high degree of root:soil contact (depending on the volume) and this can amplify soil-based responses due to the presence of plant roots. This amplification can potentially increase the sensitivity and reveal changes due to the presence of plants, which may not be apparent otherwise. Therefore, the impact of the roots on soil structure is likely to be greater on the bulk soil in a pot than in the field. In a pot, the concentration of the roots can increase rapidly and become root-bound, when most of the soil essentially has contact with the roots. The time taken to onireach a root-bound state will depend on the size of the pot and growth rate of the plant and the nature of the roots. Thus, pot experiments have artificial boundaries which constrain the appropriate experiment time. This is different from a field experiment where responses are more dispersed through the surrounding soil with no boundary limitation, and root-induced modifications may be confined to the rhizosphere only. However, a pot experiment allows specific factors to be controlled (such as environmental factors and initial soil structure) which is appropriate to ask specific questions, to determine specific effects and to test specific hypothesis. Here, for example, the impact of plants on the actual genesis of soil structure was possible by experimentally creating a soil that had been destructured at a specific scale (Chapter 4 and 5). Thus, pot experiments are useful to answer specific questions which can be linked afterward to the greater concept of soil structural genesis, however, the study of long-term effects can arguably be inappropriate in pot experiments.

6.2.2. Field experiments

Fields are subject to the prevailing weather conditions and the farming management applied to them. There are no controls on external factors such as rainfall, humidity, temperature, and light/dark cycles. These characteristics can be recorded and used to correlate data to some event (such as heavy rain, drought). In the field, plant growth has no physical boundary limitation, i.e. the spatial boundary induced by the edge of a pot is not present in a field experiment leading to a greater surface of exploration for the root system. Therefore, long-term experiments are appropriate in these circumstances: for example, to understand the effect of long-term managements on soil structural dynamics (Chapter 2). One of the disadvantages of field experiments is the disturbance applied to the soil during the sampling process. Some artefacts might be created due to sampling such as cracks created by the movement of stones (Chapter 2). However, fields are connected to the entire environment surrounding them, which is important to link with the results, because the field context can play a role in understanding the results (Chapter 3). Field experiments allow scientists to understand long-term processes. Therefore, the field experiment results can be extrapolated to larger concepts (such as soil structural dynamics) and compared to other field experiments under similar conditions.

6.3. Different plants impact soil structural genesis differently

Plants are fundamental in processes that modify the soil systems *via*: root growth, carbon inputs by rhizodeposition, root decay, and modification of hydraulic properties (Six *et al.* 2004). Plant roots enhance soil aggregation by enmeshing soil particles and release of mucilage (Tisdall and Oades 1982; Morel *et al.* 1991; Chenu and Cosentino 2011). This leads to a creation of new

micro-environments in the vicinity of roots (Dorioz *et al.* 1993). Root-exudates can be water-repellent and provide protection against desiccation in event of drought (Hallett *et al.* 2003). Moreover, mucilage is different amongst plant species, and the composition of root exudates is dependent of the genotype, age, nutrient status, pest and disease loads of the plants. For example, maize roots produce more exudate than wheat (Hütsch *et al.* 2002) and lupin root exudates invoke a greater stimulation of fungal growth than wheat exudates (Haynes and Beare 1997).

6.3.1. Diversity of root morphology

Plant root morphology depends on the species and the genotype. Different genotypes of the same plant species can show different root architectures. The nature of root architecture impacts on the soil structure differently because root morphology affects soil structure differently (Chapter 5). For example, a tap root species increases compaction in the surrounding environment of the roots due to soil displacement, whereas fibrous root species have a greater impact on rhizosphere porosity, the diversity of pore sizes and pore connectivity (Burr-Hersey *et al.* 2017; Helliwell *et al.* 2017). The choice of the different plants in Chapter 5 was based on their different inherent root architectures, i.e. tap root (tillage radish), vigorous deep-rooting (black oats), and fibrous multi-branching root (phacelia and white clover). This confirmed the hypothesis that different forms of root architecture lead to different effects on soil structure. Tillage radish and white clover did not have a significant impact on soil structure genesis due to a slower development of roots such that despite being confined to a pot situation, the influence of the roots upon soil structure was minimal.

Black oats created a porous and connected pore network compared to phacelia which increased the proportion of smaller pores (<0.31 mm) and decreased porosity and pore-connectivity. The increase of the smaller pore classes was also observed in another study which showed that root systems with high density and fine roots had a decreased macroporosity related to a shift towards small pore classes (Bodner *et al.* 2014). The inherent architecture of roots for these two species created two contrasting pore networks, however, the aggregate size distribution was congruent for both species and different from the other treatments. Black oats and phacelia decreased the proportion of aggregates >2 mm and increased the proportion of all size classes <2 mm (Chapter 4 and 5). Fine roots have been shown to be more effective in fracturing large soil aggregates (Chan and Heenan 1996). This fracturing of larger aggregates by fine roots decreases aggregation. This contradicts the hierarchical model of soil structure proposed by Tisdall and Oades (1982), but it is consistent with the later hierarchical model postulated by Oades (1984) in which plant roots and fungal hyphae increase the aggregation of macro-aggregates and then decompose which leads to the formation of micro-aggregates (Fig. 6.1.; Angers *et al.*, 1997; Six *et al.*, 2004).

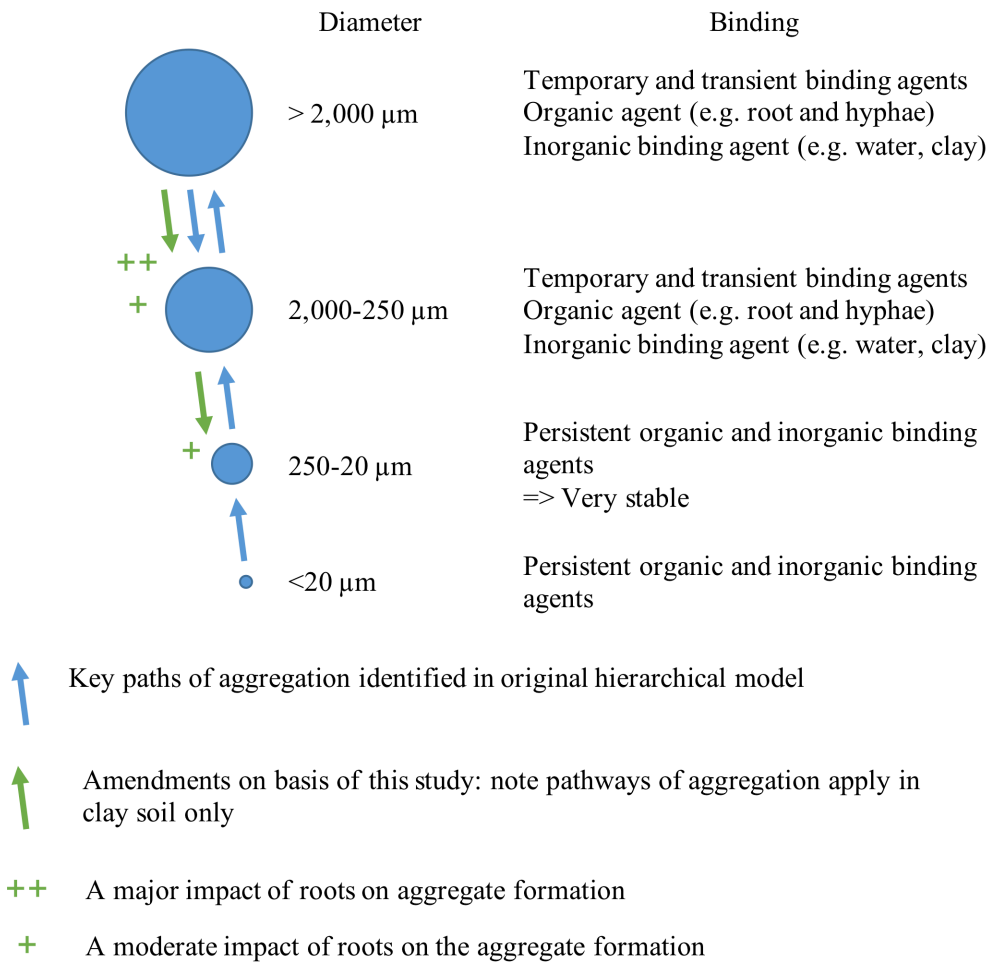


Figure 6.1. Conceptualisation of the hierarchical model of soil aggregate formation as proposed by Tisdall and Oades (1982) and Oades (1984), revisited on the basis of the findings in this thesis, specifically in the context of a clay soil.

Moreover, the penetration of roots within macropores might destabilise macro-aggregates leading to the formation of micro-aggregates (Materechera *et al.* 1994; Six *et al.* 2004). However, fine roots are more flexible, and can grow through small existing pores which leads to an increase in the stabilisation of the aggregates (Bodner *et al.* 2014). In the event of heavy rain, these aggregates might be more stable than larger aggregates, therefore, fine roots

tend to stabilise aggregation (Bodner *et al.* 2014). In the field, finer roots might create more aggregate sizes between 1-2 mm, which are joined by the root system and form macro-aggregates (>2 mm). Aggregate size distribution disconnected soil aggregates, by applying forces on the system to break down the macro-aggregates. Here, a decrease of macro-aggregation was observed which led to a formation of aggregates between 1-2 mm. The energy required to disperse soil aggregates was congruent for all the experiments and can be an indicator of the aggregate strength. However, this characteristic cannot be observed by 3D visualisation of X-ray CT. Moreover, soil texture had a greater impact upon the modification of the aggregate size distribution than plant species, meaning that the aggregate size distribution is dependent on the inherent soil properties. The 3D pore characteristics were also observed to be modified by soil texture, but plant species had contrasting effects on the pore network. The 3D characterisation of the pore network may be more sensitive to small changes compared to aggregate size distribution due to the resolution and the context dependency of the characterisation of the pore network *in situ* (Chapter 4 and 5). Indeed, the aggregate size distribution assay separates the resultant aggregates from their original spatial context in the soil system, and the population is essentially defined by the energy used to obtain that population. On the contrary, the pore network analysis encompasses the coherence of the entire system.

6.3.2. Modification of microbial community phenotype

Plant species are drivers of the microbial community structure in close association with their roots by enhancing certain groups of microbes (Ettema

and Wardle 2002; Appuhn and Joergensen 2006; Patkowska and Konopiński 2013; Finney *et al.* 2017). Multi-cropping of species also generally increases the diversity of rhizosphere communities (Ehrmann and Ritz 2013). The nature of root exudates influences the modulation of the microbial community by plants (Haynes and Beare 1997; Hütsch *et al.* 2002; Appuhn and Joergensen 2006). Here, the hypothesis that different species induced a different microbial community phenotype was validated (Chapter 5). Phacelia increased the relative proportion of saprophytic fungi and decreased the relative proportion of Gram-positive bacteria compared to all the other treatments. This confirms previous studies that showed plants enhanced fungal and Gram-negative bacterial components of the community and had no impact on the Gram-positive bacterial community, which is more influenced by the quality and composition of soil organic matter (Denef *et al.* 2009; Millard and Singh 2010; Mellado-Vázquez *et al.* 2016). PLFA profiling also revealed that the microbial community phenotype was modified for black oats, but the magnitude of the discrimination was lower than the phacelia. For white clover and tillage radish, there was no PLFA-detectable significant effect of plants on the microbial community phenotype which might be due to a lower concentration of roots for these two species, resulting in a dilution effect of rhizosphere with bulk soil (Chapter 5).

Furthermore, differences in microbial community structure can have a significant impact on soil physical structure. For example, fungal dominated communities increase porosity at a scale relevant to water storage and flow and gas exchange (Feeney *et al.* 2006; Crawford *et al.* 2012). The nature of the carbon input can modulate the microbial impact on soil structure via

contributing or hindering crack formation. This revealed the role of the microorganisms as degraders or as producers of soil binding agents (Preston *et al.* 2001). Black oat and phacelia had opposite impacts on the soil (see section 6.3.1) and enhanced different microbial community phenotypes, which raises the hypothesis that the microbial community can contribute to the modification of the soil structural genesis as well as the plant root morphologies. Notwithstanding, the soil structure can also impact the microbial community via the heterogeneous distribution of resources (Ettema and Wardle 2002). A recent study, performed on the same Highfield long-term experiment studied here, revealed that under the same soil texture, different cropping managements had no effect on the community structure (Neal *et al.* 2017). However, this study did reveal differences in gene coding profiles (Neal *et al.* 2017). There was a significant increase of genes coding for extra-cellular and outer-membrane associated enzymes under the bare fallow treatment. As shown in Chapter 2, the bare fallow treatment had a compromised soil structure: a low porosity, pore size diversity and pore-connectivity (Chapter 2); and the organic carbon and nitrogen were reported lower than under arable and grassland (Hirsch *et al.* 2009). Constrained correspondence analysis revealed that the connection of the pore system and the organic carbon were correlated with the presence of genes coding for the extra-cellular enzymes (Neal *et al.*, personal communication). Moreover, in the bare fallow treatment, there was a shift from aerobic to anaerobic for certain functional genes encoding for nitrogen and sulphur metabolism which was correlated with the low pore connectivity of the pore network (Neal *et al.*, personal communication). Indeed, a soil system with a lower porosity, pore connectivity might have a reduction of oxygen diffusion

which can cause an adaptation of the microbial community to their environment. This evidence suggests that soil structure, as well as organic carbon, can have a profound effect on the assemblage of functional genes to accommodate prevailing environments.

6.4. Plants modulate soil structure depending on their environment: optimal configuration of pore architecture.

6.4.1. The soil environment

Texture is an inherent soil property that plants cannot modify. For example, the percentage of clay within the soil is driving the cohesiveness of the matrix due to its inherent adhesive properties. Thus, clay content is an important factor for soil aggregation (Tisdall and Oades 1982; Dexter 1988), but also impacts plant growth responses (Helliwell *et al.* 2017) and soil remediation via increasing aggregation due to wet and dry cycles (Gregory *et al.* 2009). Bulk density is a characteristic that plants can modify. For example, in a compacted soil, plant roots may displace soil constituents when they are formed and grow, and biopores are formed when they decompose at the end of their life-cycle. This will decrease the bulk density of the soil. Indeed, these biopores can be re-used by other plants as preferential growth-pathways because it is easier for the roots to grow in an existing pore (Gregory 2006; Jin *et al.* 2013). Moreover, organic matter incorporated within the soil can be decomposed by organisms which leads to the creation of new pores, and thus decreases the bulk density (Metzger and Yaron 1987; Watts and Dexter 1997; Shepherd *et al.* 2002).

The initial state of soil structure could impact on the establishment of the plant roots via the heterogeneous distribution of water and nutrients. For example, in

a compromised soil structure (such as at the extreme ends of porosity, and low pore size diversity, pore connectivity), a plant root might struggle to find resources and water compared to a well-structured soil where there are flow paths allowing the water flow and nutrient through the pore networks. The initial state of soil structure can be modified by organisms living in the soil to access nutrients and water which impacts their fitness. The organisms provide other functions to the soil (such as exudation of extracellular compounds, moving of soil particles and organic matter in the soil profile) which modify the soil properties. The modification of one organism of its environment might facilitate other organisms living in the same environment to function in particular ways, including modify their surrounding environment, as well. This is essentially the concept of the 'extended phenotype' (Dawkins 1982), where the action of one organism on its surrounding environment affects both its fitness and that of other organisms in the same habitat (Chapter 1). Furthermore, in soil, organisms always function in the context of a community or system. Each organism can have on impact on its surrounding environment which can affect the other. The additive effect of all organisms living in the same environment has been described as the 'extended composite phenotype' (Phillips 2009, 2016). The modification of soil structure might also provide feedback and feedforward between the living organisms (such as plants, earthworms, fungi, bacteria) and the soil structure, as discussed in Chapter 1. For example, in the case of plants, the growing plant modifies the soil structure which might in turn render new nutrient sources available, which plant roots can use to grow more, and inevitably further modify the soil structure, etc. These modifications follow a successive sequence of feedforward and

feedbacks between the soil and the organisms within it. This concept is also behind the theory of self-organisation of soil systems. Soil systems are able to modify intrinsically without any intervention from external factors (Young and Crawford 2004; Crawford *et al.* 2012). These different successions can be observed for the two basic processes of the soil structural modification: soil structural genesis and soil structural dynamics. Soil structural genesis involves creation of soil structure from a de-structured soil in the presence of plants, whereas soil structural dynamics involve changes from an initial state which occurs from either an external event (such as weather conditions) or changes in cropping management.

6.4.2. Feedback of soil structure on the development of plants and root responses via the modification of structure

All the experiments reported in this thesis showed that plant effects on soil structural modification were different depending on soil textural characteristics (an inherent soil property) or bulk density (a dynamic soil property). These two characteristics both influence the cohesiveness of the soil. Here, non-cohesive soils have a small proportion of clay or a low bulk density (approximately 1 g cm^{-3}). There was no modification of the aggregate size distribution in the presence of plants for non-cohesive soil (Chapter 4). In such soils, in relation to pore-networks, plants decreased the porosity (Chapter 2, 4 and 5) and pore-connectivity (Chapter 2 and 5). But plants had no effect on pore-connectivity in Chapter 4, which might be due to a shorter duration of the experiment than in Chapter 5 (respectively 6 and 8 weeks of growth): plants affected the pore-connectivity only after 8 weeks of growth and no significant effect was seen at Week 6 (Chapter 5).

Plant roots develop differently depending on the cohesion of soil, for example, in non-cohesive soil, plants grow deeper and form thicker roots (Hacke *et al.* 2000; Jackson *et al.* 2000; Li *et al.* 2005). These developments are required to access water, for example, which infiltrates easily within the non-cohesive soil but is not stored and drains through the soil profile as well as nutrients (Wang *et al.* 2011; George *et al.* 2012). Therefore, a decrease of porosity and pore-connectivity could lead to a greater retention of water and nutrients at the soil surfaces via the formation of a greater proportion of storage pores in non-cohesive soil.

A cohesive soil is characteristically one with a finer texture, i.e. a greater proportion of clay and silt. The presence of plants decreased the proportion of aggregates >2000 μm and increased all the smaller aggregate size classes, with no difference between phacelia and black oat (Chapter 4 and 5). In section 6.3.1, black oats and phacelia are shown to have different effects on soil structural genesis (Chapter 5). This apparent contradiction of effects on aggregate size distribution and 3D pore characteristics might be due to the fact that both plant species impact soil structure via the modification of aggregates. The re-arrangement of aggregates might be different depending on the root morphologies as black oat has generally thicker and more structured root architecture compared to phacelia which has thinner roots with less organised structure (Bodner *et al.* 2010; Burr-Hersey *et al.* 2017). However, in a cohesive soil, the presence of plants increased or maintained soil porosity (Chapter 2, 3 and 4) and increased pore-connectivity (Chapter 2 and 3). Therefore, the presence of plants led to an increase in number and connectedness of flow

paths within the pore network, leading to a greater access to water and nutrient for plants.

Therefore, the concept of how roots influence soil structure can be modified (Fig. 6.2.a) and adapted with the overall results obtained in this study (Fig. 6.2.b). In a non-cohesive soil, plants decrease porosity and pore connectivity and increase pore size diversity. In a cohesive soil, plants increase porosity, pore size distribution and pore connectivity (Fig. 6.2.b).

Another parameter can be added to this concept, *viz*. the bulk density (Fig. 6.2.c). In chapter 5, phacelia decreased the porosity and pore-connectivity. The soil texture used was a clay soil with a bulk density of 1 g cm^{-3}. The plant response is coherent with a plant growing in a non-cohesive soil. This means that the bulk density might have a greater impact on the cohesiveness on the soil than the soil texture for the extreme ends (≤1 g cm^{-3} and >1.4 g cm^{-3})

Therefore, the bulk density can be added to the concept of cohesiveness. A non-cohesive soil can be defined by a bulk density ≤1 g cm^{-3} for any soil texture and a low proportion of clay for a bulk density comprised between 1 to 1.4 g cm^{-3}. The second value was hypothesised from another study due to non-experimental data (Harris *et al.* 2003). A cohesive soil might be defined by a bulk density >1.4 g cm^{-3} for any soil texture and a high proportion of clay for a bulk density comprised between 1 to 1.4 g cm^{-3} (Fig. 6.2.c).

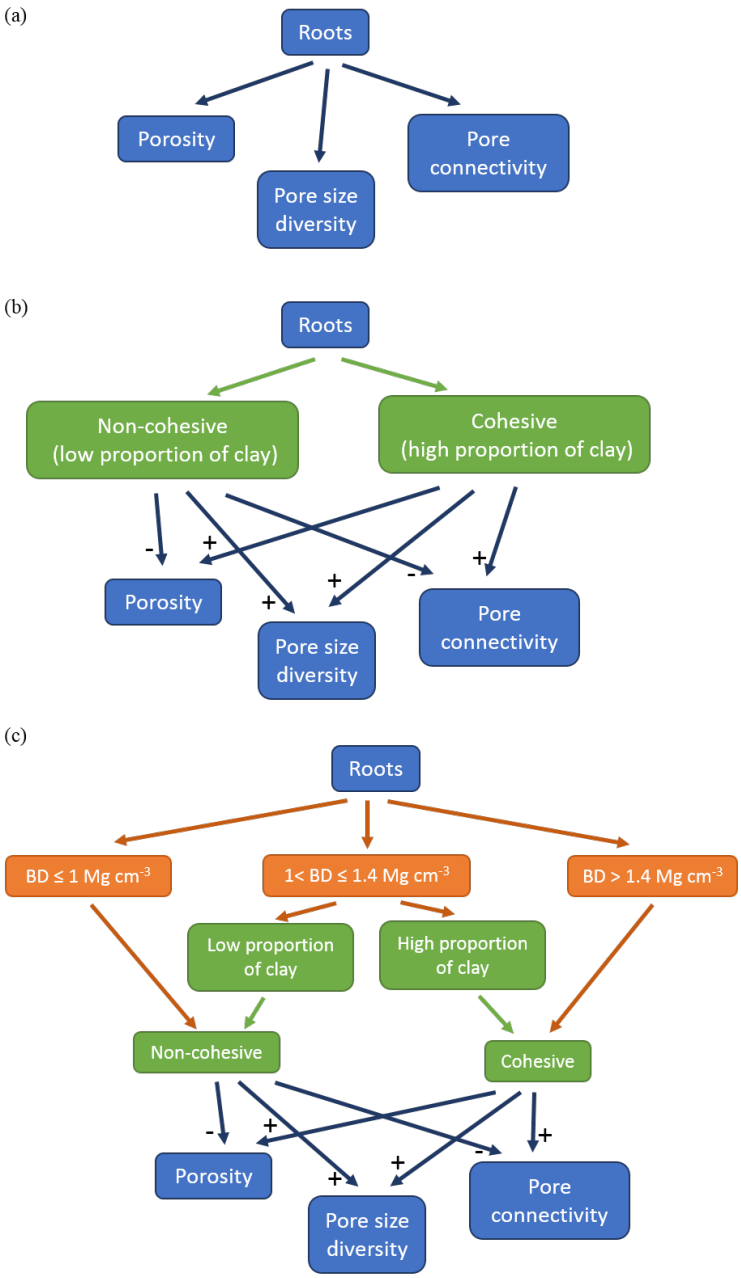


Figure 6.2.: Concept of the impact of roots upon soil structure (a) roots impact soil structure via increasing porosity, pore size diversity and pore connectivity; (b) this study revealed that roots impact soil structure differently depending on the cohesiveness of the soil; (c) modulation of concept by consideration of soil bulk density: roots impact the soil structure differently depending on the bulk density (for both extreme ends), and then depending on the soil texture. + denotes an increase and – denotes a decrease in effect.

The pot experiment (Chapter 4) revealed that depending on the soil texture, plant growth had a different effect on soil aggregate size distribution. After 6 weeks of growth, phacelia roots had no impact on this property for the sandy loam soil (Chapter 4). With a low proportion of clay particles, root and fungi, microbial exudates have no impact on the aggregation (Degens *et al.* 1994), the only agents of aggregation are via the more direct binding effects of roots and fungal hyphae (Six *et al.* 2004). Here, phacelia was not mycorrhizal, so the roots were the only binding agent in the soil, thus root impact might require more time to be manifest. For the clay soil, there was an impact of the root on the aggregates after 6 and 8 weeks of plant growth (Chapter 4 and 5). The modification of aggregates was similar for phacelia and black oat which were pot bound, and clover and tillage radish apparently had no influence, but there was much less root growth in the latter cases (Chapter 5). Plant roots decreased the proportion of aggregates >2,000 µm and increased the proportion of aggregates <1,000 µm compared to unplanted soil. This result contradicts the hierarchical models created by Tisdall and Oades (1982) and revised by Oades (1984), which postulated that plant roots and hyphae increase the proportion of aggregates >2,000 µm (Tisdall and Oades 1982) and the decay of plant roots and fungal hyphae decreased the proportion of aggregates >2000 µm and increase the formation of micro-aggregate (2,000-250 µm; Oades, 1984). Here, the unplanted soil displayed a greater proportion of aggregates >2,000 µm after 6 and 8 weeks of incubation, showing that microbial exudates, as well as clay particles, might be involved in macro-aggregate formation, and live roots broke down these aggregates to form a greater proportion of aggregates <1,000 µm (Chapter 4 and 5). The dynamics of aggregation processes show that in

cohesive soil, roots decreased the macro-aggregation toward a greater proportion of micro-aggregation, which is not due to the decay of the roots due to the duration of the experiment which was not taking the decay of the plant in consideration (Fig. 6.1.).

These results suggest that there is a relationship between the impact of plants on soil structural genesis and soil cohesiveness. The nature of the soil (such as texture, bulk density) impacts the plant-induced modification of the pore architecture depending on the initial state of soil characteristics. Plants also modify soil structure by inducing wet and dry cycles, via evapotranspiration processes. Wet and dry cycles can first disrupt aggregation via the swelling of clay particles and the compression of entrapped air in capillary pores (Grant and Dexter 1990; Denef *et al.* 2001), but the presence of roots dry the immediate environment via water uptake resulting in a greater cohesion of root exudates and clay particles (Reid and Goss 1982; Six *et al.* 2004). Therefore, development of pore networks by the plant could be considered as leading to an optimal configuration of pore architecture depending on the environmental circumstances. The concept of an 'optimal' soil structure was posited in relation to fungal growth by Harris *et al.* (2003). The optimal bulk density for optimal fungal growth was shown to be 1.4 g cm^{-3}, with an increase of the growth response with an increase of bulk density from 1 to 1.4 g cm^{-3} (Harris *et al.* 2003). The increase of bulk density (from 1.2 to 1.4 g cm^{-3}) increased the solid-pore interface by decreasing pore volume and increasing pore surface, which can be used by fungi to grow and explore the soil against the edge of the pores. A physical connection is needed between an organism and the substrate

source, ultimately manifest as an aqueous connection for both water and dissolved nutrients. Recent studies highlighted a 'hydro-patterning' phenomenon, which is a local response of root growth induced by the heterogeneous distribution of water within the soil profile (Babé *et al.* 2012; Bao *et al.* 2014; Morris *et al.* 2017). Roots grow and branch preferentially towards the water sources (Babé *et al.* 2012; Iwata *et al.* 2013; Bao *et al.* 2014). Therefore, water films within the pore space might also induce fungal growth. In a lower bulk density, the proportion of pores is greater, therefore, the fungi might grow to bridge through the pore, but this requires fungi to export energy from physically remote sources which reduce the speed of fungal growth. In a greater bulk density (>1.4 g cm^{-3}), the proportion of pores decreased which led to reduced gas and water exchange within the pores and creating potentially more physical contact points between hyphae and pore surfaces, which in turn leads to a decrease in fungal growth. Therefore, the 'optimal' bulk density for the fungal growth was 1.4 g cm^{-3} (Harris *et al.* 2003). Here, it seems that a similar observation can be made for the plant growth response i.e. that a plant impacts its surrounding environment depending on the initial state of soil structure (Fig. 6.3.). This is the concept of the 'optimal' configuration of pore architecture induced by plants.

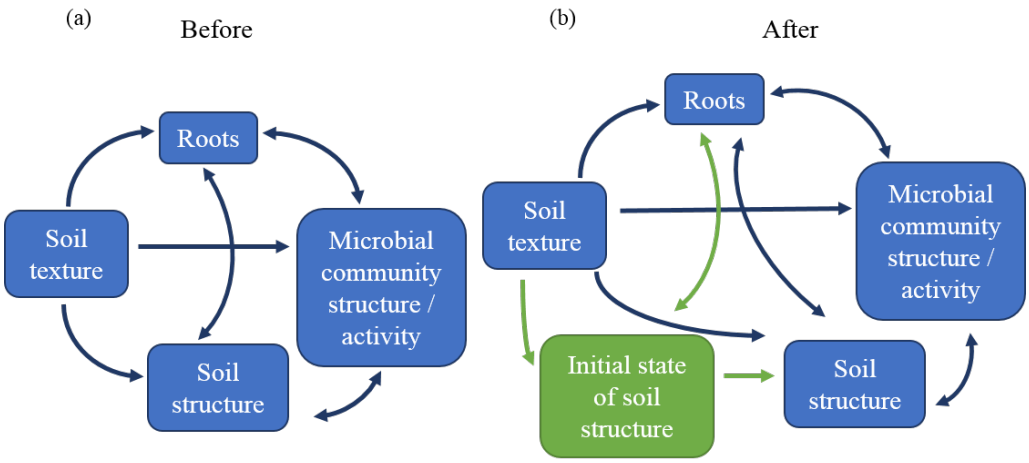


Figure 6.3.: Amendment to conceptualisation of key inter-relationships between soil based factors in soil structural genesis and dynamics, and self-organising processes, based on findings in this study. (a) Original framework as explained in introduction; (b) amendment depicting the essential role of initial state of soil structure in terms of the response of roots to initial state of soil properties (including texture and structure) and the modification of soil structure. See text for further explanation.

This notwithstanding, the optimal transport of water versus nutrients might not necessarily require the same pore configuration. Water movement through the soil profile is driven by the pore network: porosity, pore connectivity and pore size distribution are factors that control the water distribution. Therefore, water movement is impacted by the soil structure and the inherent properties of soil. Water transmission is increased in cohesive soil, via increasing transmitting pores and increasing the connectivity of the pore system by the plants (Chapter 2 and 3, following section). Water flows preferentially along roots and biopores leading to a greater dispersion of water and avoids water logging (Six *et al.* 2004). In contrast, water storage is increased in non-cohesive soil via decreasing the porosity, connectivity and increasing the proportion of storage

pores (Chapter 2 and 3, following section). In the event of drought, for example, water evaporates or leaks through the soil profile, leaving the plants without any accessible water. The creation of storage pores for water, and deeper growth through the soil profile could potentially curtail desiccation of the plant (Chapter 2; Hacke *et al.*, 2000; Jackson *et al.*, 2000; Li *et al.*, 2005).

The transport of nutrients is different due to the different nature of nutrients. For example, the relative diffusion rates of nitrogen vs phosphorus are very different: nitrate (NO_3^-), has a fast diffusion rate, compared to ammonium (NH_4^+) and particularly phosphate (PO_4^{3-}) which have a slow diffusion rate in soil (Nye and Tinker 1977). Nitrate can diffuse easily through water films, thus its diffusion is essentially similar to that of associated water. Ammonium and phosphate are less mobile within soil and greater pore-connectivity might not increase the diffusion rate of these nutrients. However, plants and fungi can access the sources of low diffusing nutrients through the existing pore network via space-filling growth arising from their extending and branching growth forms, which is potentially more efficient that waiting for the diffusion of these nutrients. Thus, there is a trade-off, for plants and fungi, between of resource the organism uses for foraging versus the amount of resource gained. In comparison, in a less connected pore network, plants can be nutrient deficient due to sources that are inaccessible to roots and slow diffusion rates of nutrient. This inaccessibility of nutrient sources might force the plant to create new pores to access the nutrients, which requires relatively more energy compared to a growth through an existing pore. Therefore, the optimal configuration of

pore architectures is a trade-off, for the plants, between an access to water and supply of nutrients.

The optimal configuration of the soil pore architecture might lead to a greater retention or flow path of water and nutrients, depending on plant needs and the environment which leads to a better adaptation to the environment surrounding the plant. Even though the environment impacts the plant responses leading to a different impact on soil structural characteristics, the presence of plants shows a consistent effect on the pore size distribution by increasing the diversity of pore sizes within the pore network, as discussed in the next section.

6.5. The presence of plants increases the diversity of pore sizes

6.5.1. Concept of diversity

It is suggested that the pore size diversity can be considered as an analogue of the concept of diversity in taxonomy. Hence, the diversity can be representative of the richness of pores, i.e. the number of individual pore sizes, and the evenness of pore sizes, i.e. the relative representation of different pore size classes. Given that the number of pore sizes is arbitrarily defined, here diversity means the evenness of the pore size distribution, apparent by the degree of linearity in the cumulative pore size distribution curve. A linear relationship indicates an equal representation of all size classes, i.e. a highly diverse network, whereas a curved relationship reveals a dominance of particular size classes.

6.5.2. Diversity of pore sizes at the millimetre-centimetre scale

The impact of plants upon soil structure differed between field (Chapter 2) and pot experiments (Chapter 2 and 3). The field experiment represented the effect of a long-term management, composed of a range of factors such as tillage, wet and dry cycles, in contrast, the pot experiments were the only effect of a plant on soil structural genesis.

After 50 years, the presence of plants resulted in contrasting effects on the pore size distribution (Chapter 2). Notably, a system involving perennial plants had greater structural heterogeneity, manifest as a wider range of pore sizes. In contrast, systems involving annual plants had a more homogeneous distribution of all pore sizes <1.2 mm but almost 30% of the pore sizes >1.2 mm. This difference between both treatments might be induced by tillage of the annual plant systems as part of its treatment which disturbed the pore size distribution and increased the proportion of pores >1.2 mm (Ambert-Sanchez *et al.* 2016). Therefore, the presence of plants apparently increased the diversity of pore sizes which might lead to a greater resistance of soil structure in a presence of stress. In the case of a physical soil disturbance, the pore size distribution will inevitably be impacted, but a greater inherent diversity of pore sizes might reduce the impact of such a loss because there are more pore sizes represented, and it is less likely that all size classes from a range would be completely lost. Therefore, the recovery of the pore size diversity is easier in the presence of a greater inherent diversity of pore sizes. The greater diversity creates a more complex pore network, which might increase the water and nutrient storage within the pore systems by increasing the storage and transmission pores (Chapter 2, 3 and 4).

In the pot experiment, the presence of phacelia impacted on the pore size distribution only after 8 weeks of growth (Chapter 4 and 5) whereas the other plant species did not show any effects (Chapter 5). Phacelia increased the formation of pore sizes between 0.05 to 0.16 mm, which are characteristically considered to be transmission pores (Metzger and Yaron 1987; Watts and Dexter 1997). Notwithstanding, the diversity of pore sizes was not drastically changed after 8 weeks of plant growth, meaning that plants might need more time to impact the pore size distribution. Moreover, the columns were kept at a constant water regime, but wet and dry cycles impact on soil structure dynamics (Bronick and Lal 2005). Water induces the clay particles swelling and the compression of entrapped air in capillary pores and the shrinkage of the clay particles in the drying process lead to creation of new pores (Grant and Dexter 1990; Denef *et al.* 2001). Therefore, the lack of wet/dry cycles in the columns might be the cause of a small modification of the pore size distribution.

6.5.3. Diversity of pore sizes at the micrometre scale

After 50 years, the presence of plants increased the diversity of pore sizes in the perennial system, compared to the annual system, the pore size distribution had approximately 70 % of pore sizes < 5.97 μm (Chapter 2). This difference could have been induced by the tillage management in the annual treatment which impacted the micro-structure. For the converted fields (Chapter 3), a rapid change was observed in the pore size distribution. Only 2 years after the conversion, perennial plants showed a greater diversity of pore sizes, to the same extent as observed after 50 years (Chapter 2 and 3), meaning that the

modification of the pore size distribution is faster than any other pore characteristics in the field, apparent after 2 years. The transmission pores are between 50-500 µm, the storage pores are defined from 0.5- 50 µm and the macro-pores >500 µm (Metzger and Yaron 1987; Watts and Dexter 1997), thus the action of plants increased the proportion of pores between 50 to 500 µm, meaning that plants increased the transmission and decreased the proportion of storage pores in the pore networks (Chapter 2 and 3).

In Chapter 5, there were no modifications observed after 8 weeks of plant growth at the micro-scale. Thus, the process of modification of pore sizes, at this scale, takes longer than 8 weeks, but less than 2 years. Further investigation is required to highlight the duration of the modification process.

6.5.4. Mechanisms generating the pore size distribution

At both scales, the presence of plants affected the pore size distribution diversity similarly, by increasing the diversity, which was in agreement with other studies that demonstrated that plants enhanced the formation of macro-pores and greater proportion of intra-aggregate pores (Horn *et al.* 1994; Dexter and Richard 2009). For the converted plots (Chapter 3), there was a difference in behavioural trend between the year 2010, 2012 and 2015. In 2012, the pore size distribution under the grassland treatment was not significantly different from the other treatments, which was different from the two surrounding years. This result reveals that the pore size distribution is dynamic over time, and is not following mechanisms operating successively year-on-year. The hypothesis is that the modification of pore size follows a non-monotonic mechanism depending on external factors to modify it. In Chapter 3, 2012 was revealed to

be the wettest year, therefore, the mechanism that modifies the pore size distribution might be based on the wet and dry cycles as well as the presence of plants. In wet conditions, clay particles swell, which might modify the microstructure of aggregates (see section 6.3.2.) and in dry conditions clay particles shrink and are absorbed with the root exudates and there is an increase of the porosity around the roots (Reid and Goss 1982; Six *et al.* 2004; Helliwell *et al.* 2017).

In the context of the field experiment, at the microscale, pore size distribution changes appear to be following rapid non-monotonic mechanisms (due to rapid changes in weather and moisture conditions). In comparison, other structural parameters (such as porosity, pore connectivity and pore surface density) have a slower response to this rapid change in conditions, and require a number of years to be affected by the conversion of managements.

6.6. Modification of soil characteristics varies in time

6.6.1. Modification of pore structure varies in time at the millimetre-centimetre scale

The modification over time at the millimetre scale can here only be assessed for the pot experiments, because for the field experiment, only one measurement at this scale was taken after 50 years under the same management. The presence of plants impacted the porosity after 8 weeks of growth for all the different plant species growing in the clay soil (Chapter 5). Moreover, in another experiment using sandy loam soil, phacelia decreased the porosity, after 2 weeks of growth (Chapter 4). The pore size distribution was modified significantly only after 8 weeks of plant growth (Chapter 5). The pore connectivity and the pore surface density were affected by the plant presence

only after 2 weeks (Chapter 4 and 5). Thus, at the millimetre scale, plant effects impacted the soil characteristics at different rates, depending on the different characteristics except for the porosity which depended on the soil texture.

6.6.2. Modification of pore structure varies in time at the micrometre scale

6.6.2.1. Long-term field experiment

The presence of plants had significant effects on porosity, pore-connectivity and pore surface density after 7 years (with a greater significance after 10 years) for the porosity, and 10 years after conversion for pore connectivity and pore surface density (Chapter 3). Therefore, the modification of the pore network in term of pore volume (porosity), pore connection and pore shape (pore surface density) requires at least 10 years after the change in cropping managements. These results were congruent with other observations at the macroscale from field converted to no-till managements: the recovery of porosity and pore-connectivity within the soil profile requires at least 10 years of conversions to no-till management (Mangalassery *et al.*, 2013). These combined results showed that soil structural recovery from a compromised state requires at least 10 years at the micro-scale but also at the macro-scale and duration of recovery was very similar for both scale. There was a consistent trend in the development of the structural properties over time, with the same impact of plants on soil structural dynamics operating successively every year. This was different from the dynamics for pore size distribution, which was faster process and appeared to be non-linear (see section 6.5.4.).

6.6.2.2. Short-term pot experiment

The pot experiments were designed to study short-term dynamics of plant effects on soil structural genesis, i.e. over 8 weeks (Chapter 5). Plants had no effects on the pore network of the aggregates after 8 weeks of growth. The aggregates were randomly selected within the sample and would have therefore included representatives from both the bulk soil and rhizosphere zones. Previous experiments showed that the first impact of the plant on soil structure was spatially confined to the rhizosphere (Helliwell *et al.* 2017). In studies on root growth in compacted soil, plants affected only the rhizosphere without any impact on the surrounding bulk density. In the rhizosphere, plants enhanced pore connectivity leading to the preferential water flow along the root (Whalley *et al.* 2005). However, Feeney *et al.* (2006), demonstrated fungal impact upon soil structural dynamics via columns divided into two chambers with a mesh: the inner chamber contained the plant and microbes, and the outer chamber only the microbes. The plant and fungal effect was apparent upon soil structural modification after 28 days with a greater impact in the inner chamber than the outer chamber revealing the greater impact of roots upon soil structural modification. However, the aggregate size selected for scanning was greater than the size selected in Chapter 5 (>2,000 µm and 2,000-1,000 µm, respectively) and the resolution was coarser (4.4 µm). This difference in aggregate sizes and resolution might be responsible for the pore characteristic modification detected by Feeney *et al.* (2006) and the absence of modification after 8 weeks of plant growth (Chapter 5). Indeed, a smaller aggregate size focuses only on the micro-structure compared to a greater aggregate size, where a bigger range of pores can be assessed (see section 6.5.3). Thus, the

modification of micro-structure within the entire column might take more time to happen. Roots can also induce transient pore clogging via growing through existing pores, so fine roots might be present within the micro-pores and when they decay they can form new biopores (macro and micro-pores; Bodner *et al.*, 2014; Ghestem *et al.*, 2011; Gish and Jury, 1983; Wuest, 2001). Moreover, the columns were kept at a constant water regime (Chapter 5), which allowed the identification of the role of plants without this factor. The following question can be the relative roles of plants versus wet and dry cycles upon soil structural genesis, despite that plants affects wet and dry cycles too (see section 6.4.2 and 5.2).

6.6.3. Variations in time- and scale-dependency

The modification of soil structure is a dynamic process, driven by different factors (such as wet and dry cycles, living organisms; sections 6.2, 6.3 and 6.4). The different experiments highlighted that the modification of soil structure varies with respect to both time and space. Especially the pot experiments showed that soil structure was impacted by the presence of plants after 6 or 8 weeks of growth at the millimetre scale (Chapter 4 and 5 respectively) whereas there was no plant effect at the microscale after 8 weeks of growth (Chapter 5). Therefore, the series of experiment here provide that the temporal effects of soil structural genesis by plants are scale-dependent. That is, effects are first apparent at larger scales (mm), and subsequently at the smaller scales (µm). The millimetre scale might be easier to impact due to the nature of binding agents (Fig. 6.1.). The binding agents are transient and temporary (Tisdall and Oades 1982), highly degradable by micro-organisms.

Plant roots grow preferentially through existing pores i.e. around the existing aggregates or through macro-pores within aggregates (Gregory 2006; Jin *et al.* 2013), in turn roots might modify the stability of the surrounding aggregates due to excretion of root mucilage and the plant-induced wet and dry cycles. The local wet and dry cycles might redistribute the binding particles from the surrounding aggregate close to the plant root (see section 6.3.2.). The modification of micro-structure might be enhanced by the secondary root systems, laterals and root hairs, which are produced after the growth of primary roots, due to this later production of root system, their impact on soil structure is delayed compared to primary roots (Pierret *et al.* 2011). Furthermore, binding agents at the micro-scale are more persistent (organic and inorganic; Tisdall and Oades, 1982), therefore, the influence of plant roots might be longer at that scale to have a significant impact on the existing binds. The original *in situ* location of the aggregates sampled for characterisation might have been important too. The first aggregates to be modified are part of the rhizosphere (see section 6.5.2.2.), but since the aggregates scanned here were randomly selected within the entire column, so they were not spatially defined. Thus, in the time frame of this experiment (8 weeks), a new hypothesis can be that only the aggregate from the rhizosphere may contain a detectable effect. This will need further investigation. The impact on the bulk soil might require a long-time experiment to see the modification. Thus, soil structural genesis is scale, location and time dependant.

Aggregates from the long-term field experiment were sampled at much longer time period (Chapter 3). The first time point was two years after conversion and aggregates were from the bulk soil. As seen in the section 6.5.2.1.,

porosity, pore connectivity and pore surface density were modified only after 10 years. The millimetre scale could not be assessed as the sample came from the archives from Rothamsted Research and only aggregates were available. However, at a millimetre scale, looking at the effect of conversion from tillage to no-till soil, porosity and pore-connectivity were also significantly increased after 10 years of conversion (see section 6.5.2.1.). Therefore, the modification of the macro and the micro structure at the field scale apparently takes approximately the same amount of time. The evolution of the soil structure was not measured at the millimetre scale (Chapter 3). This assessment can be interesting to compare the time effect at both scales, i.e. how long the different soil structural properties are affected by the presence of plants. It can be hypothesised that the millimetre scale has a more rapid recovery of the soil structural properties than the micrometre scale.

6.7. Ecological perspectives

6.7.1. Effect of cropping management upon soil structure

6.7.1.1. Bare-fallow treatment which compromised soil structure

These studies have shown that long-term fallow management compromised soil structure via a decrease or an increase in porosity (for clay and sandy soil respectively), and a decrease in pore size diversity and pore connectivity for both soils (Chapter 2). The loss of pore size diversity and connectivity led to a decrease of the permeability (Chapter 2). This loss of structure inevitably reduces the number and the connection of flow paths and increases the isolation of resources (such as water and nutrients) from living organisms. Due to the lack of organic inputs (via plant roots or managed addition of organic material), and the continuous disturbance of the soil, there was a decrease in

the content of carbon, nitrogen and living organisms in these soils (Hirsch *et al.* 2009). One of the consequences of the loss of structure and nutrient is an adaptation of the microbial community living in the soil, for example via an increase in the abundance of genes coding for extracellular enzymes ('exoenzymes') compared to intracellular enzymes ('endoenzymes') and genes coding for motility functions (Neal *et al.*, 2017; Neal *et al.*, in preparation). The energetic cost of production of extracellular enzymes is high, due to the loss of these enzymes from the cell into the environment, – there is little to no opportunity for biochemical recycling of these compounds. Moreover, given this spatial isolation of the microbial community from potential resources, organisms rely on connected water films in the soil to allow diffusive movement of exoenzymes to substrates, substrates to enzymes, and products to the cell wall for uptake. A reduction in pore connectivity therefore reduces the diffusion of the enzymes and substrates through the water film, which can lead to the starvation of the microbes. Therefore, the loss of structure applies an evolutionary selection of the genes coding different functions to low nutrient use efficiency within the microbial community.

Furthermore, the recovery of the soil structure from a compromised soil observed in Chapter 3 shows this process takes of the order of at least a decade. After 10 years of conversion, the micro-structure of aggregates showed an increase of porosity, pore size diversity and pore connectivity (Chapter 3) but the magnitude of the treatment effect was smaller than in aggregates in the long-term experiment (Chapter 2 and 3). The recovery of the meso-fauna and the microbial community was apparent after 2 years of conversion and the increase of organic matter after 4 years (Hirsch *et al.* 2017). This highlights an

intriguing relationship between the rapid recovery of living organisms and the organic matter, and the slower recovery of soil structure. The improvement of soil structure involves a slower mechanism regulating the soil structural properties via the presence of plants, addition of organic matter and the modification of soil structure by the living organisms. Moreover, it could be hypothesised that the modification of soil structural properties may regulate evolutionary processes via the increase of pore connectivity and pore size diversity which in turn increase the diffusion of substrate, or even mobile genetic elements, through the pore network. The presence of plants also increases the organic carbon inputs that can be used by the microbial community. This increases of the amount of substrates within the soil profile, which is easily accessible by the microbial community, can lead to a greater presence of endoenzymes than exoenzymes within the microbial community genotype. Genotype modification might require a similar period to adapt that the soil structural recovery.

Therefore, bare fallow management should be avoided in crop rotation, due to its detrimental effects on soil physical characteristics and replaced by a planted rotation, for example cover crops.

6.7.1.2. Addition of organic manure to conventional agricultural plot

The addition of manure to a conventional wheat field had the same effect upon soil structure as the grassland management which led, for the sandy soil, to a decrease of porosity and increase of pore size diversity (Chapter 2). This result shows that the addition of organic fertiliser (such as farmyard manure), and despite the tillage, had a substantial impact on soil structural properties.

However, the inorganic fertiliser had a similar effect as a bare fallow treatment. Therefore, the impact on the soil structure in the manured arable is due to the addition of manure and not only by the addition of any fertiliser. Manure is a product of highly decomposed materials enriched in microbiota and mixed with straws which can be at any stage of decomposition. This product is rich in nutrients and has a high C:N ratio, which is beneficial for fungal growth (Hu *et al.* 2001; Carney *et al.* 2007; Strickland and Rousk 2010). The addition of manure provides a rich substrate for the microbial community living within the soil, which might enhance the microbial activity. This microbial community enhancement might in turn impact on soil structural dynamic, by modifying soil structure via the growth of bacterial colonies or fungal hyphae and the exudation of extracellular-polymeric substances. In a high C:N ratio, fungal growth is enhanced (Hu *et al.* 2001; Carney *et al.* 2007; Strickland and Rousk 2010) which will lead to a greater impact on soil structural dynamic via the enmeshment of soil particle by hyphae (Tisdall and Oades 1982).

This relationship between the addition of manure and soil structure was observed only in a sandy soil, further studies are required to explore this relationship in the context of other soil types. In the context of a sandy soil, the addition of manure would be highly recommended as organic fertiliser due to its beneficial effects on soil structural properties and reducing the use of inorganic fertiliser which had a detrimental effect.

6.7.1.3. Grassland

This study revealed that perennial plants had a substantial impact on soil structural dynamics via the increase and decrease of porosity (clay and sandy

soil, respectively), the increase of pore size diversity and pore connectivity (Chapter 2 and 3). The increase of pore size diversity and pore connectivity was associated with an increase of permeability (Chapter 2). This enhancement of soil structure leads to a greater proportion of storage and transmission pores which are essentials for the water and nutrients diffusion or retention. Therefore, the presence of plants optimises the number and connection of flow paths of water and nutrients, which might reduce in turn water loss and nutrient leaching through soil profile. Moreover, no managed disturbance of the soil profile and the diversity of plants increased the soil organic matter and the presence of living organisms (Hirsch *et al.* 2009).

Therefore, the perennial plants are beneficial to the soil structure and can be incorporated in crop rotation, instead of the bare fallow management, for example.

6.7.1.4. Cover crops association

These studies highlighted that soil texture impacts on the precise influence of a plant species on soil structural genesis (Chapter 4) and different plant species have differential effects upon soil structural properties and microbial community phenotype (Chapter 5). Here, there was a focus of the impact of a plant alone on soil structural genesis to highlight the differences between plant species which could be induced by the morphology of the roots (see section 6.3.1), the rhizodeposition and the microbial community associated with the plant species (see section 6.3.2). Black oat and phacelia showed the greatest and contrasting impact upon soil structural genesis via the maintenance of porosity and pore connectivity for the black oat and the decrease of both

porosity and pore connectivity for the phacelia. This difference might be induced by the root morphology (see section 6.3.1) and by the microbial community phenotype altered by both species (see section 6.3.2). White clover and tillage radish showed a smaller impact upon soil structural genesis which might be due to a slower growth for the white clover and difficulty of growth for the tillage radish, as it could grow rapidly in another experiment (Burr-Hersey *et al.* 2017). These experiments were limited by the number of plant species and soil textures studied. Moreover, in practice, growers use complementary mixtures of cover crops but they are limited to certain species due to the UK policy, and they do not currently consider the effect of soil texture on the impact of the roots upon soil structure (P. Brown, personal communication). This is due to a lack of knowledge, which would require further research to screen new plant species that can have a beneficial impact on soil structural dynamics and the effect of the different plant species on different soil texture. Moreover, these results highlight that different species can have a differential time to establish. This needs to be accounted for if different crop species mixtures i.e. that if a plant species has a slower growth than the neighbouring plant, the growth of the first plant might be inhibit due to the allelopathic effect. Further research is needed to highlight the compatibility of plant growing together, and their combined impact on soil structural genesis.

6.7.2. Ways that plants modulate soil structure to remediate soil

These studies show that the initial state of soil structure is important for responses and impacts of plants on soil structural genesis and dynamics. There is an apparent cycle of feedback and feedforward between the plant roots and

the soil structure (see sections 6.3 and 6.4). Thus, plants are one of the important factors for soil pedogenesis by creating and modifying soil structure and adding organic matter to the soil via their mucilage excretion. This highlights the primary importance of plants in the concept of self-organisation of soil system by providing energy to the system (via plant growth and secretion of mucilage). Plants impact on its surrounding environment to enhance its fitness: for example, to access and retain more water, nutrients which could have implications for the surrounding organisms. Thus, plants are one of the important factors in the extended composite phenotype, as in natural systems there are many plant species cohabiting (see section 6.4.1). The diversity of plants can modify the plant effects on the surrounding neighbours via inhibiting or enhancing certain traits from the neighbouring plants which is the allelopathic effect (Ehrmann and Ritz 2013). More studies are required to understand the complementarity of species in different context of soil textures.

Moreover, plant impacts can be variable depending on the soil structure (Chapter 5) and the soil texture (Chapter 2 and 4). The impact of the same plant upon soil structural genesis depending on soil textures was driven by the initial state of soil structure (such as bulk density, soil structure) and the inherent soil properties (such as soil texture, nutrient status). Plants are influenced by the gravity, light and hydro-patterning (Morris *et al.* 2017), meaning that plants can sense their surrounding environments. Here, depending on soil textural characteristics plants affected the soil structure differently, by increasing or decreasing porosity (sandy loam and clay soil, respectively; Chapter 4). These modifications can have functional impacts such as: increase of porosity leads to

greater water and nutrient diffusions through the soil profile compared to a decrease of porosity which increase the water and nutrient retention. In non-cohesive soils, the loss of water and nutrients through leaching process can be reduced by the presence of plants by increasing the retention capacity of the soil system (see section 6.4.2). This shows that the impact of plants to modify soil structure leads to a greater adaptation of the soil environment for the plant fitness. Therefore, plants are highly adaptable to their environment and can induce variable effects upon the soil structural and biological characteristics.

Chapter 7: Conclusions

7.1. Main findings

- A long-term bare fallow treatment can have a significant effect on soil structural dynamics, via decreasing and increasing the porosity in clay and sandy soil respectively, and decreasing for both types soil pore size diversity, pore connectivity, pore surface density and permeability. This suggests a bare fallow treatment should be avoided in crop rotations due to the detrimental effect upon soil structure, and replaced by planted treatments.

- Long-term grassland has a profound effect on soil structure by increasing and decreasing the porosity (clay and sandy loam soils, respectively), and increasing pore size diversity, pore connectivity, pore surface density and permeability. These results validated the hypothesis that perennial plants invoke a greater porosity and a wider range of pore sizes in soils.

- In the sandy soil, the application of organic fertilizer rather than inorganic fertilizer has a significant influence on soil structure via decreasing the porosity, increasing the pore size diversity and permeability. The application of manure to a conventional arable field had the same effects upon soil structural dynamics as the grassland treatment. This partially validated the hypothesis that presence of plant matter improves soil structural properties. However here, the addition of manure was the main factor driving the soil structural improvement.

- Recovery of soil structure from a compromised soil apparently requires a decadal-scale time frame after the management conversion. Pore size diversity was the first soil structural parameter to be affected by the change to grassland treatment (2 years since conversion) and was also

affected by the weather conditions, which partially validated the hypothesis that plants induce a rapid recovery. Porosity, pore surface density and pore connectivity required at least 10 years to be significantly affected by the management conversion, however the magnitude of the plant effects upon soil structural dynamics was smaller than the effects of plants observed in the long-term experiment, which is partially not validated the fast recovery of soil structural characteristics.

- The growth of one plant species in two contrasting soil textures revealed a contrasting effect depending on the soil texture characteristics, manifest by a very different aggregate size distribution and the contrasting effect of plants on both textural soils. This validates the hypothesis that a plant can modify soil aggregation properties and pore network differently depending on the inherent soil texture.

- Four cover crop species have differential effects on soil structural genesis, which validated our hypothesis. Moreover, the different cover crops modulate the microbial community phenotype by influencing different microbial groups. This supports the hypothesis that plants are drivers of the self-organisation of the soil systems via modifying soil structure and the microbial community structure.

- A symbiotic relationship usually describes a mutually beneficial interaction between two living organisms. Here, we posit that soil structure and roots could validly be argued to have a symbiotic relationship, which is a beneficial interaction between root and soil structure, due to their close interactions. Without plant inputs and impacts, soil structure might not be modified, as plants are the primary mechanism by which energy is added to the soil system. Moreover, soil is fundamental for the establishment and growth of plants and can modify plant responses. This thesis revealed the close interactions between plants and soil structure: with the hypothesis that plants induce

the formation of an ‘optimal pore architecture’, which enhances the growth of plants and associated microbes. Thus, the interaction of plants and soil structure might also be considered as beneficial for the soil system i.e. a greater optimisation of soil structure in order to enhance plants and microbial growth. Therefore, this relationship is considered as a symbiotic relationship.

7.2. Future work

- The concept of the ‘optimal pore architecture’, which is the potential optimisation of the spatial environment by the root growth, developed in Chapter 2 (Chapter 2) and in the general discussion requires further study. The concept can be extended to the soil texture and the bulk density, from the experiment developed by Harris *et al.* (2003), where they showed the effect of bulk density on fungal growth. A similar experiment can be realised for plants: linking the role of the bulk density and the soil textural properties on plant effects upon soil structural genesis. The hypotheses are: (1) for a very low or high bulk density, the soil texture had no influence upon plant impact upon soil structure; (2) with an intermediate bulk density, the soil textures influence the plant effects upon soil structure.

- Soil structural genesis appeared to be scale, location, and time dependent. This requires further investigation looking at the magnitude of plant effects on soil structural genesis and dynamics at the millimetre scales (with cores) and microscales (with aggregates from the bulk soil and the rhizosphere zone). The location of the aggregates might help to visualise the first modification of the microscale in short time experiment. Moreover, analysis of soil structural properties at the same time point might reveal which scale is impacted first. From these studies, the hypothesis is that the macroscale is the first scale to be modified, then the microscale in the rhizosphere zone and finally the aggregates from the bulk density.

- Cover crops can be used to enhance soil structural properties. However, the individual impacts of cover crops are not known and there are many more species that can be considered than analysed here. Moreover, the impact of soil texture on the different cover crops species requires also further investigation. This knowledge would be informative for the farmers and growers to prescribe the cover crop species which would enhance soil properties accordingly to their needs.

- Cover crops are amenable to being in mixtures, but the understanding of belowground interactions between differing species is hardly known at all (Ehrmann and Ritz 2013). Allelopathic effects of cover crops on their neighbours can be beneficial or detrimental depending on the compatibility of the plant species contained in the mixtures. Here, white clover displayed a slower growth than phacelia and black oat, therefore in a mixture of these three species, white clover might be inhibited by the presence of phacelia and black oat as it requires a long time to establish and grow. These relationships need further investigation to highlight the potential complementary of plant species.

- In the conversion plot, the soil structural properties required at least 10 years of management conversion before showing any evidence of plant effects. Hirsch *et al.* (2017) showed that there was an increase of the microbial groups only after two years of management conversion. However, there has been no associated work on the evolution of the gene coding functions in the converted plot. It could be hypothesised that the diversity of gene coding for different functions requires a long period before showing a treatment effect. This investigation could reveal the effect of time on the evolutionary process for the microbial community genotype.

Collated references

This reference list collates all cited references in the entire thesis

Abdollahi L, Munkholm LJ, Garbout A (2014) Tillage system and cover crop effects on soil quality: II. Pore characteristics. Soil Sci Soc Am J 78:271. doi: 10.2136/sssaj2013.07.0302

Alameda D, Villar R (2012) Linking root traits to plant physiology and growth in *Fraxinus angustifolia Vahl*. seedlings under soil compaction conditions. Environ Exp Bot 79:49–57. doi: 10.1016/j.envexpbot.2012.01.004

Altieri MA (1999) The ecological role of biodiversity in agroecosystems. Agric Ecosyst Environ 74:19–31. doi: 10.1016/S0167-8809(99)00028-6

Ambert-Sanchez M, Mickelson SK, Ahmed SI, *et al.* (2016) Evaluating soil tillage practices using X-Ray computed tomography and conventional laboratory methods. Trans ASABE 59:455–463. doi: 10.13031/trans.59.11308

Angers DA, Recous S, Aita C (1997) Fate of carbon and nitrogen in water-stable aggregates during decomposition of $^{13}C^{15}N$-labelled wheat straw *in situ*. Eur J Soil Sci 48:295–300. doi: 10.1111/j.1365-2389.1997.tb00549.x

Appuhn A, Joergensen RG (2006) Microbial colonisation of roots as a function of plant species. Soil Biol Biochem 38:1040–1051. doi: 10.1016/j.soilbio.2005.09.002

Arvidsson J (1998) Effects of cultivation depth in reduced tillage on soil physical properties, crop yield and plant pathogens 9, 79–85.

Ashworth AJ, DeBruyn JM, Allen FL, *et al.* (2017) Microbial community structure is affected by cropping sequences and poultry litter under long-term no-tillage. Soil Biol Biochem 114:210–219. doi: 10.1016/j.soilbio.2017.07.019

Babé A, Lavigne T, Séverin JP, *et al.* (2012) Repression of early lateral root initiation events by transient water deficit in barley and maize. Philos Trans R Soc B Biol Sci 367:1534–1541. doi: 10.1098/rstb.2011.0240

Bacq-Labreuil A, Crawford J, Mooney SJ, *et al.* (2018) Effects of cropping systems upon the three-dimensional architecture of soil systems are modulated by texture. Geoderma 332:73–83. doi: 10.1016/J.GEODERMA.2018.07.002

Ball BC (1981) Pore characteristics of soils from two cultivation experiments. J. Soil 32, 483–498.

Bao Y, Aggarwal P, Robbins NE, *et al.* (2014) Plant roots use a patterning mechanism to position lateral root branches toward available water. Proc Natl Acad Sci 111:9319–9324. doi: 10.1073/pnas.1400966111

Bartlett M, Ritz K (2011) The zoological generation of soil structure. In: Ritz K, Young I (eds) The architecture and biology of soils: life in inner space. CABI, Wallingford, Oxfordshire OX10 8DE, UK, pp 71-85

Batey T (2009) Soil compaction and soil management - a review. Soil Use Manag 25:335–345. doi: 10.1111/j.1475-2743.2009.00236.x

Baveye PC, Baveye J, Gowdy J (2016) Soil “ecosystem” services and natural capital: critical appraisal of research on uncertain ground. Front Environ Sci 4:1–49. doi: 10.3389/fenvs.2016.00041

Bellù LG, Liberati P (2006) Inequality analysis: the Gini index. doi: 10.1016/0306-4573(92)90089-I

Bengough AG, Bransby MF, Hans J, *et al.* (2006) Root responses to soil physical conditions; growth dynamics from field to cell. J Exp Bot 57:437–447. doi: 10.1093/jxb/erj003

Blackwell PS, Ringrose-Voase AJ, Jayawardane NS, *et al.* (1990) The use of air-filled porosity and intrinsic permeability to characterise macropore structure and saturated hydraulic conductivity of clay soils. J Soil Sci 41, 215–228.

Blackwell PS, Wells EA (1983) Limiting oxygen flux densities for oat root extension. Plant Soil 73:129–139. doi: 10.1007/BF02197762

Blake JJ, Spink JH, Dyer C (2003) Factors affecting cereal establishment and its prediction. HGCA Res Rev 51:1–53

Blanchart E, Albrecht A, Alegre J, *et al.* (1999) Effects of earthworms on soil structure and physical properties. Lavelle, P, Brussaard, L, Hendrix, P (Eds), Earthworm Manag Trop CAB Int Wallingford, UK 139–162

Bodner G, Himmelbauer M, Loiskandl W, Kaul H-P (2010) Improved evaluation of cover crop species by growth and root factors. Agron Sustain Dev 30:455–464. doi: 10.1051/agro/2009029

Bodner G, Leitner D, Kaul HP (2014) Coarse and fine root plants affect pore size distributions differently. Plant Soil 380:133–151. doi: 10.1007/s11104-014-2079-8

Bossuyt H, Denef K, Six J, *et al.* (2001) Influence of microbial populations and residue quality on aggregate stability. Appl Soil Ecol 16:195–208

Bottinelli N, Henry-des-Tureaux T, Hallaire V, *et al.* (2010) Earthworms accelerate soil porosity turnover under watering conditions. Geoderma 156:43–47. doi: 10.1016/j.geoderma.2010.01.006

Bottinelli N, Jouquet P, Capowiez Y, *et al.* (2015) Why is the influence of soil macrofauna on soil structure only considered by soil ecologists? Soil Tillage Res 146:118–124. doi: 10.1016/j.still.2014.01.007

Bronick CJ, Lal R (2005) Soil structure and management: A review. Geoderma 124:3–22. doi: 10.1016/j.geoderma.2004.03.005

Brown GG, Barois I, Lavelle P (2000) Regulation of soil organic matter dynamics and microbial activity in the drilosphere and the role of interactions with other edaphic functional domains. Eur J Soil Biol 36:177–198. doi: 10.1016/S1164-5563(00)01062-1

Burr-Hersey JE, Mooney SJ, Bengough AG, *et al.* (2017) Developmental morphology of cover crop species exhibit contrasting behaviour to changes in soil bulk density, revealed by X-ray computed tomography. PLoS One 12:1–18. doi: 10.1371/journal.pone.0181872

Buyer JS, Sasser M (2012) High-throughput phospholipid fatty acid analysis of soils. Appl Soil Ecol 61:127–130

Buyer JS, Teasdale JR, Roberts DP, *et al.* (2010) Factors affecting soil microbial community structure in tomato cropping systems. Soil Biol Biochem 42:831–841. doi: 10.1016/j.soilbio.2010.01.020

Caesar-TonThat TC, Cochran VL (2000) Soil aggregate stabilization by a saprophytic lignin-decomposing basidiomycete fungus I. Microbiological aspects. Biol Fertil Soils 32:374–380. doi: 10.1007/s003740000263

Cameron DD (2010) Arbuscular mycorrhizal fungi as (agro)ecosystem engineers. Plant Soil 333:1–5. doi: 10.1007/s11104-010-0361-y

Cammeraat ELH, Risch AC (2008) The impact of ants on mineral soil properties and processes at different spatial scales. J Appl Entomol 132:285–294. doi: 10.1111/j.1439-0418.2008.01281.x

Canti MG (2003). Earthworm activity and archaeological stratigraphy: A review of products and processes. J Archaeol Sci 30, 135–148. https://doi.org/10.1006/jasc.2001.0770

Capowiez Y, Bottinelli N, Sammartino S, *et al.* (2015) Morphological and functional characterisation of the burrow systems of six earthworm species (*Lumbricidae*). Biol Fertil Soils 51:869–877. doi: 10.1007/s00374-015-1036-x

Capowiez Y, Samartino S, Cadoux S, *et al.* (2012) Role of earthworms in regenerating soil structure after compaction in reduced tillage systems. Soil Biol Biochem 55:93–103. doi: 10.1016/j.soilbio.2012.06.013

Carney KM, Hungate BA, Drake BG, Megonigal JP (2007) Altered soil microbial community at elevated CO_2 leads to loss of soil carbon. Proc Natl Acad Sci U S A 104:4990–4995. doi: 10.1073/pnas.0610045104

Carter M (2001) Organic matter and sustainability. In: Rees RM, Ball BC, Campbell CD, Watson CA (eds) Management of Soil Organic Matter. CAB International, pp 9–22

Casanova-Katny MA, Torres-Mellado GA, Palfner G, Cavieres LA (2011) The best for the guest: High Andean nurse cushions of *Azorella madreporica* enhance arbuscular mycorrhizal status in associated plant species. Mycorrhiza 21:613–622. doi: 10.1007/s00572-011-0367-1

Cercioglu M, Anderson SH, Udawatta RP, Haruna SI (2018) Effects of cover crop and biofuel crop management on computed tomography-measured pore parameters. Geoderma 319, 80–88. https://doi.org/10.1016/j.geoderma.2018.01.005

Chan KY, Heenan DP (1999) Microbial-induced soil aggregate stability under different crop rotations. Biol Fertil Soils 30:29–32. doi: 10.1007/s003740050583

Chan KY, Heenan DP (1996) The influence of crop rotation on soil structure and soil physical properties under conventional tillage. Soil Tillage Res 37:113–125. doi: http://dx.doi.org/10.1016/0167-1987(96)01008-2

Chauvel A, Grimaldi M, Barros E, *et al.* (1999) Pasture damage by an Amazonian earthworm. Nature 398:32–33. doi: 10.1038/17946

Chen G, Weil RR (2010) Penetration of cover crop roots through compacted soils. Plant Soil 331:31–43. doi: 10.1007/s11104-009-0223-7

Chenu C (1993) Clay- or sand-polysaccharide associations as models for the interface between micro-organisms and soil: water related properties and microstructure. Geoderma 56, 143–156. https://doi.org/10.1016/0016-7061(93)90106-U

Chenu C, Cosentino D (2011) Microbial regulation of soil structural dynamics. In: Ritz K, Young I (eds) The architecture and biology of soils: life in inner space. CABI, Wallingford, Oxfordshire OX10 8DE, UK, pp 37–70

Chenu C, Le Bissonnais Y, Arrouays D (2000) Organic matter influence on clay wettability and soil aggregate stability. Soil Sci Soc Am J 64:1479. doi: 10.2136/sssaj2000.6441479x

Chenu C, Stotzky G (2002) Interactions between microorganisms and soil particles: an overview. In: Huang PM, Bollag JM, Senesi N (eds) Interactions between soil particles and microorganisms. John Wiley & Sons, Ltd, pp 4–40

Clode PL, Kilburn MR, Jones DL, *et al.* (2009) *In situ* mapping of nutrient uptake in the rhizosphere using nanoscale secondary ion mass spectrometry. Plant Physiol 151:1751–1757. doi: 10.1104/pp.109.141499

Coleman K, Jenkinson DS, Croker GJ, *et al.* (1997) Simulating trends in soil organic carbon in long-term experiments using RothC-26.3. Geoderma 81:29–44

Crawford JW (1994) The relationship between structure and the hydraulic conductivity of soil. Eur. J. Soil Sci. 45, 493–502.

Crawford JW, Deacon L, Grinev D, *et al.* (2012) Microbial diversity affects self-organization of the soil-microbe system with consequences for function. J R Soc Interface 9:1302–1310. doi: 10.1098/rsif.2011.0679

Cripps CL, Eddington LH (2005) Distribution of mycorrhizal types among alpine vascular plant families on the Beartooth plateau, rocky mountains, U.S.A., in reference to large-scale patterns in arctic–alpine habitats. Arctic, Antarct Alp Res 37:177–188. doi: 10.1657/1523-0430(2005)037[0177:DOMTAA]2.0.CO;2

Czarnes S, Hallett PD, Bengough a. G, Young IM (2000) Root- and microbial-derived mucilages affect soil structure and water transport. Eur J Soil Sci 51:435–443. doi: 10.1046/j.1365-2389.2000.00327.x

d'Humieres D, Ginzburg I, Krafczyk M, *et al.* (2002) Multiple-relaxation-time lattice Boltzmann models in three dimensions. Philos Trans R Soc A Math Phys Eng Sci 360:437–451. doi: 10.1098/rsta.2001.0955

Dawkins R (1982) The Extended Phenotype. Oxford University Press

Daynes CN, Field DJ, Saleeba JA, *et al.* (2013) Development and stabilisation of soil structure via interactions between organic matter, arbuscular mycorrhizal fungi and plant roots. Soil Biol Biochem 57:683–694. doi: 10.1016/j.soilbio.2012.09.020

Decaëns T (2000) Degradation dynamics of surface earthworm casts in grasslands of the eastern plains, of Colombia. Biol Fertil Soils 32:149–156. doi: 10.1007/s003740000229

Degens BP, Sparling GP, Abbott, K. L (1994) The contribution from hyphae, roots and organic carbon constituents to the aggregation of a sandy loam under long-term clover-based and grass pastures. Eur J Soil Sci 45:459–468. doi: 10.1111/j.1365-2389.1994.tb00531.x

Denef K, Roobroeck D, Manimel Wadu MCW, *et al.* (2009) Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biol Biochem 41:144–153. doi: 10.1016/j.soilbio.2008.10.008

Denef K, Six J, Bossuyt H, *et al.* (2001) Influence of dry-wet cycles on the interrelationship between aggregate, particulate organic matter, and microbial community dynamics. Soil Biol Biochem 33:1599–1611. doi: 10.1016/S0038-0717(01)00076-1

Dexter AR (2004) Soil physical quality Part I. Theory, effects of soil texture, density, and organic mailer, and effects on root growth. Geoderma 120:201–214. doi: 10.1016/j.geodermaa.2003.09.005

Dexter AR (1988) Advances in characterization of soil structure. Soil tillage Res 11:199–238

Dexter AR, Richard G (2009) The saturated hydraulic conductivity of soils with n-modal pore size distributions. Geoderma 154:76–85. doi: 10.1016/j.geoderma.2009.09.015

Dorioz JM, Robert M, Chenu C (1993) The role of roots, fungi and bacteria on clay particle organization. An experimental approach. Geoderma 56:179–194. doi: 10.1016/0016-7061(93)90109-X

Duchene O, Vian JF, Celette F (2017) Intercropping with legume for agroecological cropping systems: Complementarity and facilitation processes and the importance of soil microorganisms. A review. Agric Ecosyst Environ 240:148–161. doi: 10.1016/j.agee.2017.02.019

Edwards CA, Lofty JR (1977) Biology of Earthworms. 2nd edn Richard Clay Group, UK

Ehlers W (1975) Observations on earthworm channels and infiltration on tilled and untilled loess soil. Soil Sci 119:242–249

Ettema CH, Wardle DA (2002) Spatial soil ecology. Trends Ecol Evol 17:177–183

Feeney DS, Crawford JW, Daniell T, *et al.* (2006) Three-dimensional microorganization of the soil-root-microbe system. Microb Ecol 52:151–158. doi: 10.1007/s00248-006-9062-8

Felten D, Emmerling C (2009) Earthworm burrowing behaviour in 2D terraria with single- and multi-species assemblages. Biol Fertil Soils 45:789–797. doi: 10.1007/s00374-009-0393-8

Fernandez AL, Sheaffer CC, Wyse DL, *et al.* (2016) Structure of bacterial communities in soil following cover crop and organic fertilizer incorporation. Appl Microbiol Biotechnol 100:9331–9341. doi: 10.1007/s00253-016-7736-9

Finney DM, Buyer JS, Kaye JP (2017) Living cover crops have immediate impacts on soil microbial community structure and function. J Soil Water Conserv 72:361–373. doi: 10.2489/jswc.72.4.361

Fischer C, Roscher C, Jensen B, *et al.* (2014) How do earthworms, soil texture and plant composition affect infiltration along an experimental plant diversity gradient in grassland? PLoS One 9:e98987. doi: 10.1371/journal.pone.0098987

Fitter AH, Heinemeyer a, Staddon PL (2000) The impact of elevated CO2 and global climate change on arbuscular mycorrhizas: a mycocentric approach. New Phytol 147:179–187. doi: 10.1046/j.1469-8137.2000.00680.x

Frostegård A, Bååth E (1996) The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol Fertil Soils 22:59–65. doi: 10.1007/s003740050076

Gaunt J, Sohi S, Yang H, *et al.* (2001) A procedure for isolating sustainable, soil organic matter fractions suitable for modelling. In: Rees RM, Ball BC, Campbell CD, Watson CA (eds) Management of Soil Organic Matter. CAB International, pp 90–95

George SJ, Harper RJ, Hobbs RJ, Tibbett M (2012) A sustainable agricultural landscape for Australia: A review of interlacing carbon sequestration, biodiversity and salinity management in agroforestry systems. Agric Ecosyst Environ 163:28–36. doi: 10.1016/j.agee.2012.06.022

Ghestem M, Sidle RC, Stokes A (2011) The influence of plant root systems on subsurface flow: implications for slope stability. Bioscience 61:869–879. doi: 10.1525/bio.2011.61.11.6

Gish T, Jury W (1983) Effect of plant roots and root channels on solute transport. Trans ASAE 440–444

Grant CD, Dexter AR (1990) Air entrapment and differential swelling as factors in the mellowing of moulded soil during rapid wetting. Aust J Soil Res 28:361–369. doi: 10.1071/SR9900361

Gregory AS, Dungait JAJ, Watts CW, *et al.* (2016) Long-term management changes topsoil and subsoil organic carbon and nitrogen dynamics in a temperate agricultural system. Eur J Soil Sci 67, 421–430. https://doi.org/10.1111/ejss.12359

Gregory AS, Watts CW, Griffiths BS, *et al.* (2009) The effect of long-term soil management on the physical and biological resilience of a range of arable and grassland soils in England. Geoderma 153:172–185. doi: 10.1016/j.geoderma.2009.08.002

Gregory PJ (2006) Roots, rhizosphere and soil: The route to a better understanding of soil science? Eur J Soil Sci 57:2–12. doi: 10.1111/j.1365-2389.2005.00778.x

Griffiths BS, Ritz K, Bardgett RD, *et al.* (2000) Ecosystem response of pasture soil communities to fumigation-induced microbial diversity reductions:

an examination of the biodiversity - ecosystem function relationship. Oikos 90:279–294. doi: 10.2307/3547138

Hacke UG, Sperry JS, Ewers BE, *et al.* (2000) Influence of soil porosity on water use in *Pinus taeda*. Oecologia 124:495–505

Hallett PD, Gordon DC, Bengough AG (2003) Plant influence on rhizosphere hydraulic properties: Direct measurements using a miniaturized infiltrometer. New Phytol 157:597–603. doi: 10.1046/j.1469-8137.2003.00690.x

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4:

Hanson I, Djohari J, Orr J, *et al.* (2009) New observations on the interactions between evidence and the upper horizons of the soil, in: Ritz, K., Dawson, L., Miller, D. (Eds.), Criminal and Environmental Soil Forensic. pp. 239–251.

Harris K, Young IM, Gilligan CA, *et al.* (2003) Effect of bulk density on the spatial organisation of the fungus *Rhizoctonia solani* in soil. FEMS Microbiol Ecol 44:45–56

Haygarth PM, Ritz K (2009) The future of soils and land use in the UK: Soil systems for the provision of land-based ecosystem services. Land use policy 26:S187–S197. doi: 10.1016/j.landusepol.2009.09.016

Haynes RJ, Beare MH (1997) Influence of six crop species on aggregate stability and some labile organic matter fractions. Soil Biol Biochem 29:1647–1653. doi: 10.1016/S0038-0717(97)00078-3

Haynes RJ, Swift RS, Stephen RC (1991) Influence of mixed cropping rotations (pasture-arable) on organic matter content, water stable aggregation and clod porosity in a group of soils. Soil Tillage Res 19:77–87. doi: 10.1016/0167-1987(91)90111-A

Helfrich M, Ludwig B, Potthoff M, Flessa H (2008) Effect of litter quality and soil fungi on macroaggregate dynamics and associated partitioning of litter carbon and nitrogen. Soil Biol Biochem 40:1823–1835. doi: 10.1016/j.soilbio.2008.03.006

Helliwell JR, Miller AJ, Whalley WR, *et al.* (2014) Quantifying the impact of microbes on soil structural development and behaviour in wet soils. Soil Biol Biochem 74, 138–147. https://doi.org/10.1016/j.soilbio.2014.03.009

Helliwell JR, Sturrock CJ, Mairhofer S, *et al.* (2017) The emergent rhizosphere: imaging the development of the porous architecture at the root-soil interface. Sci Rep 7:14875. doi: 10.1038/s41598-017-14904-w

Hendrickx JMH, Flury M (2001) Uniform and preferential flow mechanisms in the vadose zone. In: C.Hendrickx D, H. JM, Fl M, C. D (eds) Conceptual models of flow and transport in the fractured Vadose zone. National Academies Press, Washington, D.C., pp 149–187

Hirsch PR, Gilliam LM, Sohi SP, *et al.* (2009) Starving the soil of plant inputs for 50 years reduces abundance but not diversity of soil bacterial communities. Soil Biol Biochem 41:2021–2024. doi: 10.1016/j.soilbio.20

Hirsch PR, Jhurreea D, Williams JK, *et al.* (2017) Soil resilience and recovery: rapid community responses to management changes. Plant Soil 412:283–297. doi: 10.1007/s11104-016-3068-x

Hodge CAH, Burton RGO, Corbett WM, *et al.* (1984) Soils and their use in eastern England. Bull. 13. Soil Survey of England and Wales, Harpenden, United Kingdom.

Holden PA (2011) How do the microhabitats framed by soil structure impact soil bacteria and the processes that they regulate? In: Ritz K, Young I (eds) The architecture and biology of soils: life in inner space. CABI, Wallingford, Oxfordshire OX10 8DE, UK, pp 118–148

Hole FD (1981) Effects of animals on soil. Geoderma 25:75–112. doi: 10.1016/0016-7061(81)90008-2

Horn R, Taubner H, Wuttke M, Baumgartl T (1994) Soil physical properties related to soil structure. Soil Tillage Res 30:187–216. doi: 10.1016/0167-1987(94)90005-1

Hu S, Chapin FS, Firestone MK, *et al.* (2001) Nitrogen limitation of microbial decomposition in a grassland under elevated CO2. Nature 409:188–191. doi: 10.1038/35051576

Hütsch BW, Augustin J, Merbach W (2002) Plant rhizodeposition - An important source for carbon turnover in soils. J Plant Nutr Soil Sci 165:397–407. doi: 10.1002/1522-2624(200208)165:4<397::AID-JPLN397>3.0.CO;2-C

Iwata S, Miyazawa Y, Fujii N, Takahashi H (2013) MIZ1-regulated hydrotropism functions in the growth and survival of Arabidopsis

thaliana under natural conditions. Ann Bot 112:103–114. doi: 10.1093/aob/mct098

Jackson RB, Sperry JS, Dawson TE (2000) Root water uptake and transport : using physiological predictions. Trends Plant Sci 5:

Jin K, Shen J, Ashton RW, *et al.* (2013) How do roots elongate in a structured soil? J Exp Bot 64:4761–4777. doi: 10.1093/jxb/ert286

Jones CG, Lawton JH, Shachack M (1994) Organisms as ecosystem engineers. Oikos 69:373–386

Jones CG, Lawton JH, Shachak M (1997) Positive and negative effects of organisms as physial ecosystem engineers. Ecology 78:1946–1957

Johnston AE, Poulton PR, Coleman K, *et al.* (2017) Changes in soil organic matter over 70 years in continuous arable and ley-arable rotations on a sandy loam soil in England. Eur J Soil Sci 68, 305–316. https://doi.org/10.1111/ejss.12415

Jouquet P, Dauber J, Lagerlöf J, *et al.* (2006) Soil invertebrates as ecosystem engineers: Intended and accidental effects on soil and feedback loops. Appl Soil Ecol 32:153–164. doi: 10.1016/j.apsoil.2005.07.004

Jouquet P, Huchet G, Bottinelli N, *et al.* (2012) Does the influence of earthworms on water infiltration, nitrogen leaching and soil respiration depend on the initial soil bulk density? A mesocosm experiment with the endogeic species Metaphire posthuma. Biol Fertil Soils 48:561–567. doi: 10.1007/s00374-011-0652-3

Jouquet P, Traoré S, Choosai C, *et al.* (2011) Influence of termites on ecosystem functioning. Ecosystem services provided by termites. Eur J Soil Biol 47:215–222. doi: 10.1016/j.ejsobi.2011.05.005

Karlen DL, Mausbach MJ, Doran JW, *et al.* (1997) Soil Quality: A Concept, Definition, and Framework for Evaluation. Soil Sci Soc Am J 61:4. doi: 10.2136/sssaj1997.03615995006100010001x

Kézdi A (1974) Handbook of soil mechanics. Soil Physics, vol 1, Elsevier S

Kravchenko AN, Negassa WC, Guber AK, *et al.* (2014) Intra-aggregate pore structure influences phylogenetic composition of bacterial community in macroaggregates. Soil Sci Soc Am J 78, 1924. https://doi.org/10.2136/sssaj2014.07.0308

Kravchenko AN, Negassa WC, Guber AK, Rivers ML (2015) Protection of soil carbon within macro-aggregates depends on intra-aggregate pore characteristics. Sci Rep 5:1–10. doi: 10.1038/srep16261

Kristiansen SM, Schjønning P, Thomsen IK, *et al.* (2006) Similarity of differently sized macro-aggregates in arable soils of different texture. Geoderma 137:147–154. doi: 10.1016/j.geoderma.2006.08.005

Lavelle P (2001) Soil Ecology. Kluwer Acad Publ Dordrecht, Netherlands

Lavelle P (1997) Faunal activities and soil processes: adaptive strategies that determine ecosystem function. Adv Ecol Res 27:93–132

Lavelle P, Barois I, Martin A, *et al.* (1987) Management of earthworm populations in agro-ecosystems: A possible way to maintain soil quality? In: Clarholm M, and Bergstom L (eds) Ecology of Arable Land: Perspectives and Challenges. Kluwer Academic, Boston, Massachusetts

Lavelle P, Blanchart E, Martin A, *et al.* (1992) Impact of soil fauna on the properties of soils in the humid tropics. Myths and Science of Soils of

the tropics. Soil Sci Soc Am Am Soc Agron Special publication:157–185

Lavelle P, Spain A, Blouin M, *et al.* (2016) Ecosystem engineers in a self-organized soil: a review of concepts and future research questions. Soil Sci 181:

Le Bissonnais Y (1996) Aggregate stability and assessment of soil crustability and erodibility : I . Theory and methodology. Eur J Soil Sci 47:425–437. doi: 10.1111/j.1365-2389.1996.tb01843.x

Lee K, Foster R (1991) Soil fauna and soil structure. Aust J Soil Res 29:745. doi: 10.1071/SR9910745

Lee KE (1985) Earthworms: Their Ecology and Relationships with Roils and Land Use. Academic Press Sydney 411 pp

Lehman RM, Acosta-Martinez V, Buyer JS, *et al.* (2015) Soil biology for resilient, healthy soil. J Soil Water Conserv 70:12A–18A. doi: 10.2489/jswc.70.1.12A

Li Y, Xu H, Cohen S (2005) Long-term hydraulic acclimation to soil texture and radiation load in cotton. Plant, Cell Environ 28:492–499. doi: 10.1111/j.1365-3040.2005.01291.x

Lipiec J, Medvedev V V, Birkas M, *et al.* (2003) Effect of soil compaction on root growth and crop yield in Central and Eastern Europe. Int Agrophysics 17:61–69

Lobry de Bruyn LA (1999) Ants as bioindicators of soil functions in rural environments. Agric Ecosyst Environ 74:425–441

Luijendijk E, Gleeson T, (2015) How well can we predict permeability in sedimentary basins? Deriving and evaluating porosity-permeability

equations for non-cemented sand and clay mixtures. Geofluids 15, 67–83. https://doi.org/10.1111/gfl.12115

Luo L, Lin H, Schmidt J (2010) Quantitative Relationships between Soil Macropore Characteristics and Preferential Flow and Transport. Soil Sci Soc Am J 74:1929. doi: 10.2136/sssaj2010.0062

Mangalassery S, Sjögersten S, Sparkes DL, *et al.* (2013) The effect of soil aggregate size on pore structure and its consequence on emission of greenhouse gases. Soil Tillage Res 132:39–46. doi: 10.1016/j.still.2013.05.003

Martin SL, Mooney SJ, Dickinson MJ, West HM (2012) Soil structural responses to alterations in soil microbiota induced by the dilution method and mycorrhizal fungal inoculation. Pedobiologia (Jena) 55:271–281. doi: 10.1016/j.pedobi.2012.06.001

Masle J, Passioura J (1987) The effect of soil strength on the growth of young wheat plants. Aust J Plant Physiol 643–656. doi: 10.1071/PP9870643

Materechera SA, Alston AM, Kirby JM, Dexter AR (1992) Influence of root diameter on the penetration of seminal roots into a compacted subsoil. Plant Soil 144:297–303. doi: 10.1007/BF00012888

Materechera SA, Kirby JM, Alston AM, Dexter AR (1994) Modification of soil aggregation by watering regime and roots growing through beds of large aggregates. Plant Soil 160:57–66

Mellado-Vázquez PG, Lange M, Bachmann D, *et al.* (2016) Plant diversity generates enhanced soil microbial access to recently photosynthesized carbon in the rhizosphere. Soil Biol Biochem 94:122–132. doi: 10.1016/j.soilbio.2015.11.012

Metzger L, Yaron B (1987) Influence of sludge organic matter on soil physical properties. In: Stewart BA (ed) Advances in Soil Science Vol 7. Springer-Verlag, New York Inc., pp 141–163

Millard P, Singh BK (2010) Does grassland vegetation drive soil microbial diversity? Nutr Cycl Agroecosystems 88:147–158. doi: 10.1007/s10705-009-9314-3

Miller RM, Fitzsimons MS (2011) Fungal growth in soils. Eds K Ritz I Young Archit Biol Soils Life Inn Sp Chapter 8:149–163

Miller RM, Jastrow JD (1990) Hierarchy of root and mycorrhizal fungal interactions with soil aggregation. Soil Biol Biochem 22:579–584. doi: http://dx.doi.org/10.1016/0038-0717(90)90001-G

Morel JL, Habib L, Plantureux S, Guckert A (1991) Influence of maize root mucilage on soil aggregate stability. Plant Soil 136:111–119

Morris EC, Griffiths M, Golebiowska A, *et al.* (2017) Shaping 3D Root System Architecture. Curr Biol 27:R919–R930. doi: 10.1016/j.cub.2017.06.043

Müller-Inkmann M, Fründ HC, Hemker O (2013) An experimental setup to assess earthworm behaviour in compacted soil. Biol Fertil Soils 49:363–366. doi: 10.1007/s00374-012-0730-1

Naveed M, Moldrup P, Schaap MG, *et al.* (2016) Prediction of biopore- and matrix-dominated flow from X-ray CT-derived macropore network characteristics. Hydrol. Earth Syst. Sci. 20, 4017–4030. https://doi.org/10.5194/hess-20-4017-2016

Neagoe A, Iordache V, Bergmann H, Kothe E (2013) Patterns of effects of arbuscular mycorrhizal fungi on plants grown in contaminated soil. J Plant Nutr Soil Sci 176:273–286. doi: 10.1002/jpln.201200079

Neal AL, Rossmann M, Brearley C, *et al.* (2017) Land-use influences phosphatase gene microdiversity in soils. Environ Microbiol 19:2740–2753. doi: 10.1111/1462-2920.13778

Nye RH, Tinker PB (1977) Solute Movement in the Soil Root System. Blackwell Science Ltd

Oades JM (1993) The role of biology in the formation, stabilization and degradation of soil structure. Geoderma 56, 377–400. https://doi.org/10.1016/0016-7061(93)90123-3

Oades JM (1984) Soil organic matter and structural stability: mechanisms and implications for management. Plant Soil 76:319–337. doi: 10.1007/BF02205590

Or D, Phutane S, Dechesne A (2007) Extracellular polymeric substances affecting pore-scale hydrologic conditions for bacterial activity in unsaturated soils. Vadose Zo J 6:298. doi: 10.2136/vzj2006.0080

Pagliaia M, Marsilib A, Servadiob P, *et al.* (2003) Changes of some physical properties of a clay soil following the passage of rubber tracked and wheeled tractors of medium power. Soil Tillage Res. 73, 119–129.

Pagliai M, Vignozzi N (2002) The soil pore system as an indicator of soil quality. Adv GeoEcology 35:69–80

Pagliai M, Vignozzi N, Pellegrini S (2004) Soil structure and the effect of management practices. Soil Tillage Res 79:131–143. doi: 10.1016/j.still.2004.07.002

Paradelo M, Katuwal S, Moldrup P, *et al.* (2016) X-ray CT-Derived Soil Characteristics Explain Varying Air, Water, and Solute Transport

Properties across a Loamy Field. Vadose Zone J 15:0. doi: 10.2136/vzj2015.07.0104

Patkowska E, Konopiński M (2013) The role of oat, common vetch and tansy phacelia as cover plants in the formation of microorganisms communities in the soil under the cultivation of root chicory (*Cichorium intybus* var. *sativum* Bisch.) and salsify (*Tragopogon porrifolius* var. *sativus* (Gaterau) Br.). Acta Sci Pol, Hortorum cultus 12:179–191

Paton TR, Humphreys GS, Mitchell PB (1995) Soils: a New Global View. ULC Press London

Pelosi C, Grandeau G, Capowiez Y (2017) Temporal dynamics of earthworm-related macroporosity in tilled and non-tilled cropping systems. Geoderma 289:169–177. doi: 10.1016/j.geoderma.2016.12.005

Peth S, Horn R, Beckmann F, *et al.* (2008) Three-dimensional quantification of intra-aggregate pore-space features using synchrotron-radiation-based microtomography. Soil Sci. Soc. Am. J. 72, 897. https://doi.org/10.2136/sssaj2007.0130

Phillips JD (2009) Soils as extended composite phenotypes. Geoderma 149:9

Phillips JD (2016) Landforms as extended composite phenotypes. Earth Surf Process Landforms 41:16–26. doi: 10.1002/esp.3764

Pierret A, Hartmann C, Maeght J-L, Pages L (2011) Biotic Regulation: Plants. In: Ritz K, Young I (eds) The architecture and biology of soils: life in inner space. CABI, Wallingford, Oxfordshire OX10 8DE, UK, pp 86–103

Pires LF, Borges JAR, Rosa JA, *et al.* (2017) Soil structure changes induced by tillage systems. Soil Tillage Res 165:66–79. doi: 10.1016/j.still.2016.07.010

Preston S, Wirth S, Ritz K, *et al.* (2001) The role played by microorganisms in the biogenesis of soil cracks: Importance of substrate quantity and quality. Soil Biol Biochem 33:1851–1858. doi: 10.1016/S0038-0717(01)00113-4

Qian YH, Dhumieres D, Lallemand P (1992) Lattice BGK models for Navier-Stokes equation. Europhys Lett 17:479–484

Rabot E, Wiesmeier M, Schlüter S, Vogel HJ (2018) Soil structure as an indicator of soil functions: A review. Geoderma 314:122–137. doi: 10.1016/j.geoderma.2017.11.009

Read DB, Bengough AG, Gregory PJ, *et al.* (2003) Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. New Phytol 157:315–326

Reicosky DC, Forcella F (1998) Cover crop and soil quality interactions in agroecosystems. J Soil Water Conserv 53:224–229

Reid JB, Goss MJ (1982) Interactions between soil drying due to plant water-use and decreases in aggregate stability caused by maize roots. J Soil Sci 33:47–53. doi: 10.1111/j.1365-2389.1982.tb01746.x

Richards PJ (2009) *Aphaenogaster* ants as bioturbators: Impacts on soil and slope processes. Earth-Science Rev 96:92–106. doi: 10.1016/j.earscirev.2009.06.004

Rillig MC, Wright SF, Eviner VT (2002) The role of arbuscular mycorrhizal fungi and glomalin on soil aggregation comparing the effects of five plant species. Plant Soil 238:325–333

Ritz K, Young IM (2004) Interactions between soil structure and fungi. Mycologist 18:52–59. doi: 10.1017/S0269915XO4002010

Ritz K, Young IM (2011) Architecture and Biology of Soils. CABI, Wallingford

Roberson EB, Firestone MK (1992) Relationship between desiccation and exopolysaccharide production in soil *Pseudomonas* sp. Appl Environ Microbiol 58:1284–1291

Rossi R, Amato M, Pollice A, *et al.* (2013) Electrical resistivity tomography to detect the effects of tillage in a soil with a variable rock fragment content. Eur. J. Soil Sci. 64, 239–248. https://doi.org/10.1111/ejss.12024

Sapkota TB, Mazzoncini M, Barberi P, *et al.* (2012) Fifteen years of no till increase soil organic matter, microbial biomass and arthropod diversity in cover crop-based arable cropping systems. Agron Sustain Dev 32:853–863. doi: 10.1007/s13593-011-0079-0

Saxton KE, Rawls WJ, Romberger JS, Papendick RI (1986) Estimating generalized soil-water characteristics from texture. Soil Sci Soc Am J 50:1031–1036

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675

Scott DA, Baer SG, Blair JM (2017) Recovery and relative influence of root, microbial, and structural properties of soil on physically sequestered

carbon stocks in restored grassland. Soil Sci Soc Am J 81:50. doi: 10.2136/sssaj2016.05.0158

Shepherd KD, Walsh MG (2002) Development of reflectance spectral libraries for characterization of soil properties. Soil Sci Soc Am J 66:988. doi: 10.2136/sssaj2002.9880

Shepherd MA, Harrison R, Webb J (2002) Managing soil organic matter – implications for soil structure on organic farms. Soil Use Manag 18:284–292. doi: 10.1079/SUM2002134

Shipitalo MJ, Bayon Le R (2004) Quantifying the effect of Earthworms on Soil Aggregation and Porosity. In: Edwards CA (ed) Earthworm Ecology. CRC Press, Boca Raton, pp 183–200

Singer MJ, Southard RJ, Warrington DN, Janitzky P (1992) Stability of synthetic sand-clay aggregates after wetting and drying cycles. Soil Sci Soc Am J 56:1843. doi: 10.2136/sssaj1992.03615995005600060032x

Six J, Bossuyt H, Degryze S, Denef K (2004) A history of research on the link between (micro) aggregates, soil biota, and soil organic matter dynamics. Soil Tillage Res 79:7–31. doi: 10.1016/j.still.2004.03.008

Six J, Frey SD, Thiet RK, Batten KM (2006) Bacterial and fungal contributions to carbon sequestration in agroecosystems. Soil Sci Soc Am J 70:555. doi: 10.2136/sssaj2004.0347

Stahl PD, Klug MJ (1996) Characterization and differentiation of filamentous fungi based on fatty acid these include : characterization and differentiation of filamentous fungi based on fatty acid composition. Appl Environ Microbiol 62:4136–4146

Strickland MS, Rousk J (2010) Considering fungal:bacterial dominance in soils – Methods, controls, and ecosystem implications. Soil Biol Biochem 42:1385–1395. doi: 10.1016/j.soilbio.2010.05.007

Su YZ, Yang R, Liu WJ, Wang XF (2010) Evolution of soil structure and fertility after conversion of native sandy desert soil to irrigated cropland in arid region, China. Soil Sci 175:246–254. doi: 10.1097/SS.0b013e3181e04a2d

Sudd JH, Franks NR (1987) The behavioural ecology of ants, Blackie. Chapman & Hall, New York, USA

Tisdall JM, Oades JM (1982) Organic matter and water-stable aggregates in soils. J Soil Sci 33:141–165

Tracy SR, Black CR, Roberts JA, *et al.* (2012) Quantifying the effect of soil compaction on three varieties of wheat (*Triticum aestivum* L.) using X-ray Micro Computed Tomography (CT). Plant Soil 353:195–208. doi: 10.1007/s11104-011-1022-5

Treonis AM, Ostle NJ, Stott AW, *et al.* (2004) Identification of groups of metabolically-active rhizosphere microorganisms by stable isotope probing of PLFAs. Soil Biol Biochem 36:533–537. doi: 10.1016/j.soilbio.2003.10.015

Valentine TA, Hallett PD, Binnie K, *et al.* (2012) Soil strength and macropore volume limit root elongation rates in many UK agricultural soils. Ann Bot 110:259–270. doi: 10.1093/aob/mcs118

van Breemen N (1993) Soils as biotic constructs favoring net primary productivity. Geoderma 57:183–211. doi: 10.1016/0016-7061(93)90002-3

van Klink R, Schrama M, Nolte S, *et al.* (2015) Defoliation and soil compaction jointly drive large-herbivore grazing effects on plants and soil arthropods on clay soil. Ecosystems 18:671–685. doi: 10.1007/s10021-015-9855-z

Vergani C, Graf F (2015) Soil permeability, aggregate stability and root growth: a pot experiment from a soil bioengineering perspective. Ecohydrology 9:830–842. doi: 10.1002/eco.1686

Vergani J, Ritz K (2013) Plant: Soil interactions in temperate multi-cropping production systems. Plant Soil 376:01-29. doi: 10.1007/s11104-013-1921-8

Vogel HJ, Kretzschmar A (1996). Topological characterization of pore space in soil - Sample preparation and digital image-processing. Geoderma 73, 23–38. https://doi.org/10.1016/0016-7061(96)00043-2

Vogel HJ, Weller U, Schlüter S (2010) Quantification of soil structure based on Minkowski functions. Comput Geosci 36:1236–1245. doi: 10.1016/j.cageo.2010.03.007

Wang Y, Zhang B, Lin L, Zepp H (2011) Agroforestry system reduces subsurface lateral flow and nitrate loss in Jiangxi Province, China. Agric Ecosyst Environ 140:441–453. doi: 10.1016/j.agee.2011.01.007

Watts CW, Dexter AR (1997) The influence of organic matter in reducing the destabilization of soil by simulated tillage. Soil Tillage Res 42:253–275. doi: 10.1016/S0167-1987(97)00009-3

Watts CW, Whalley WR, Longstaff D, *et al.* (2001) Aggregation of a soil with different cropping histories following the addition of organic materials. Soil Use Manag 17:263–268. doi: Doi 10.1079/Sum200189

Whalley WR, Dumitru E, Dexter AR (1995) Biological effects of soil compaction. Soil Tillage Res 35:53–68

Whalley WR, Riseley B, Leeds-Harrison PB, *et al.* (2005) Structural differences between bulk and rhizosphere soil. Eur J Soil Sci 56:353–360. doi: 10.1111/j.1365-2389.2004.00670.x

Whitmore AP, Schröder JJ (2007) Intercropping reduces nitrate leaching from under field crops without loss of yield: A modelling study. Eur J Agron 27:81–88. doi: 10.1016/j.eja.2007.02.004

Wright SF, Upadhyaya A (1998) A survey of soils for aggregate stability and glomalin, a glycoprotein produced by hyphae of arbuscular mycorrhizal fungi. Plant Soil 198:97–107. doi: 10.1023/A:1004347701584

Wuest SB (2001) Soil biopore estimation: Effects of tillage, nitrogen, and photographic resolution. Soil Tillage Res 62:111–116. doi: 10.1016/S0167-1987(01)00218-5

Young IM, Crawford JW (2004) Interactions and self-organisation in soil-microbe complex. Science (80-) 304:1634–1637

Zhang X, Crawford JW, Deeks LK, *et al.* (2005) A mass balance based numerical method for the fractional advection-dispersion equation: Theory and application. Water Resour Res 41, 1-10. https://doi.org/10.1029/2004wr003818

Zhang X, Crawford JW, Flavel RJ, Young IM (2016) A multi-scale Lattice Boltzmann model for simulating solute transport in 3D X-ray micro-tomography images of aggregated porous materials. J Hydrol 541, 1020–1029. https://doi.org/10.1016/j.jhydrol.2016.08.013

Zhang X, Lv M (2007) Persistence of anomalous dispersion in uniform porous media demonstrated by pore-scale simulations. Water Resour Res 43, 1–11. https://doi.org/10.1029/2006WR005557

Zelles L (1997) Phospholipid fatty acid profiles in selected members of soil microbial communities. Chemosphere 35:275–294

Zelles L (1999) Identification of single cultured micro-organisms based on their whole-community fatty acid profiles, using an extended extraction procedure. Chemosphere 39:665–682