Effect of Biochar on Strawberry Growth, Soil Properties and Ecosystem Gas Exchange

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Abstract

Biochar creates a resistant soil carbon pool that is carbon-negative, provides long-lasting improvements in soil fertility and serves as a net withdrawal of atmospheric carbon dioxide stored in highly stable soil carbon stocks. The enhanced nutrient retention, improved soil fertility and water holding capacity of biochar-amended soil not only reduces the total fertilizer requirements, but also the climate and environmental impact of croplands with generally increased production. I hypothesized that biochar increases plant growth by ameliorating negative soil physicochemical, and enhancing microbial, properties with special relation to nutrient availability and contributes actively to modify ecosystem gas exchange. Moreover, I hypothesized that the rate of biochar application influences the rate of biochar surface oxidation, nature and mineralization of functional groups, when it was added to soil for a long period of time in a controlled environment. The present study focused on determining the potential of a wood-based, high temperature (1100°C) biochar, to increase strawberry plant growth and ecosystem gas exchange in topsoil and its influence on soil quality. The results discussed in this thesis were obtained from a long-term investigation conducted under controlled conditions and is novel because of its duration (18 months), and because of the use of biochar derived from demolition wood. There is currently much interest in utilising biochar as a soil amendment for increased soil health and for carbon sequestration and European and International voluntary standards for biochar safety are under review in the UK post-Brexit. All work on biochar to date, has utilised biochar from virgin wood or agricultural residues. To the best of my knowledge, this is the first study to quantify effects of biochar derived from demolition wood on soil health. The importance of

this is twofold; firstly, the stock of virgin wood for biochar production is limited, therefore it is important to be aware of any dangers of 'diluting' virgin wood with unapproved feedstock during production, and secondly, it is possible that biochar from such feedstocks might be acceptable for restoration programmes of already contaminated land. Biochar (0, 2.5, 5, 10 and 15% w/w) was mixed with topsoil, added to 14 L pots and maintained in a growth room at 20/16°C (16 hours day/night) and 50 % relative humidity for 18 months. Pots were either planted or left bare and soils in planted and unplanted pots were regularly sampled for microbiological and soil chemical determinations and plant growth measured. Biochar addition did not affect strawberry shoot growth or carbon or nitrogen content, but the 2.5% addition of biochar slightly increased root biomass, whilst the highest concentration (15%), reduced biomass relative to the 2.5% amendment, but not to the control. In the strawberry shoot, K, P, Zn, Cu, and As concentrations increased with biochar addition, while Pb content decreased with increasing rate of biochar compared to the control. Other than these, none of the shoot or root elements analysed exhibited clear biochar-driven trends. Neither leaf conductance nor leaf temperature were affected by biochar amendment. However, biochar amendment generally reduced ecosystem respiration (Re), net ecosystem exchange (NEE), gross ecosystem exchange (GEE) and soil enzyme activities.

Biochar had no effect on microbial biomass nitrogen and carbon. CO_2 and CH_4 fluxes in soil were generally reduced by biochar amendment, but presence or absence of strawberry plants had no effect. However, soil water content, pH and Olsen P concentrations all increased with biochar amendment, as did soil nitrate concentrations in unplanted soils (but not as markedly in the presence of plants).

Bulk density of the soil deceased in line with increasing biochar addition. Results from FTIR analysis showed that when this high temperature wood biochar was applied to soil, due to microbial and plant mediated transformation, it becomes more aromatic because of the loss of aliphatic and labile compounds and broadening of aromatic bands. The maximum number of functional groups (aliphatic, aromatic and carbohydrates) was recorded in the control soil (0 % biochar) both with and without plants. Aromatics (C-C and CH) were more prevalent than oxygen containing compounds (carboxyl and carbonyl), or aliphatic compounds and there were very few hydrocarbons. Shifts in the spectra for all wave numbers were observed in planted biochar-amended soils compared to control (0 % biochar). After 12 months, a marked decrease in spectral bands between 500 and 4000 cm^{-1} was noted in treatments with 2.5 %, 10 % and 15 % biochar.

Overall, the use of biochar made from demolition wood ought to be avoided in agricultural settings. However, in contaminated areas, concentrations up to the lowest used in this study may be beneficial if pH changes or improvements in bulk density are desired.

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Sumyya Razzaq Khan

Dedication

I dedicate this effort in loving memory of my parents,

DR. ABDUL RAZZAQ KHAN

MRS. NUSRAT RAZZAQ

Who are the real symbols of trust, respect and infinite love,

My Loving Husband,

MUHAMMAD IRFAN

And Blessings of Allah, my kidoos,

SARAAN* and *RAYAAN

Abbreviations

FTIR	Fourier transform infrared spectroscopy
BC	Biochar
IR	Infrared
CEC	Cation exchange capacity
EC	Electrical conductivity
AES	Agricultural and environmental sciences
ICPMS	Inductively coupled plasma mass spectrometry
LOI	Loss on ignition
AMR	Acid molybdate reagent
WFPS	Water filled pore space
MQ	MilliQ
TCD	Thermal conductivity detector
FID	Flame ionization detector
ECD	Electron capture detector
GHG	Green house gas emission
IRGA	Infrared gas analyzer

NEE	Net ecosystem exchange
GEP	Gross ecosystem photosynthesis
SOM	Soil organic matter
ATR	Attenuated total reflectance

Chapter 1 General Introduction

1.1 Overview

In this Chapter, the literature relating to biochar was reviewed. Emphasis was placed on the concept of biochar, historical background, production methods, and uses and benefits for soil, plants and the environment. At the end of this Chapter the main aims and objectives of my research are stated, with a brief description of thesis organization.

1.2 Biochar

Biochar is a finely grained product similar to charcoal, resulting from incomplete combustion of biomass in a low oxygen environment through pyrolysis, torrefaction or gasification. It is high in organic carbon content and largely recalcitrant to decomposition (Rumpel *et al*., 2006; Sohi, 2012; Ahmed *et al*., 2020; Garcia *et al*., 2020; Dubey *et al*., 2020; Ahmad *et al*., 2022). However, biochar possesses a labile component and is subject to change by microbial activities (Zimmerman, 2010). The feedstock used to produce biochar comes from agriculture green wastes (manure, crop residues, grasses, trees and wood from virgin trees or demolition sites), industrial green wastes and urban sludge (Lehmann *et al*., 2006; Sevilla and Fuertes, 2011; Norah *et al*., 2015; Shen *et al*., 2016). Its co-products from pyrolysis (bio-oil and syngas) can be used as green energy (Lehmann *et al*., 2008).

1.3 Historical Background

The agricultural soils of Terra Preta de Indio Amazonia were first explored by Francisco de Orellana in 1542 but their fertility status was not appreciated until the 1990s (Laird *et al*. 2009). Due to their extraordinary fertility compared to adjacent nutrient poor Oxisols within the same region (Figure 1.1), they were the focus of international attention and have been the subject of a growing pool of literature (e.g. Glaser *et al*., 2001; Lehmann *et al*., 2003). These soils have a characteristically high nutrient content, such as nitrogen, phosphorus, potassium and calcium, and appreciably greater amounts of stable soil organic matter (Laird *et al*., 2009), up to 150 g kg^{-1} created by pre-Columbians from 500 to 2500 years BP by unknown techniques (Smith, 1980; Woods and McCann, 1999; Petersen *et al*., 2001). Terra Preta soils were believed to be associated with the presence of archaeological artifacts like ceramics, pots, stone tools, half combusted wood logs and charcoal particles (Costa *et al*., 2004).

The organic matter present in these soils was constituted mainly of black carbon, known for its recalcitrant microbial and chemical nature (Haumaier and Zech 1995; Skjemstad *et al*., 1996; Golchin *et al*., 1997; Schmidt *et al*., 1999; Schmidt and Noack, 2000; Chan and Xu 2009) which has a same aromatic polycyclic carbon structure that is a characteristics component of charred plant residues (Glaser *et al*., 1998). Lehmann *et al*., (2003) concluded that the growing interest in the use of charcoal as a soil amendment is extensively based on the exposure of hidden Terra Preta potential and benefits to environment and society. This has led to great interest in use of biochar as a soil amendment, although many studies have failed to replicate the positive benefits observed in Amazonia, possibly because the management

system of the area (cropping, fallow, burning) is integral to the perceived benefits of the biochar.

Figure 1.1 Amazonian Dark Earth. (Source: Glaser *et al*., 2001)

1.4 Production of Biochar

Biochar may be produced as a co-product via a number of different techniques, e,g. pyrolysis (slow pyrolysis; flash pyrolysis; fast pyrolysis), gasification, microwave heating, hydrothermal carbonization and torrefaction (Ukanwa *et al*., 2019; Ahmed *et al*., 2020).

1.4.1 Pyrolysis

Pyrolysis is relatively old, efficient, environmental friendly and low cost method to generate three types of energy products: (i) Solid (biochar), (ii) liquid (bio-oil) and (iii) gas (syngas) (Table 1.1) (Liu *et al*., 2015) by using relatively low temperatures (200-1200°C) in limited or complete absence of oxygen (Laird *et al*., 2009; Neves *et al*., 2011; Yaashikaa *et al*., 2020; Hu *et al*., 2021). However, the primary

composition and properties will depend on various factors like details of processing (e.g. temperature, condensation, and retention time), degradability, nature and type of raw material (Collison *et al*., 2009; Ahmed *et al*., 2020). The yield and properties of biochar can be controlled to suit agronomic and carbon management needs through manipulation of production technology, especially temperature and size, type and nature of feedstock (Zimmerman, 2012).

Table 1.1 Elemental balance of pyrolytic conversions (kg).
(Source: Day *et al*., 2005)

In		**Out**	
100 kg of Biomass		**32 kg of Biochar**	**68 kg of Syngas + Bio oil**
C	46	22	24
H	6.3	1.1	5.2
O	42.5	3.7	38.8
N	2.2	2.2	
Ash	3.0	3.0	

1.4.2 Pyrolysis Mechanism

The basic mechanism of pyrolysis is complex and divided by Demirbas (2003) into five stages: Removal of free moisture; removal of CO and CO_2 after the decomposition of complex carbohydrates like hemi-cellulose; start of an exothermic phase that generates heat and ultimately results in increased temperature; volatile loss of methane and ethane and finally, complete decomposition occurs as the process continues.

Day *et al*., (2005) explained the effects of different temperature zones on structural and chemical reforming of biomass during the multi-step process of pyrolysis (Figure 1.2).

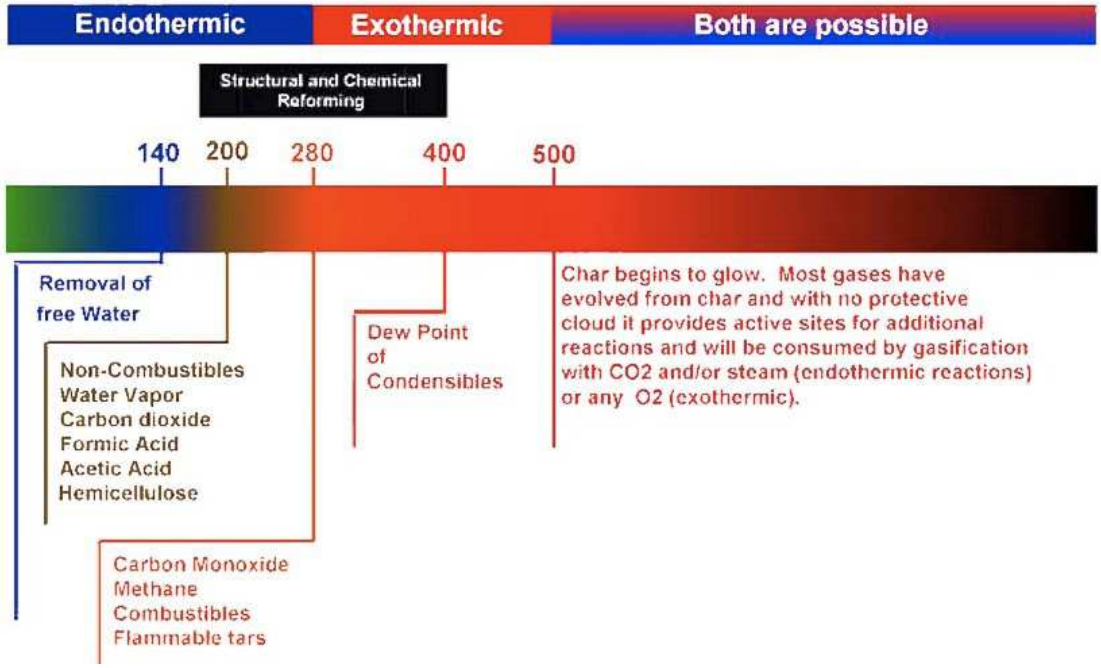


Figure 1.2 Conversions and removal of feedstock components in different temperature zones of pyrolysis (Source: Day *et al*., 2005)

They concluded that properties of char can vary according to the composition of the biomass combusted. Once started, the process continues without additional heat and if oxygen is present, or the material is exposed to its exothermic environment for long, it will result in the ultimate product, biochar. In general, the process is more important than the feedstock in determining the proportion of char, syngas and bio-oil produced. Pyrolysis is mainly of three types; slow, fast and flash depending on conditions such as retention time, rate of heating and final temperature.

According to Brown (2009) slow pyrolysis is characterized by the production of almost equal amounts of oils, chars and gases because of the prolonged exposure of

biomass to moderate heating (at 20 °C min^{-1} to 100 °C min^{-1}) and an average temperature of 600 °C is reached. Slow pyrolysis will result in complete breakdown and rearrangement of the molecular structure of biochar biomass. The yield of solid product, biochar produced in slow pyrolysis is higher than that of the other co-products (bio-oil and syngas) (Tripathi *et al*., 2016; Bamdad *et al*., 2018).

Fast pyrolysis is characterized by quick heating of biomass at very high heating rates of about 100 °C s^{-1} to 1000 °C s^{-1} with 650 °C temperature on average for a short period of time (Williams, 2005). The products of fast pyrolysis consist mainly of liquid bio-oils (60-75%) rather than syngas and biochar (Mohan *et al*., 2006; Laird *et al*., 2009; Suttibak *et al*., 2012).

Flash pyrolysis is an advanced and modified modern form of fast pyrolysis, characterized by high pressure and production of low quality bio-oil. In this process, biomass heating is done at high temperature for a very short time (Wang *et al*., 2005; Yu *et al*., 2007; Canabarro *et al*., 2013; Tripathi *et al*., 2016).

Gasification involves complex biomass conversion in a series of chemical reactions at specific temperatures (500-1400˚C) and pressure to change the molecular structure of biochar in the presence of gasifying agents, which are O_2, air, water vapors and CO_2 (Baruah and Baruah, 2014; Loha *et al*., 2014). The process involves four consecutive steps; feedstock drying, partial combustion or oxidation, pyrolysis in low or complete absence of oxygen and reduction of charred biomass (Srirangan *et al*., 2012; Parthasarathy and Narayanan, 2014).

The production quality of end-products (fuel, biochar and tar) depends on the type of feedstock, reactor type, gasifying agent as well as conditions (temperature and

pressure). The end products of gasification can be used for the generation of energy or manufacturing of many chemicals (Iisa *et al*., 2019; Brown *et al*., 2020; Ahmed *et al*., 2020).

In torrefaction, low calorific value biomass is treated thermally for a few minutes to a couple of hours at relatively low temperature (200-300˚C) under inert conditions (Guizani *et al*., 2016; Ullah *et al*., 2017). As a product of the torrefaction process, chars and ashes (solid), water, acetic acids, alcohols (liquids) and CO_2, CO, CH_4 and H_2 (gases) are produced (Huang *et al*., 2012; Chen *et al*., 2015).

In hydrothermal carbonization biochar or hydrochar is generated from biomass in the presence of low oxygen, water, high pressure and temperature (120-300˚C) for one hour or more (Meyer *et al*., 2011). Hydrothermal carbonization has gained interest in recent years due to its efficiency and convenience (Reza *et al*., 2014; Roland *et al*., 2014; Kim *et al*., 2016). The products of carbonization are bio-oil and CO_2 in addition to the biochar and coal like solid end product (Axel and Felix, 2010; Kang *et al*., 2012; Chen *et al*., 2017).

1.5 Properties of Biochar

The exact carbon composition, nutrient and ash content, liming value and recovery of biochar mainly depend upon the available resources and processing conditions of pyrolysis. Slow pyrolysis chars produced in the presence of steam tend to be acidic (because carboxylic acid groups are activated). However, fast pyrolysis chars produced in the absence of steam tend to be very basic in reaction.

Biochar influences many soil processes and functions because it exhibits a large specific surface area, holding negatively charged organic functional groups on its

surfaces (Cheng *et al*., 2008; He *et al*., 2021; Khan *et al.*, 2021), is alkaline in reaction and has a porous structure (Glaser *et al*., 2002; Downie *et al*., 2009).

The ratio of volatile to stabilised carbon in biochar is controlled by the proportion of cellulose, hemicelluloses and lignin content of biomass that is oxidized anaerobically in pyrolysis, in turn determining the quality of biochar and its potential use.

1.5.1 Effect of pyrolysis temperature on morphology of biochar

Biochar is mainly amorphous in nature, with variable proportions of conjugative aromatic crystals (Qadeer *et al*., 1994), complexes of organic (aromatic-aliphatic) volatile compounds, pores, voids and cracks. On pyrolysis, these crystallites start releasing volatiles by creating wide pores sometimes larger than those of graphite (Laine and Yunes, 1992).

With increasing temperatures the amorphous structure of biochar becomes more ordered with exposed edges and faces, Figure 1.3 (Downie *et al*., 2009).

1.5.2 Effect of pyrolysis temperature on elemental composition of biochar

During pyrolysis at high temperatures the carbon content increased while oxygen and hydrogen decreased in a range of different biochars produced from different biomass such as oak wood, hazelnut shells and wheat straw (Demirbas, 2006). The decrease in oxygen and hydrogen content (Figure 1.4 a and b) following pyrolysis may be due to breakdown of weaker bonds within the biochar structure at higher temperatures e.g. 500-600 °C (Della Rocca *et al*., 1997).

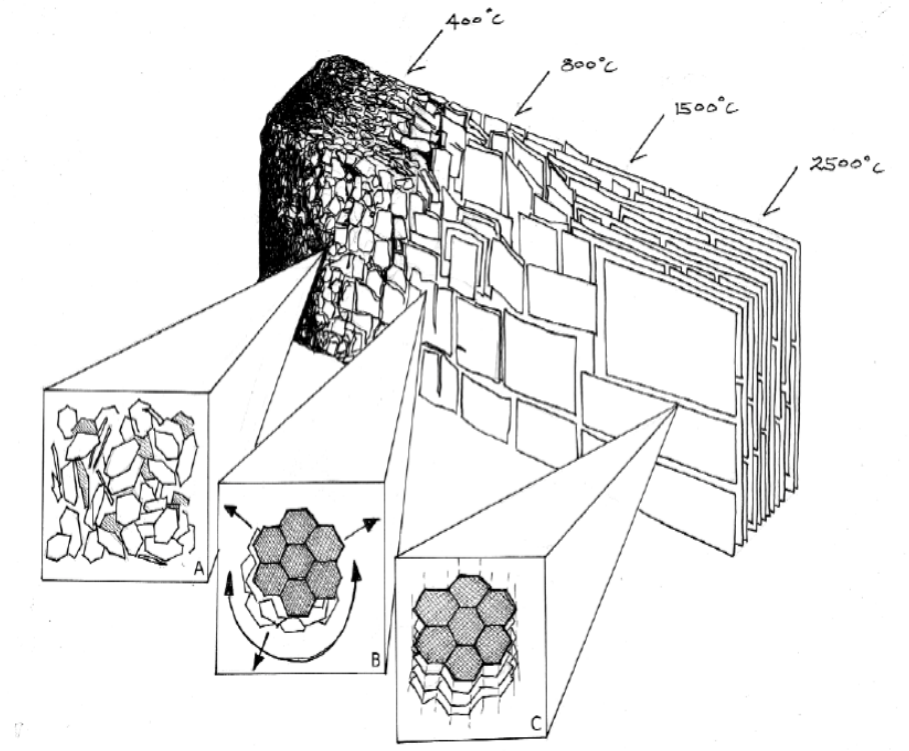


Figure 1.3 Changes in C, O and H content in biochar structure when exposed to high temperatures. (Source: Downie *et al.,* 2009)

Feedstocks with a high lignin content undergoing slow and moderate temperature pyrolysis give a high biochar yield; however biomass with a high content of cellulose combusted at higher temperature by fast pyrolysis, produces a greater proportion of bio-oil and volatile gas with low biochar yield (Demirbas, 2001, 2004 and 2006).

1.6 Sources of biochar

All forms of biomass can be converted to biochar, but the following are preferred because their high lignin content generally renders them unsuitable for composting or for anaerobic digestion: Forest thinning, woody materials, agricultural wastes like

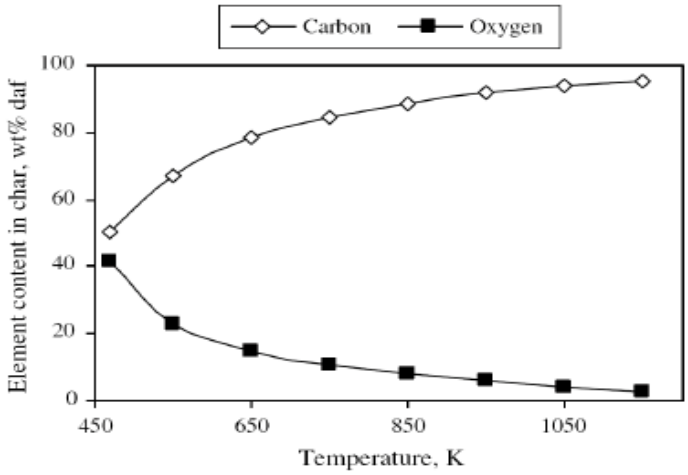


Figure 1.4 (a) Effect of temperature on oxygen and carbon (% wt. on the basis of dry matter ash free) content of biochar produced from plant biomass
(Source; Demirbas, 2008)

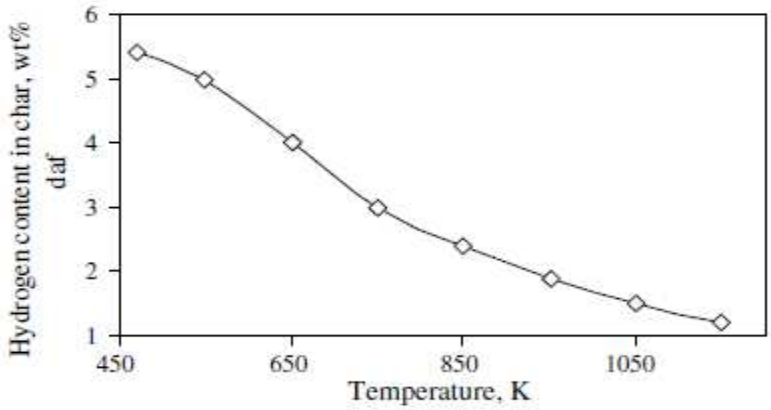


Figure 1.4 (b) Effect of temperature on hydrogen (% wt. on the basis of dry matter ash free) content of biomass (wheat straw, oakwood) biochar
(Source; Demirbas, 2008)

nut shells, olive husk, corncob and tea waste (Demirbas 2004; Ioannidou and Zabaniotou, 2007), crop residues (e.g. stover, leaves, stems, and grain husks), paper mill sludge (Van Zwieten *et al*., 2010), green waste (Chan *et al*., 2007), animal manures, bone meal and other waste products (Downie *et al*., 2007; Lima *et al*., 2008; Chan *et al*., 2008).

Zanzi *et al*., (1996) described an empirical formula for the calculation of the amount of biochar produced from pyrolysis of biomass as follows:

$$\text{Bio-char yield (wt\% daf)} = \frac{(a_b/a_c) - (a_b/100)}{1 - (a_b/100)} \times 100,$$

Where a_b is wt% ash in dry biomass and a_c is wt % ash in dry biochar and daf means dry ash-free.

The elemental composition of biochar depends on the nature and type of feedstock and processing conditions of pyrolysis. The important characteristics of biochar due to the feedstock (either from plant or animal origin) from which it was originally generated are presented in Tables 1.2 and 1.3.

Total carbon content in plant-based biochar was higher than in waste-based biochar, however, total hydrogen and oxygen contents were higher in food waste biochars. Most of the time, it was reported that carbon content in biochar was increased by 34.1% after pyrolysis treatment relative to the raw biomass stocks (Shinogi and Kanri, 2003).

When biomass is converted into biochar, there is a significant difference in ash content among various biomass feedstocks. Wood and crop wastes had the lowest ash contents with low biochar yield and it mostly contains lignin, cellulose and hemicelluloses (Cao and Harris, 2010). The ash content of biochar has a great influence on soil fertility, plant growth and yield, because most of the inorganic elements are left in the ash fraction along with essential metal nutrients.

Table 1.2 Pyrolysis temperatures, surface area, ash content (%), volatile matter (%) and nutrient (%) of some selected plant oriented biochars (Source: Spokas *et al*., 2010)

Biomass	Pyrolysis	Surface area	%C	%O	%N	%VM	%Ash	%Fixed C
Activated charcoal Coconut shell	450 °C	977	83	<0.1	0.4	2	15	83
Hardwood sawdust	500 °C fast	10	67	13	0.3	29	15	55
Macadamia nut	600 °C flash	7	93	2	0.7	17	2	81
Hardwood chip	550 °C slow	66	71	21	0.1	35	5	61
Distillers grain	350 °C slow	0.3	69	7	7.5	45	11	43
Distillers grain	400 °C slow	0.3	69	6	7.4	38	12	50
Corn cob	350 °C slow	<0.1	79	13	0.7	33	3	64
Corn cob	400 °C slow	<0.1	83	9	0.6	25	4	71
Mixed wood	400 °C slow	4	80	12	0.8	27	4	70
Mixed wood	450 °C slow	27	81	11	0.8	24	4	73
Wood pellets	400-500 °C slow	2	73	19	0.2	12	6	81
Wood waste	400-500 °C slow	34	32	<0.1	0.3	20	67	13
Peanut shell	481 °C slow	1	59	3	12	40	15	45

Table 1.3 Nutrient (%), ash content (%), bulk density (g cm^{-3}) and specific surface area ($m^2 g^{-1}$) with special reference to pyrolysis temperature of some selected biochars produced from animal waste materials in comparison with traditional coal, coconut shell and wood biochar
(Source: Lima *et al*., 2010)

Sample	Bulk Density (g cm^{-3})		pH		Ash		*BET ($m^2 g^{-1}$)		%			
	700°C	800°C	700°C	800°C	700°C	800°C	700°C	800°C	C	H	N	S
Broiler cake	0.54	0.53	8.6	9.4	45.2	51.2	318	281	43.9	1.02	2.84	0.35
Broiler litter	0.60	0.62	8.1	9.1	49.2	51.8	238	199	-	-	-	-
Turkey cake	0.53	0.46	9.2	9.0	40.4	41.1	147	168	39.9	1.05	3.43	0.37
Turkey litter	0.57	0.55	8.1	9.3	43.5	44.8	179	206	-	-	-	-
Dairy	0.56	0.59	7.2	8.4	71.0	68.0	131	77	25.2	0.15	1.08	0.00
Coal	0.42	0.43	4.2	4.7	2.5	-	4	12	86.8	1.08	1.85	0.06
Coconut shell	0.61	-	6.6	-	1.8	-	35	-	82.1	1.33	0.19	0.09
Wood	0.38	-	5.1	.	1.4	.	301	-	85.1	1.76	0.31	0.22

**BET – Brunauer-Emmett-Teller analysis for measuring specific surface area.*

1.7 Potential Uses of Biochar

Research into biochar is growing internationally because of its wide applicability (as a fuel), its multi-functional efficiency (feed stocks generating solid char, liquid vapours and syngas), in addition to being a potentially useful soil amendment and carbon sink (Glaser *et al*., 2002 a, b; Lehmann *et al*., 2003, 2006; Shen *et al*., 2016). Kwapinski *et al*., (2010) pointed out some factors that will limit the efficiency and application of biochar under some specific circumstances (Du *et al*., 2012; Jiang *et al*., 2014). These factors are: Nutrient composition of feedstock or source, release and availability of nutrients from biochar, percentage of volatile matter, elemental composition, macro- and micro-structures of biochar (function of pyrolysis temperature and method), physico-chemical nature and properties, and limited understanding of agricultural effects of biochar application (Beesley *et al*., 2011; Zhang *et al*., 2013; Bian *et al*., 2014) .

1.7.1 Biochar as a soil amendment

Biochar showed variable responses (Glaser *et al*., 2002) when applied as a soil amendment due to variation in inherent properties as a result of different sources and pyrolysis conditions (Baldock and Smernik 2002; Downie *et al*., 2009; Major *et al*., 2010). Lehmann *et al*. (2006) granted biochar a status of soil conditioner and fertilizer after considering the findings of several investigations on effects of biochar additions to soil physical properties such as aggregation, water holding capacity, strength and chemical properties (e.g. pH and CEC) (Yamato *et al*., 2006; Liang *et al*., 2006 ; Chan *et al*., 2007; Novak *et al*., 2009); in addition to biological properties (e.g. modifying the microbial community and serving as habitat for microflora) (Pietikäinen *et al*., 2000).

It was concluded that not all biochars behave the same in all soils; depending on the biochar source (Kuzyakov *et al*., 2009), production method (Amonette *et al*., 2009), and soil (Kolb *et al*., 2009). These variations defined the different adsorption behaviour and biological activity of biochar, due to widely varying pH, surface area, pore size distribution, and charge properties (Brewer *et al*., 2009; Gaskin *et al*., 2009). However, common features seem to include an initial stimulation of biological activity (Kolb *et al*., 2009; Smith *et al*., 2010) and a subsequent persistence of C (Kuzyakov *et al*., 2009).

1.7.2 Effect of biochar on soil chemical properties

Biochar possesses the potential of increasing availability of nutrients for plants because of its high sorption affinity for organic and inorganic compounds (Kleineidam *et al*. 2002; Lehmann *et al*., 2003; Nguyen *et al*. 2004). The availability of nutrients can also be affected by biochar through increased cation exchange capacity (CEC), altered soil pH or by a contribution of nutrients from the biochar. For an enhanced nutrient retention and supply following biochar amendment, one potential mechanism is increasing CEC by up to 50% as compared to that of un-amended soils. Due to its greater surface area as well as negative surface charge, biochar has more ability of adsorbing and retaining cations in an exchangeable form as compared to other forms of soil organic matter (Yu *et al*. 2006). Due to its unique macro- and micro-crystalline structures, biochar potentially absorbs and lessens nutrient leaching from soil and forms stable ionic electrostatic complexes with ion species from soil solution (Major *et al*., 2010).

In freshly produced biochar, there is little ability of cation retention which results in minimal CEC (Lehmann 2007), but it increases with exposure time in soil due to surface oxidation (Cheng *et al*., 2006). It supports the findings of high CEC observed in Amazonian Anthrosols (Liang *et al*., 2006).

1.7.3 Effect of biochar on soil physical properties

The physical properties of soil such as structure pore size distribution and density can be altered by incorporation of biochar with implications on soil aeration, water holding capacity, plant growth and soil workability (Downie *et al*., 2009). There is evidence which suggests that overall net soil surface area is increased by biochar application (Chan *et al*., 2007) and as a result it may improve retention of soil water and nutrients (Downie *et al*., 2009) and soil aeration specifically in fine textured soils (Kolb, 2007). The bulk density for biochar is much lower compared to that of mineral soils (~0.3 g m^{-3} for biochar and 1.3 g m^{-3} of typical soil); therefore biochar application can reduce overall total bulk density of the soil which is desirable generally for plant growth (Brady and Weil, 2004).

The improvement in soil moisture retention is an indirect result of alterations in soil aggregation and structure after application of biochar (Brodowski *et al*., 2006). Soil aggregation can be affected by interaction of biochar with SOM, minerals and microorganisms; however, long term effects on soil aggregation are determined by the surface charge characteristics and their development over the time.

1.7.4 Effect of biochar on soil microbial properties

The effects of biochar on the biological activity of soil need greater study to evaluate the potential repercussions of wide application of such material due to its variability in terms of production methodology and wide range of substrate choices. Current research on effects of biochar on soil microorganism and their activities suggests that there is an initial stimulating effect that diminishes over time (Kuzyakov *et al.,* 2009), as the labile component is metabolized (Smith *et al.,* 2010), although one study did report enhanced biological N fixation in biochar-amended soils (Rondon *et al.,* 2007).

1.7.5 Effect of biochar on soil biochemical properties

Enzyme assays of char-amended soils suggested that the effects of biochar on soil enzyme activities are variable, depending on the soil, and on the particular enzyme. The enzyme responses vary in direction and magnitude which reflects that, biochar can stimulate overall microbial activity in the short-term (Smith *et al*., 2010; Bailey *et al*., 2011), but is unpredictable in the long term. This early boost or stimulation is possibly limited to a specialized subset of the microbial community (Kolb *et al*., 2009), which results in some increased enzyme activities. Decreased activities may be due to sorption or blocking of either enzyme or substrate. In some cases, biochar stimulates soil enzyme activities, to a much greater degree than soil assays would indicate, given that substrate reactivity can be impeded by biochar exposure.

1.7.6 Biochar and soil quality

Biochar can be used as soil modifier to improve soil quality and crop productivity in various types of soils (Blackwell *et al*., 2009) because of distinct characteristics like long residence time, conditioning effect, carbon storage and filtration and percolation of soil water (Lehmann and Joseph 2009). This has been successfully demonstrated in highly weathered or degraded soils, due to agricultural activities (Kimetu *et al*., 2008).

1.7.7 Biochar as an alternative agricultural management technique

Biochar may become an essential component of both conventional and sustainable agriculture due to its potential benefits for not only increasing soil productivity, but also for reducing impacts of extensive agriculture on soil, water and the environment. Biochar production from a wide range of sources not only enhances existing soil management techniques, but also adds value to waste management practices (McHenry, 2009). However, the extent of the

contribution is not currently sufficient to make biochar an alternative to existing use of inorganic and organic fertilizers, but it can be considered comparable in facilitated agriculture (Lehmann and Joseph, 2009).

1.7.8 Biochar as a plant growth regulator

The use of biochar as a plant growth regulator depends on its chemical (Maia *et al*., 2011; Novotny *et al*., 2015; Figueredo *et al*., 2017) and physical properties (Lehmann *et al*., 2015; Paustian *et al*., 2016). Application of biochar as an alternative agricultural practice modifies and improves soil fertility, nutrient availability (Nelson *et al*., 2011; Prendergast-Miller *et al*., 2014; Olmo *et al*., 2016), soil moisture retention (Obia *et al*., 2016 a, b), reduces plant nutrient stresses, enhances aggregate stability (Burrell *et al*., 2016; Fungo *et al*., 2017a), increases soil microbiological diversity (Rutigliano *et al*., 2014; Xu *et al*., 2016) and regulates the emission of green house gases (Smith, 2016; Fungo *et al*., 2017b).

Increased crop growth (Reynolds *et al*., 2003; Marris, 2006) and yield of various crops such as cowpea (Yamato *et al*., 2006), soybean (Tagoe *et al*., 2008), maize (Yamato *et al*., 2006; Rodríguez *et al*., 2009), and rice (Haefele, 2007; Haefele *et al*., 2008; Asai *et al*., 2009) has been reported following biochar application.

1.7.9 Biochar as a potential biofuel

The utilization of biomass resources in the area of energy production has gained interest in countries whose economies are based on agriculture and forestry. Moreover, agricultural residues offer an attractive option, in favour of the efforts to develop energy recovery processes with few exemptions. Among all the complex biochemical and thermo-chemical conversion techniques and processes, pyrolysis has received considerable attention due to its

flexibility in selection and optimization of process conditions to produce the desired maximized end product.

Karaosmanoglu *et al*., (2000) produced carbon rich, reactive and pollution-free biochar with low specific surfaces by slow pyrolysis of rapeseed straw at varying temperatures. Biochar biofuels are classed as zero-emissions fuel that are safely utilized for farm energy needs and electricity demands (Day *et al*., 2005).

1.7.10 Effect of biochar on nitrogen dynamics

Biochar additions increase soil carbon but with little evidence of it also directly increasing the nitrogen pool; although alterations in N-dynamics have been recorded (Granatstein *et al*., 2009; Kolb *et al*., 2009). Van Zwieten *et al*. (2010) discussed possible reasons relating to the influence of biochar on nitrogen transformations and leaching in the soil system. These were: modifying soil structure, pH, and cation exchange capacity; regulating the availability and distribution of electron acceptors (O_2, NO_3^-) and donors (NH_4^+, dissolved organic matter); inducing reduction of N_2O to N_2 and oxidation of biochar and minerals; influencing microbial biomass, enzymes and processes associated with N cycling (mineralization, immobilization, nitrification and denitrification) in soil (Šimek and Cooper 2002; Yanai *et al*., 2007).

1.7.11 Biochar stability and carbon sequestration potential

The polycyclic aromatic structure of biochar makes it chemically and biologically stable and inert, allowing it to persist in the environment for centuries (DeLuca *et al*., 2006). Lehman *et al*., (2006) concluded that carbon stabilization through pyrolysis of biomass is a promising technique to sequester carbon for a large time scale and far outweighs the short term losses as shown in Figure 1.5.

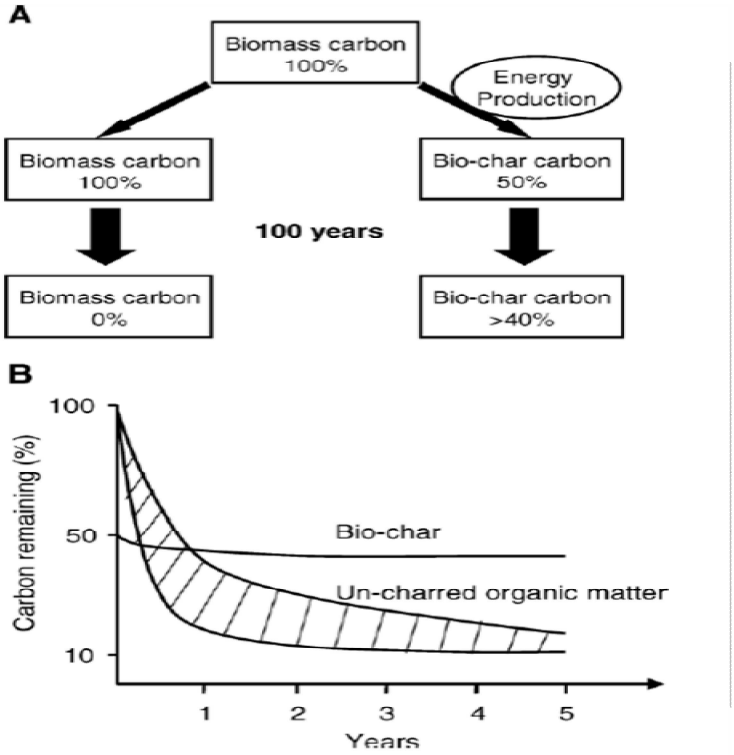


Figure 1.5 Stabilization of biomass carbon and bio-char stability over time.
(Source: **Lehmann et *al*., 2006**)

Biochar has been postulated as one of its kind among all the other naturally governed phenomena for carbon sequestration (Smith *et al*., 2010; Noguera *et al*., 2010), by reducing atmospheric CO_2 enrichment resulting from anthropogenic activities and by storing almost four times more carbon in the soil than is present in the atmosphere (Laird, 2008; Novak *et al*., 2009). Even a slight increase (2%) in soil carbon content could offset about 70-80 % of all greenhouse gas emissions going into the environment. Besides its chemical structure, biochar has a unique porous physical structure which contributes to a large surface area, increasing its capacity to retain dissolved organic matter (Lehmann and Rondon, 2005) reducing C losses from soil.

1.7.12 Biochar as a tool for remediation

Heavy metal immobilization attributed to biochar addition, has gathered much interest as a potentially cost-effective in-situ remediation technique (Beesley *et al*., 2015; Kumar *et al*. 2016 ; Lonappan *et al*., 2018; Mosa *et al*., 2018; Kumar *et al*., 2018). Biochar has been highly recommended as an effective sorbent for immobilizing, adsorbing and sequestering a number of heavy metals including chromium (Cr), cadmium (Cd), lead (Pb), nickel (Ni), copper (Cu), mercury (Hg), and zinc (Zn) from soils and water (Beesley *et al*., 2015; Kumar *et al*., 2016) from exchangeable and reducible fractions in contaminated soils over a large range of pH (Ahmad *et al*., 2018). Surface area, pH, functional chemistry, and pore size of biochar all play a role in metal-biochar specific and non-specific interactions, adsorption, precipitation and complexation reactions (Archanjo *et al*., 2017; Beiyuan *et al*., 2017; Kumar *et al*., 2018).

1.8 Negative Impacts of Biochar on soil Properties

Some of the possible negative impacts on soil, plants and the environment associated with land application of biochar are discussed as follows:

- Biochar is composed of different sized fractions (from macro-particles to highly active nano-particles) which behave differently in soil. The highly reactive nano-particles may carry contaminants down the soil profile (Hale *et al*., 2012; Oleszczuk *et al*., 2013; Chen *et al*., 2017); affect dissemination and transport of phosphorus in soils (Yao *et al*., 2012); reduce nutrient retention in the rhizosphere and pose a potential risk to groundwater (Chen *et al*., 2018).

- Due to the presence of heavy metals and contaminants in biochar, some studies have reported an increase in salinity and sodicity when biochar is applied at high rates (Zhang *et al*., 2016; Blok *et al*., 2017; Luo *et al*., 2017).

- The significant challenges to the widespread use of biochar for decontamination of degraded and salt affected soils are associated with high cost of biochar production, transport, and application (Blackwell *et al*., 2009).

- The strong hydrophobicity of biochar has negative effects on water penetration (Saifullah *et al*., 2018), soil structural stability (Mukherjee and Lal, 2014), hydraulic conductivity and soil aggregate stability (Jeffery *et al*., 2015a, 2015b).

- Due to the high adsorptive capacity of biochar, decreased plant growth was reported because biochar is highly effective at retaining nutrients, which may prove detrimental for plant uptake (Gaskin *et al*., 2010).

- Stunted plant growth was reported, where biochar was applied in the complete absence of fertilizers (Gundale and DeLuca, 2007; Asai *et al*., 2009) in plough layer.

- The high C: N ratio of fresh biochar (up to 400), caused imbalanced nitrogen immobilization, potentially leading to nitrogen deficiency when applied alone (Asai *et al*., 2009; Chan and Xu, 2009; Lehmann and Joseph, 2009). This likely happened because of rapid mineralization of the labile carbon fraction, contributing to the reduction in soil and plant available nitrogen.

1.9 Knowledge Gaps in biochar research

1. Biochar production and utilization are primitive and ancient technologies. Current food and climate challenges need a comprehensive and modern evaluation of biochar's potential.
2. Activating biochar is another field, where biochar is extensively used to remove specific contaminants. This will further led to expand the biochar usage options.

3. Biochar characterization is critical for determining the biochar's role and efficiency in various areas of elemental analysis, surface functional groups, stability, and structure. Current technologies like Scanning Electron Microscopy (SEM), Fourier Transform Infrared Spectroscopy (FTIR), Thermo Gravimetric Analysis (TGA), X-Ray Diffraction (XRD), Brunauer Emmett Teller (BET), Nuclear Magnetic Resonance (NMR), and Raman spectroscopy are recommended to characterize biochar.
4. Focus should be given to distinguish biochar from other organic materials in the soil with the help of new techniques like SEM and FTIR that determine the morphology and surface chemistry of biochar.
5. Optimization of pyrolysis conditions like operating parameters, heating rate, temperature and residence time with the biomass source is ideal for its maximum utilization.
6. In order to minimize the heterogeneity of biochar, standardization of pyrolysis conditions over the range of biomass sources is required that will further determine the suitability of biochar for a given application.
7. Biochar’s long mean residence times in soils (100s of years) differentiate it from other conventional soil amendments (such as manures and fertilizers). All management practices should be designed for long period of times in order to get maximum benefit from biochar.
8. Extensive research is needed to understand short term, midterm and long term effects of biochar additions to field in various environments (forest, temperate and tropical)
9. Biochar proven character as soil quality improver accompanied with a big knowledge gap regarding the variable mechanism of either positive or negative agronomic effect, with variations in soil type, liming, nutrient solubility, availability and water retention.

1.10 Research Aims /Rationale

The main aim of the research was to evaluate the effects of different concentrations of biochar amendment on plant growth and on soil chemical, physical and biological effects and interactions. Much published work focuses on short-term pot experiments, whilst here, the investigation was conducted over a continuous 13 month period utilising large containers in which strawberry plants were grown for the whole duration. This work therefore gives a detailed insight into the short- and long-term effects of biochar amendment on soil functioning and plant growth.

It is increasingly clear that biochar may play an important future role in carbon sequestration and application to land may help to satisfy this role, whilst simultaneously acting as a soil improver and enhancing soil carbon stocks. Producing biochar that satisfies the International Biochar Standards and the European Biochar Certificate is important if food for human consumption is to be grown on biochar-amended land. However, production of suitable biochar from virgin wood has cost implications and it is important to evaluate the effects of biochar made from sources that may not meet the suggested regulatory standards. This project therefore evaluated the effects of biochar produced using demolition wood. The purpose of this was to determine if biochar made from 'cheaper' biomass is safe to apply to soils without adversely affecting soil ecosystem functioning. If so, such biochar may be utilised on reclamation sites or marginal agricultural land. The nature of the experimental work enabled every aspect of the plant-soil-microbial ecosystem to be evaluated from a functional perspective.

The **specific objectives** of the research were to:

(1) Characterise the biochar produced from demolition wood and compare it to the International and European Biochar Standards (Chapter 3).

(2) Determine the effects of a range of biochar concentrations on plant growth and physiological responses (Chapter 4).

(3) Quantify the effects of biochar on soil chemical, physical and microbiological properties and functions (Chapter 5 and 6).

(4) Measure the effects of biochar and presence of plants on soil organic matter composition (carbon) using FTIR (Chapter 7).

1.11 Hypothesis

The testable hypotheses for the research experiment were:

1. Biochar generated from demolished contaminated wood would be beneficial and safe for environment, soil and plant? (Chapter 3, biochar characterization and comparison with international standards)
2. Biochar is either stimulator or inhibitor for living entities (Plant and microbes) by assessing plant growth and enzyme activities (Chapter 4 and 5)
3. What are the parameters which are most affected by Biochar, Time and Plant? (Chapter 4, 5,6 and 7)
4. How presence or absence of strawberry plant contribute or stimulate the effect of biochar on soil properties? (Chapter 5 and 6, soil microbial, biochemical, physical and chemical properties)
5. Biochar contributes towards carbon sequestration by reducing the amount of CO_2 released from soil (Chapter 5)
6. Whether biochar is source or sink for soil total and available nutrients (Chapter 6)

7. Whether time and plant are able to produce significant changes in surface chemistry of biochar when exposed for 370 days under green house controlled conditions? (Chapter 7, FTIR studies of biochar and biochar amended soil)

1.12 Thesis Organization

The thesis layout and organisation is as follows:

Chapter 1 discussed the background of biochar, its introduction and potential uses as soil improver and plant growth regulator, pyrolysis and its effects on biochar nature and behaviour with special emphasis on those biochar characters, which determine its usage and functionality.

Chapter 2 provided an overview of general materials and methods used in the experiment to characterize soil, biochar and biochar amended soils in the start of experiment or during the whole experiment after predefined time intervals.

Chapter 3 discussed the characterization of biochar and its comparison with European, British and International standards of characterization.

Chapter 4 presented the results of strawberry plant physiological growth parameters and nutrient concentration of root and shoot were presented in this Chapter.

Chapter 5 discussed the effect of biochar on soil microbial, biochemical properties in absence or presence of strawberry plants. Soil data obtained for soil respiration (CO_2 and CH_4 flux) were presented in this Chapter.

Chapter 6 discussed the effect of biochar on soil physical and chemical properties, total and available soil nutrients in one year experimental period with or without strawberry plants.

Soil data obtained after every three months for physical and chemical parameters, analysis were presented in this Chapter.

Chapter 7 determined plant mediated changes in carbon chemistry of soil biochar mixtures as a function of time. These changes were measured by comparing the spectra obtained by FTIR of initial and final soil, biochar and soil–biochar samples.

Chapter 8 provides general discussion and interpretation of the results obtained and integrates the findings.

Chapter 9 provides general conclusions according to the aims and objectives of the research study.

Chapter 2 Materials and Methods

2.1 Overview

The methods described in this Chapter were performed throughout on the biochar, soil and plant material used in, and derived from, the main experiment which formed the basis of the work. Strawberry plants were grown in large pots for 53 weeks in soil amended (or not) with biochar. A parallel set of experimental pots were maintained throughout without plants. A range of plant, soil and microbial parameters were measured at regular intervals throughout the experimental period in order to give a detailed and integrated understanding of the biological and chemical effects of adding biochar to soil. The first measurements were taken after one month and thereafter every three months. Plants were harvested at the end of experiment.

2.2 Experimental Setup

Two-week-old strawberry plantlets/runners (*Fragaria* x *ananassa* 'Florence' were purchased from Black Moor Fruit Nursery, (Hampshire, UK) in December (2011) and transplanted to 14 L plastic pots filled with either soil only or soil amended with a range of biochar concentrations. These pots were exposed to chilling for two weeks and then transferred to a growth room in January (2012). Parallel pots were established, but without planting and these were left bare throughout. Within the growth room, light and temperature were controlled **(**20^{o}C/16^{o}C day/night; 16 hour day; 50% humidity) during the experimental period. Pots were set up in a randomised block design with five replicate blocks.

Since the topsoil used for growing the strawberries was purchased, all pots were amended with a solution made from field soil and water in order to enhance the populations of

indigenous microorganisms. Plants were not given supplemental nutrients and pots were maintained at 60% water filled pore space throughout the experimental period, and gravimetrically kept at a constant moisture level on daily basis.

A few plants exhibited limited flowering during the study with no more than three blooms on a plant. Those flowers and dead leaves were removed to prevent fruiting and avoid any disease infestation respectively.

2.2.1 Treatment structure

Five biochar application rates were used: 0 (control), 2.5, 5, 10 and 15 % (w/w equivalents). The rates were calculated as a soil: biochar ratio and in order to maintain the same final soil pot volume for all treatments, the volume of soil was adjusted as required. Plants were grown in replicate pots with each biochar amendment. Additional pots with 0, 10 and 15% biochar were established, and these were maintained in the same manner as the other pots, although these were not planted and remained plant-free throughout.

The eight treatments were arranged in a randomized block design with five replicates to study the effect of biochar, time and planting on the soil (and/or plant) system.

2.2.2 Strawberry as test plant

Strawberry is a horticultural crop in the family Rosaceae with the genus name Fragaria, with over twenty species. Strawberries are commercially cultivated in 76 countries, in broad range of low temperature conditions (Simpson, 2018). Strawberry has clear and classic growth stages and leaf developments. Strawberry is an easy to manage green house plant because of its shallow root system and little woody tissue above the ground. The lack of woody tissue makes the plant short and vascular as the stem do not thickens for tall growth (Raja *et al*.,

2018; Wei *et al*., 2020). Nutrient and water management is comparatively easy as compared to other plants. Good management of soil, water and essential nutrients makes favourable conditions for strawberry growth and yield. (Tang *et al*., 2020; Lee *et al*., 2020; Choi, 2021).

2.2.3 Biochar

Biochar used in the study was produced by gasification by O-Gen UK Ltd. (Stoke-on-Trent) at 1100 ^{o}C. The feedstock was demolition wood. O-Gen was formed as a company in 2005 to develop a gasification plant in order to meet local electricity demand produced from locally available waste. Wood waste varies from waste timber, chipboard, construction and demolition wood, furniture treated or untreated waste, packaging and pallets, industrial and commercial waste with preservatives and municipal waste in their gasification plant .timber wood waste contain nails, screws and other contaminants.

2.2.4 Topsoil

Soil used in the study was a sandy-loam top soil obtained from HomeBase, Loughborough, UK. Soil analysis data are presented in Table 2.2.

Table 2.2 Basic characteristics of topsoil used in the experiment

Characteristics	**Topsoil**
pH	7.20
Moisture content (% of dry weight)	52.59
Organic matter (% of dry weight)	16.75
Total nitrogen (%)	0.23
Total carbon (%)	10.38
Total sulphur (%)	0.074
C:N ratio	43.90

Total and available elemental data of the topsoil are presented in Table 2.3.

Table 2.3 Nutrient profile of topsoil used in the experiment

Characteristic	Available Nutrients mg kg^{-1}	Total Nutrients mg kg^{-1}
Na	71.9 ± 5.2	2228.9 ± 205.0
Mg	171.6 ± 12.7	2096.5 ± 27.2
K	86.7 ± 41.6	10608.8 ± 90.1
Ca	1949.9 ± 468.4	2010.0 ± 108.5
	mg kg^{-1}	**mg kg^{-1}**
Al	0.04 ± 0.07	22779.5 ± 199.3
Cr	0.002 ± 0.005	20.6 ± 0.40
Mn	10.2 ± 2.4	333.4 ± 4.40
Fe	0.3 ± 0.06	14132.2 ± 322.8
Co	0.005 ± 0.0003	4.9 ± 0.09
Ni	0.04 ± 0.01	12.8 ± 1.1
Cu	0.06 ± 0.010	14.8 ± 0.4
Zn	0.2 ± 0.1	73.2 ± 4.2
As	0.008 ± 0.001	9.1 ± 0.3
Se	0.001 ± 0.0004	0.40 ± 0.1
Mo	0.003 ± 0.0006	0.9 ± 0.1
Cd	0.003 ± 0.1	0.2 ± 0.003
Cs	0.01 ± 0.002	1.8 ± 0.02
Ba	29.5 ± 4.2	262.0 ± 7.4
Pb	0.004 ± 0.002	23.8 ± 0.04

Extraction and analytical procedures for available and total nutrients are given below.

2.2.4.1 Soil sampling from experimental pots

Soil samples were taken from experimental pots on days 30, 90, 180 and 370 days after establishment. The first soil sampling from the growing pots was done after one month of onset of study to provide baseline values of soil properties. The three further samples provided information about temporal changes in soil chemistry and microbiology.

Table 2.4 Categories of treatments used in the experiment to compare the effects of biochar, planting and time (number of days) as factors on different parameters

Name	Major Effects	Description
Category 1	Plant	Yes
Planted	Biochar	Five rates of application (Control, 2.5, 5, 10 and 15 %)
	Time	Four soil samplings (after 30, 90, 180 and 370 days)
Category 2	Plant	No
Unplanted	Biochar	Three rates of application (Control, 10 and 15 %)
	Time	Four soil samplings (after 30, 90, 180 and 370 days)
Category 3	Plant	Yes or No
Comparison of planted and unplanted	Biochar	Three rates of application (Control, 10 and 15 %)
	Time	Four soil samplings (after 30, 90, 180 and 370 days)

Soil samples were taken from pots by using narrow soil augers. Firstly, the soil surface was cleared of debris. The augers were then pushed vertically into the soil to a depth of 30 cm with the help of a hammer. Three soil samples were collected from random points in each pot. Care was taken to minimize disturbing growing roots in pots where there were plants. The three sub-samples from individual pots were homogenized and sieved to < 4 mm to remove roots. Soil samples were analyzed for selected physical, chemical and microbial properties on the same day of sampling (Table 2.5).

Table 2.5 Soil physical, chemical and microbial characteristics measured in samples taken from experimental pots.

Soil characteristic	Fresh soil sample	Oven dried ground soil samples
Physical	Moisture, bulk density, water filled pore space	
Chemical	Loss on ignition, pH, NH_4-N, NO_3-N, Olsen-P	CNS, FTIR, TEs*
	Extraction for exchangeable nutrients	
Microbial	Enzyme assays (dehydrogenase, betaglucosidase, phosphatase),	
	Microbial biomass carbon and nitrogen	

**TEs – total elements following acid digestion*

The rest of each soil sample was dried at 45 ^{o}C prior to acid digestion and analysis of total nutrients, grinding for total CNS and FTIR. After sieving, subsample of dried soil was ground using an agate ball mill at 300 rpm for 4 minutes. Dry samples were stored in the dark at room temperature in plastic zip lock bags to avoid any contamination.

2.3 Soil Characterization

Soil chemical properties (pH, loss on ignition, nitrate, ammonium, Olsen-P, and CNS), soil physical properties (gravimetric water content, soil bulk density, water filled pore space, saturation percentage, porosity and aggregate size distribution) and

soil microbial and biochemical properties (microbial biomass carbon, microbial biomass nitrogen, and enzyme activity (dehydrogenase, beta-glucosidase and phosphatase)) were quantified at different time intervals during the duration of the plant growth. Full analytical details follow.

2.3.1 Soil chemical properties

The following procedures were used for the determination of above mentioned soil characteristics:

2.3.1.1 Soil pH

pH of sieved (< 2 mm) fresh soil was measured after preparing soil samples in a soil: deionised water ratio of 1:2.5, in Oak Ridge polycarbonate centrifuge tubes. Replicate samples were shaken on an end-over-end shaker for 30 minutes. A pH meter was calibrated using buffers of pH 4.01 and 7.00 and pH was recorded when the reading was stable.

2.3.1.2 Organic matter content

Soil organic matter content was estimated using the method of loss on ignition (Sutherland, 1998). Approximately 5 g of < 2 mm was ignited in a muffle furnace at 550°C for 8 hr. The LOI was determined gravimetrically and expressed as a percentage.

2.3.1.3 Ammonium and nitrate nitrogen

Six g of fresh soil sample was weighed into centrifuge tubes with 40 mL potassium chloride KCl (2N) and shaken on an end-over-end shaker for 30 minutes. After shaking, these samples were filtered through Whatman No. 42 filter paper. This extractant was used for both ammonium and nitrate analysis.

For ammonium determination, 1 mL of extractant was diluted with ultrapure water and then 1 mL of each, nitrophenol prusside and 13% sodium hypochlorite was added. After placing in a water bath for 30 minutes at 25°C, sample absorbances were measured at 635 nm using a spectrophotometer after the calibration of instrument with standards.

For nitrate nitrogen, a selected volume of KCl extractant solution was placed on a shaker with 3 mL ammonium chloride, 1 mL borax solution and 0.6 g of spongy cadmium to reduce nitrate to nitrite. One mL of both sulphanilamide solution and N-1-napthylethylenediamine di-hydrochloride were added to a 50 mL volumetric flask with 7 mL of reduced solution. After standing for 10-15 minutes the nitrite ion complexed to form a red azo-species in solution that was measured at 543 nm (Jones, 1984).

2.3.1.4 Available phosphorus

Phosphorus concentrations of soil samples were determined using the Olsen-P colorimetric method (Olsen and Sommers, 1982). Two g of soil sample were weighed into a 50 mL screw cap centrifuge tube in triplicate with 30 mL of 0.5 M sodium bicarbonate and approximately 5 g of low-phosphate charcoal for extraction. The centrifuge tubes were placed on an end-over-end shaker for 30 minutes before centrifuging at 2500 g for 15 minutes. Blank extractions containing charcoal and sodium bicarbonate without soil were also included in triplicate. If the supernatant was brown in colour, then addition of a further quantity of charcoal was recommended followed by re-suspension and centrifugation for 10 minutes. After shaking, each sample was filtered through Whatman No.42 filter paper.

Calibration standards (200, 400, 600, 800 and 1000 μg L^{-1} P) were prepared from a working phosphate standard (10 mg P L^{-1}) by adding 0, 1, 2, 3, 4 and 5 mL in 50 mL volumetric flask along with 4 mL of the acid molybdate reagent (AMR) and freshly prepared ascorbic acid solution. Five mL aliquots from soil samples and blanks were treated the same, except additional acid increment (2 mL of 3M H_2SO_4). The final volume was made with deionised water and 20 minutes were allowed for colour development, before measuring absorbance at 880 nm.

2.3.1.5 Total nutrients in soil by acid digestion

Approximately 200 mg samples of finely ground dried soil were digested in PFA vials within a block digester with 2 mL of concentrated HNO_3 (69% AR) and 1 mL of $HClO_4$ at 80°C for 8 hr and then at 100°C for a further 2 hr. An aliquot (2.5 mL) of HF was then added and the samples were heated to 120°C for 8 hr. A further 2.5 mL of HNO_3 and 2.5 mL ultrapure water were then added to the dry residue and the vessels heated at 50°C for 30 minutes. The digested soil samples were kept in 5% HNO_3 and the total concentrations of major and minor nutrients were determined by ICPMS with some blanks as well.

2.3.1.6 Available nutrients in soil

After soil sampling all soil samples were sieved to <4 mm and unwanted material such as plant debris removed. Fresh samples were weighed into centrifuge tubes with a specific volume of 1 M NH_4NO_3 (ratio was 1g:5 mL) and samples were shaken for 30 minutes (Rowell, 1994). Following centrifugation (2200 g) for 30 minutes and filtration (< 0.22 μm), the supernatant solutions were diluted 1-in-10 with 2% nitric acid before analysis by ICPMS.

2.3.1.7 Total carbon, nitrogen and sulphur

Approximately 15 mg of oven dried and ball milled soil samples were weighed into a silver capsule with 5 mg of vanadium pentoxide (V_2O_5). Total CNS content of each soil sample was measured by using CNS analyser.

2.3.2 Soil physical properties

2.3.2.1 Soil moisture

Soil moisture was determined gravimetrically and calculated from weight loss after oven drying the samples at 105ºC until constant weight and then expressed on a dry weight basis.

2.3.2.2 Soil bulk density

Soil was sampled in metal tins of known diameter and height on the final sampling date. Three samples were collected from every pot in order to obtain a homogenized sample. Soil was trimmed with a flat bladed knife to remove excess soil for the top, bottom and sides of the tins. After weighing the compact soil sample in the tin, it was oven dried at 105°C until a constant weight was achieved. Once dried, soil water content, soil volume and bulk density was calculated as follows:

$$\textbf{Soil water content}\ (g\ g^{-1}) = \frac{\text{(weight of moist soil - weight of oven dry soil)}}{\text{weight of oven dry soil}}$$

$$\textbf{Soil bulk density}\ (g\ cm^{-3}) = \frac{\text{oven dry weight of soil}}{\text{volume of soil/container}}$$

2.3.2.3 Water filled pore space (WFPS)

Oven dried soil samples were re-wetted through capillary action after noting their oven dry weights (Haney, Brinton and Evans, 2008). Soil samples with metal tins were placed in beakers with perforated cling film in the bottom of the beaker to prevent soil loss. The disposable plastic beaker was filled with water. The wetted soils were weighed after the water

reached the surface (i.e. soil appeared moist at the surface). Water filled pore space were calculated by the following equation (Haney and Haney, 2010):

$$\text{Soil water content (g g}^{-1}\text{)} = \frac{\text{Weight of moist soil} - \text{Weight of oven-dried soil}}{\text{Weight of oven-dried soil}}$$

$$\text{Soil bulk density (g cm}^{-3}\text{)} = \text{Oven-dried weight of soil / Volume of soil}$$

$$\text{Soil porosity (\%)} = \text{Soil bulk density / 2.65}$$

$$\text{Volumetric water content (g cm}^{-3}\text{)} = \text{Soil water content} \times \text{Bulk density}$$

$$\text{WFPS (\%)} = \text{Volumetric water content} \times 100 / \text{Soil porosity}$$

2.3.3 Soil microbial and biochemical properties

The following soil microbial and biochemical properties were measured:

2.3.3.1 Microbial biomass carbon and nitrogen

Fresh soil samples were used for the estimation of microbial biomass carbon and nitrogen by the chloroform fumigation-extraction technique described by Vance *et al*., (1987). Fifteen grams of fresh soil samples were incubated in a desiccator with chloroform and soda lime for 24 hours. These fumigated samples and unfumigated control samples were extracted in 60 mL of 0.5 M K_2SO_4.

Microbial biomass carbon and nitrogen in the extracts was analysed using a Shimadzu CN analyser (TOC-V CPH Shimadzu). The results were corrected using the value of 0.45 for carbon and 0.54 for nitrogen. Microbial biomass carbon was then determined as follows.

Microbial biomass carbon is then determined as:

$$C_M = \left\{\left(\frac{TOC_F}{W_{soil\,F}}\right) - \left(\frac{TOC_B}{W_{soil\,B}}\right)\right\} \times \left(\frac{V_{ext}}{k_{EC}}\right)$$

Where

C_M = Microbial biomass carbon (mg C kg^{-1} soil)

TOC_F = TOC measured in the fumigated soil extract (µg mL^{-1}).

TOC_B = TOC measured in the blank soil extract (µg mL^{-1}).

V_{ext} = Volume (mL) of the K_2SO_4 extract (50 mL)

W_{soil} = Dry weight equivalent of soil (g), F or B

k_{EC} = a coefficient to convert 'chloroform-labile' carbon to microbial biomass carbon, the value of 0.45 is commonly accepted (Jenkinson *et al*., 2004). The value of 0.54 was used as the equivalent k_{EN}.

2.3.3.2 Dehydrogenase activity

Fresh soil samples (5 g) were suspended in 5 mL of 1% solution of 2, 3, 5-triphenyltetrazolium chloride (TTC) solution and incubated for 16h at 25°C. The triphenyl formazan (TPF) produced was extracted with acetone (25 mL) after vigorous shaking for 2 hours in the dark.

After filtration in a darkened room, the intensity of TPF (expressed as µg TPF.g^{-1} dm 16 h^{-1}) was measured photometrically at 546 nm in comparison with calibration standards (modified method by Thalmann, 1968).

$$\text{µg TPF.g}^{-1}\text{ dm 16h}^{-1} = \frac{(S-C)*100}{5 \text{ x \%dm}}$$

Where S was mean sample value (µg TPF); C for control sample value (µg TPF);
5 was initial soil weight in grams; 100. $\%^{-1}$ dm was a factor used to calculate dehydrogenase activity for dry matter.

2.3.3.3 β- Glucosidase activity

The β-glucosidase activity was measured by following the modified method of Hoffman and Dedeken (1965). Approximately 5 g of fresh soil sample was incubated at 37°C with 20 mL of acetate buffer (2M) and 10 mL of β-glucosido-saligenin (salicin) as the substrate (35 mM) for 3 h. Saligenin was released from the substrate and formed a blue indophenol dye at pH 9 2,6-dibromchinone-4-chlorimide (Schinner *et al.*, 2012). The colour extinction was determined colorimetrically at 578 nm in comparison with phenol standards of known concentrations.

β-glucosidase activity was expressed as the amount of saligenin released per gram of dry matter during the incubation time.

$$\mu g \text{ saligenin } g^{-1} \text{ dm } 3\ h^{-1} = \frac{(S - C).30.40.100}{3.5.\ \%\ dm}$$

Where, S stands for mean sample value expressed in µg saligenin; C for mean control value expressed in µg saligenin; 30 was the volume of incubation filtrate (mL); 40 was the factor for dilution of the filtrate; 3 is the amount of filtrate (mL); 5 was initial soil weight (g) and 100. $\%^{-1}$ dm is factor for soil dry matter.

2.3.3.4 Phosphatase activity

After the addition of a buffered p-nitrophenyl phosphate solution, soil samples were incubated for 1 h at 37°C. The p-nitrophenol released by phosphomonoesterase activity was

extracted and coloured with sodium hydroxide and determined photometrically at 400 nm. The concentration of pNP in samples and control was calculated from the calibration curve. This was a slightly modified method of Tabatabai and Bremner (1969) and Eivazi and Tabatabai (1977).

Phosphomonoesterase activity was expressed as µg p-nitrophenol (pNP) per gram dry matter and incubation time.

$$\mu g\ NP.g^{-1}\ dm.\ h^{-1} = \frac{(S - C).10.100}{\%dm}$$

Where, S was sample mean value (µg pNP); C was control mean value (µg pNP); 10 was the factor for dilution of extract and 100. $\%^{-1}$ dm was factor for soil dry matter.

2.4 Plant Measurements

Plants were destructively harvested at the end of the experiment to obtain plant physiological and nutrient concentration data in order to identify differences between treatments. Above-ground plant biomass was cut as close as possible to the soil surface using stainless steel scissors and loosely stored in paper bags. Following the harvest, roots were separated from the soil and cleaned of all substrate by physical shaking and washing with three successive rinses of water.

Care was taken in recovering all root tissue by draining all wash water, between rinses, through a 500 µm sieve.

Stem tissue comprised the main trunk of the plant. Primary branches included all woody tissue branching directly from the stem. Secondary branch tissue was denoted as all branches other than primary branch tissue. Plant roots, shoots and leaves were dried in a forced air

ventilated oven at 40°C for 72 to 96 h. Dry weights were measured when drying was complete. Following drying, samples were cut into 1– 2 cm pieces and ground using a mill. Ground samples were stored in the dark at room temperature.

2.4.1 Plant physiological parameters

Shoot and root biomass of strawberry plants were measured by weighing them after oven drying at 80 °C for 2-3 days until a constant weight was reached. The number of stems and leaves were counted immediately after harvest.

2.4.2 Total carbon and nitrogen content of strawberry plants

All plant material (root and shoot) was analyzed for total carbon (C) and total N concentration using a CN analyzer. Accurate sample dry weights were noted for each sample to back-calculate total N and C contents.

2.4.3 Total nutrient content of strawberry roots and shoots

Approximately 200 mg of finely ground plant material was weighed into a digestion vessel with 6.0 mL of concentrated HNO_3 and placed into a microwave rotor for digestion. Digested samples were diluted to 20 mL with ultrapure water and stored at room temperature. Immediately prior to analysis, samples were further diluted (1in10) with ultrapure water.

Total nutrient concentrations were measured by ICPMS analysis. All concentrations were converted to mg kg^{-1} on oven dry weight basis.

2.4.4 Leaf conductance

Stomatal conductance was measured using a leaf porometer, through which the rate of exchange of carbon dioxide (CO_2) or water vapour was quantified in between leaf stomata and air. The leaf porometer expressed the stomatal conductance by putting the conductance of the leaf placed on the sensor in series with two known conductance elements. After determining the humidity difference across one of the known conductance elements, the water vapour flux is known, which expresses the conductance of the leaf.

2.4.5 Leaf temperature

Leaf temperature I_G was calculated as follows:

$$I_G = (T_{dry} - T_{leaf})/(T_{leaf} - T_{wet})$$

Where T_{leaf} is the measured leaf temperature, T_{dry} and T_{wet} are temperatures of dry and wet filter papers under the same conditions. I_G is theoretically proportional to stomatal conductance (Jones 1999; Grant *et al*., 2010).

2.5 Greenhouse Gas Estimation from Soil

Gas collection for flux estimation was performed using the closed chamber technique (Sjögersten *et al*., 2011) with specially built PVC chambers. Plastic chambers had an inner diameter of 6.5 cm and 16.2 cm height with a total volume of 1 L. Each chamber had a sampling port equipped with rubber septa (Fisherbrand, Loughborough, UK). The soil surface was cleared on each pot before the installation of the chamber. Once installed and prior to the collection of gas samples, the chamber headspace was homogenised by repeatedly pumping the air within the chamber with a 20 mL syringe.

Gas samples were collected from each chamber after 0, 20, and 40 and 60 minutes using a 20 mL syringe equipped with a thin needle. Gas samples were injected into pre-evacuated 12 mL borosilicate glass vials sealed with a screw cap-septum (Exetainer; LABCO, UK), leaving each vial with overpressure.

On the day of analysis, gas samples were taken with a syringe inserted into the vials and were analysed for concentrations of CO_2 and CH_4 using a gas chromatograph equipped with a thermal conductivity detector (TCD), flame ionization detector (FID) and an electron capture detector (ECD) (GC-2014, Shimadzu). Nitrogen was used as the carrier gas. The fluxes of these samples were calculated using linear regression of the gas concentration against sample time. The GHG data were converted to mass per volume and mass per weight basis by the use of the ideal gas equation and the molecular mass of each gas (Denef *et al*., 2007).

$$n = \frac{PV}{RT}$$

Where n = number of moles of CO_2 or CH_4,

P is atmospheric pressure (≈1 atm),

V is the volume of head space (dm^{-3}),

R is the ideal gas constant (0.08205746 L atm K^{-1} mol^{-1}) and

T is the temperature of sampling (273.15 + room temperature in ^{o}C).

$$E = \frac{nm}{at} \times 1000$$

Where E= flux of each gas in mg m^{-2} hr^{-1},

n = number of moles of CO_2 or CH_4,

m = molar weight of CO_2 (44.01) and CH_4 (16.04),

a = area of the soil core used and t is the time in hour. The gas flux was also expressed on a per mass basis of soil.

2.6 Plant Photosynthesis and Ecosystem CO_2 flux

Ecosystem CO_2 fluxes were measured with a custom-built plastic bell chamber (30 cm diameter, 12 L volume) placed directly on the plant pot rim and attached to an EGM-4 Infra Red Gas Analyzer (IRGA) (PP Systems, Hitchin, UK). The IRGA was an open dynamic system with an air-flow of 14 L min^{-1} with specific CO_2 pressure and concentration. Data were collected for CO_2 concentration every 8 seconds and 30 seconds between reference air samples and chamber air with an average of one minute.

Net ecosystem exchange (NEE) and ecosystem respiration (R_e) were measured for each pot. The system was placed on a pot for 5 minutes to allow equilibration and left for a further 25 minutes to collect data. A hood was placed on the chamber to exclude light (and photosynthesis). The measurements during equilibration were excluded and mean values calculated from measurement periods for NEE and Re.

Gross ecosystem photosynthesis (GEP) was estimated by subtracting the mean pot R_e by the mean pot NEE fluxes for each measurement period. Positive flux values indicate a release of CO_2 to the atmosphere while negative CO_2 flux values indicate an uptake on CO_2. To assess the effects of biochar over a period of time, three separate measurements were undertaken 5 weeks apart over a 1-week period. Each replicate block was measured during one day so possible changes over the week could be accounted for.

2.7 FTIR Spectroscopy

To identify the dynamics of soil organic carbon resulting from biochar amendment and plant growth, Fourier-transform infrared spectroscopy (FTIR) was performed on soil/substrate samples with 4 cm^{-1} resolution measuring the absorbance from 4000 to 500 cm^{-1} with 120 scans per sample (Tensor 27 SN 1683; Bruker, Austria). Dried and ground samples were placed directly on a crystal and a flat tip powder press was used to achieve even distribution and contact.

A correction was made to the spectra for the attenuated total reflection to allow for differences in depth of beam penetration at different wavelengths in order to obtain composite spectral bands. All spectra were also corrected for attenuation by water vapour and CO_2 by considering surrounding air as a background at ambient temperature (23 ± 1 °C). Minor differences in the amplitude and baseline between runs were corrected by normalisation of the data by subtraction of minimum value followed by division by the average of all data points per sample.

In addition to experimental substrates from the experiment, biochar samples were sieved into different size fractions and their available nutrient profile obtained (discussed in Chapter 2) and also analysed by FTIR to obtain spectra for each size class. The biochar fractions were: 1mm; 2 mm; 53 µm; 106 µm; 212 µm; 300 µm; 425 µm; 500 µm and 710 µm.

2.8 Statistical Analyses

All the collected data were analysed by statistical software Genstat (17th Edition, VSN International Ltd, UK) using biochar, plant and time as factors. For most of the soil parameters which were estimated at four different time intervals during the experiment, analysis was by repeated measures analysis of variance (ANOVA). The treatment means

were compared for significance at the $P < 0.05$ level using the Fisher's Least Significant Difference (LSD). The remaining parameters, mostly plant related, were analysed by general ANOVA using biochar amendment and plant (±) as the main factors and means were compared using a Tukey test for significance if a one-way ANOVA was conducted or by LSD if a two-way ANOVA was performed. Standard errors of means were calculated and presented with respective data in figures.

F statistics and P values were presented to show the significant and non-significant effects of biochar, plant, time and their interactions. In the planted category, five biochar levels (control, 2.5, 5, 10 and 15 %), were compared for four sampling times (30, 90, 180 and 370 days after establishing the experiment). In the unplanted category, only three biochar levels were compared over sampling times. In the comparison category, three levels of biochar (control, 10 and 15 %) were chosen to compare the effects of presence and absence of strawberry plants and identify the interaction between plant, biochar and time.

For FTIR, all statistical analyses were performed using Genstat (Version15). Linear Mixed Model (REML) was used to compare intensities of aliphatics, aromatics and carbohydrates observed at different wave numbers among different biochar levels. Per cent biochar amendment and plant (±) were included as fixed factors and blocking was included as a random factor during analysis.

Chapter 3 Biochar Characterization

3.1 Overview

This chapter is about the characterization of the biochar used in this study. The biochar was generated by O-Gen UK Ltd. as a by-product of gasification of demolition wood, a process used for power generation from waste material. The basic properties of biochar used in the study was presented and discussed with the help of information gathered from previous work published all over the world. In the end comparison of O-GEN biochar was made with EBC, BQM and IBI standards to predict the exact potential use of O-GEN in future.

3.2 Introduction

Biochar is a carbon-rich (70–80 %), black solid fine grained substance produced from partial thermal decomposition of wastes in low or the complete absence of oxygen (Lin *et al*., 2012; Jiang *et al*., 2021; Liao *et al*., 2022). Biochar is eco-friendly and cost effective material, when compared with other carbonaceous materials (activated carbon, grapheme). It can be produced from range of products, easily available, cheap biomass feedstock and from different industrial wastes like agriculture, forestry and dairy (Sevilla and Fuertes, 2011; Norah *et al*., 2015; Ahmed *et al*., 2020; Banik *et al*., 2021). Biochar can be produced as a chemical by-product with less energy expenditure in production of high energy hydrocarbons and bio oil (Chandler and Resende, 2019; Wang *et al*., 2018; Zheng *et al*., 2020; Khan *et al*., 2020).

The biochar used in study, was supplied by O-Gen Ltd, Stoke-on-Trent, UK. The biochar was formed by pyrolysis at 1100^{o}C under nitrogen and the feedstock was wood originating from

demolition sites. Wood-based biochar was selected because it is the most widely investigated biochar material (Dias *et al*., 2010; Calvelo Pereira *et al*., 2011; Jindo *et al*., 2012a, 2012b; Amoakwah *et al*., 2022). The potential for using the biochar as a soil improver is the focus of this PhD and is relevant to the recent interest in using biochar as a means of sequestering carbon. It is generally accepted that for field application, biochar should meet the standards suggested by the International Biochar Initiative or the European Biochar Certification Scheme, but to date this obligation is not a legal one.

3.3 Aims and Objectives/Hypothesis

The aims of the study were:

1. To characterize biochar produced from demolished wood by determining basic properties, particle size analysis and elemental analysis
2. Comparison of O-gen biochar with international biochar standards (IBI, EBC and BQM) in order to determine application of such char to soil

We hypothesized that biochar generated from demolished wood is safe for application to plant, soil and ecosystem.

3.4 Materials and Methods

The pH, moisture content, ash content (LOI), C, N and extractable and total elements were determined as described in Chapter 2. Specific to the biochar analysis is particle size distribution.

3.4.1 Particle Size Distribution

The biochar was a combination of different size fractions (Table 3.4) and the relative proportions of each fraction were determined through physical sieving. A 100 g biochar sample was taken and after removing nails and other contaminants, and sieved through a series of sieves; <8 mm, 4 mm, 2 mm, 1 mm, 0.7 mm, 0.5 mm, 425 μm, 500 μm, 300 μm, 212 μm, 106 μm. The sample collected in each sized sieve was weighed and the percentage of particular size fraction present in the total biochar sample (100 g) was calculated on w/w basis.

3.5 Results

3.5.1 Properties of the wood biochar

The data for selected biochar properties are presented in Table 3.1. The pH value of O-Gen biochar was pH 8.5, typical of high temperature pyrolysis biochar with an organic matter content of 52.5%. Total carbon, nitrogen and sulphur content were 58.24%, 2.21% and 7.89%. Moisture content was 11.4%. Due to the high carbon and low nitrogen percentage, C:N ratio was calculated as 26.35.

Table 3.1 Basic properties of wood biochar

Characteristics	Biochar
pH	8.5
Moisture content (% of dry weight)	11.4
Organic matter (% of dry weight)	52.5
Total Nitrogen (%)	2.21
Total carbon (%)	58.24
Total Sulphur (%)	7.89
C:N ratio	26.35

3.5.2 Particle Size Distribution

The biochar, mainly comprised of particle size fractions ranging from 1-8 mm (18.9 %, 4-8 mm, 17.3 %, 2-4 mm, 14.3 %, 1-2 mm) followed by 10.3 % of 300 µm-425 µm sized particles.

Table 3.2 Particle size distribution of O-Gen Biochar

Biochar Size Fraction	%
<8mm	4.9
4-8mm	18.9
2-4mm	17.3
1-2mm	14.3
0.71-1mm	6.7
0.5-0.71mm	5.8
425µm-500µm	5.6
300µm-425µm	10.3
212µm-300µm	6.4
106µm-212µm	6.2
53µm-106µm	3.7

3.5.3 Elemental analysis

Among the extracted elements with ammonium nitrate from different fractions of biochar, major cations were presented here (Figure 3.1) due to their importance in the soil-plant system. Ca and K were extracted in the highest concentrations, while Mg was the lowest. Smaller sized fractions had higher contents of exchangeable cations than larger sized biochar fractions on an equivalent weight basis.

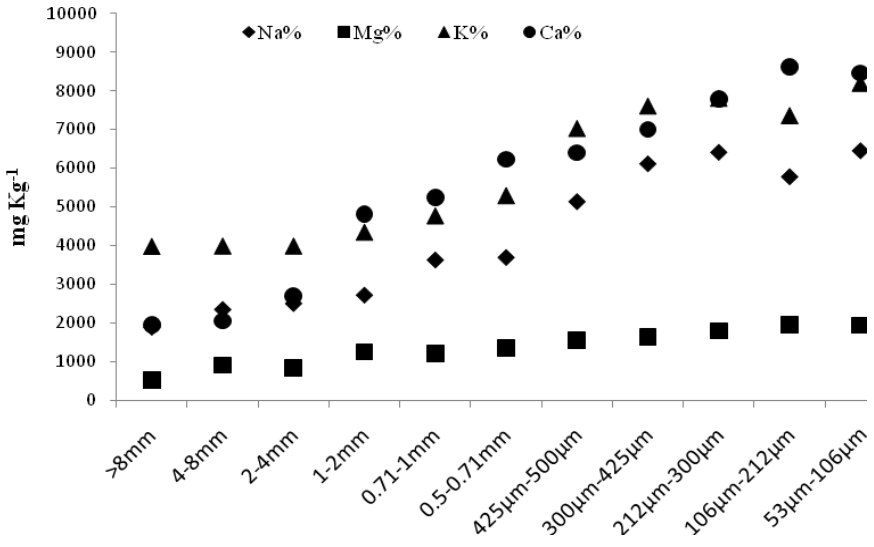


Figure 3.1 Extractable nutrient percentages of different biochar size fractions

The total contents of nutrients and trace metals (after acid digestion) are shown in Figures (3.2, 3.3 and 3.4). The nutrients (Ca, K, Na and Mg) were present in the highest concentrations whilst the trace metals, including Cu, Zn, Al, Fe, Ba, Mo and Mn, were relatively low. Concentrations of toxic metals in biochars, including Pb, Cd, Co, Cr, Sr, Cs, and Ni, were also noted.

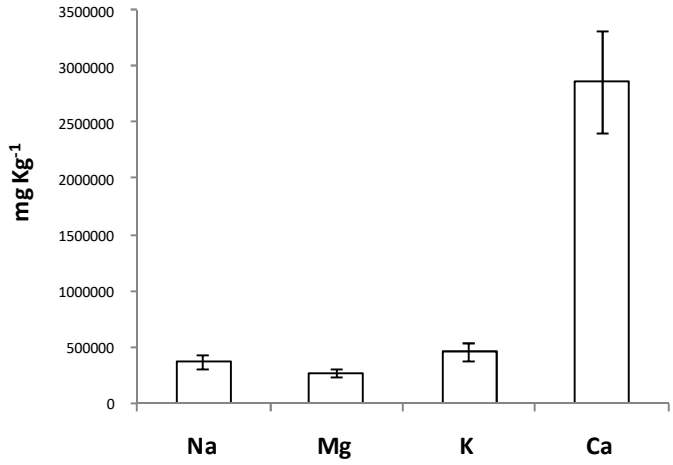


Figure 3.2 Acid digested macronutrients profile of O-Gen Biochar

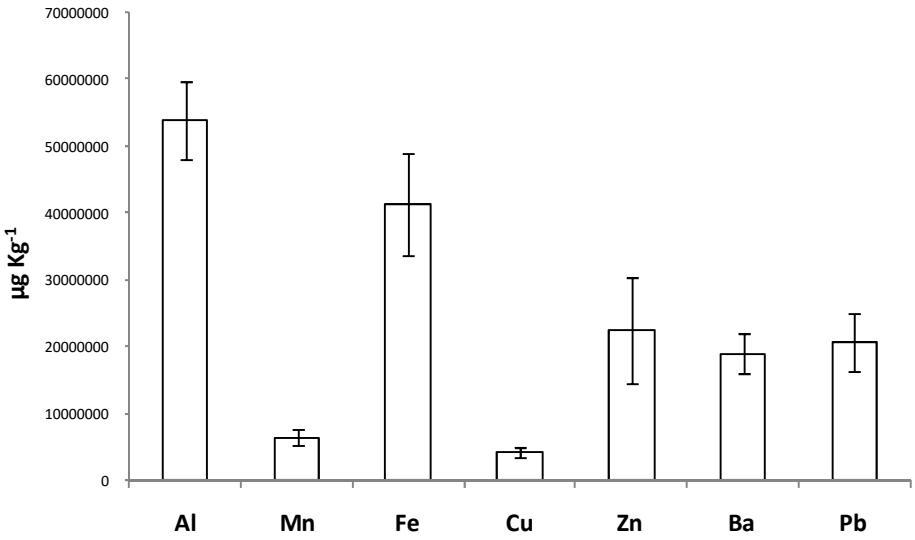
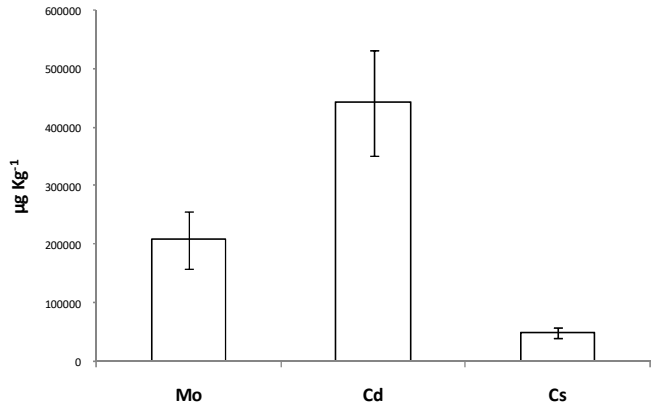


Figure 3.3 Acid digested micronutrients profile of O-Gen Biochar

3.6 Biochar standards and Certification

Biochar certification and standards were established mainly for research purposes to create uniformity, so that comparisons can be made across different biochars and laboratories.



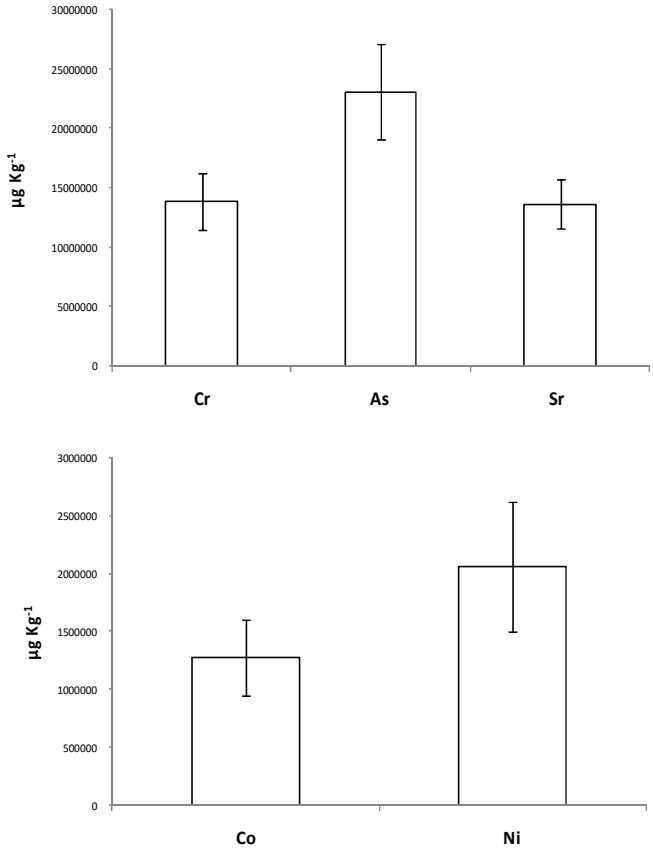


Figure 3.4 Acid digested micronutrients profile of O-Gen Biochar

Biochar certification programs have been established by the following agencies:

- IBI International Biochar Initiative (2012)
- EBC European Biochar Certificate (2012)
- BQM Biochar Quality Mandate v 1.0 – intended for UK implementation draws on both IBI and EBC

Table 3.3 EBC, BQM and IBI standards for biochar

Characteristics	EBC	BQM	IBI
Total carbon (%)	>50%	>10%	>60% - >10%
Pb µg kg^{-1}	150(120)	500(60)	121-300
Cd µg kg^{-1}	1.5(1)	39(3)	1.4-3.9
Cu µg kg^{-1}	100	1500(40)	143-6000
Ni µg kg^{-1}	50(30)	600(10)	47-420
Hg µg kg^{-1}	1	17(1)	1-17
Zn µg kg^{-1}	400	2800(150)	416-7400
Cr µg kg^{-1}	90(80)	100(15)	93-1200
As µg kg^{-1}	13	100(10)	13-100
Se µg kg^{-1}	--	100(5)	2-200
Co µg kg^{-1}	--	--	34-100
Mo µg kg^{-1}	--	75(10)	5-75
Mn µg kg^{-1}	--	3500	--

3.7 Discussion

The efficiency and success of biochar as a soil amendment is highly dependent on its characteristics, pyrolysis temperature and feedstock. The biochar, used in this study originated from demolition wood and is predominantly composed of all typical plant characters like high organic C with macro- and micro-nutrients in addition to toxic elements and contaminants retained from the starting feedstock. The C content of biochar is particularly in an aromatic form after high temperature pyrolysis, which is resistant to degradation and decay when added in soil (Amonette and Joseph, 2009).

Moisture content of the biochar was 11.4 % and is possibly a reflection of the storage method prior to collection. It was reported in the literature that biochar can hold over 2.0-2.7 times its mass of water (almost 200-300%) (Yu *et al*., 2013). Biochar addition in soil increases water holding capacity of soil (Chan *et al*., 2007; Laird *et al*., 2010; Basso, 2012), because of its

unique pores, variation in particle size, high surface area and mulching effect (Novak *et al*., 2009; Zolue, 2013).

Like most of the biochars, O-Gen biochar has alkaline pH, which helps in creating more favourable soil habitat for microbes and plant by reducing acidity in rhizosphere (Spokas *et al*., 2012; Butnan *et al*., 2015). Biochar has a distinct capacity to adsorb cations and anions from solutions, polar and non-polar organic compounds (Zheng *et al*., 2020). Biochar, when applied as a soil amendment, behaves as a liming agent resulting in increased pH and nutrient availability for different soil types (Glaser *et al*. 2002; Lehmann and Rondon 2006).

Biochars in comparison to their original feedstock have higher surface area, oxygen containing functional groups; pore surface, capacity to retain cations (Singh *et al*., 2010; Clough *et al*., 2013) and these properties can be controlled by regulating the pyrolysis conditions and time of biochar exposure. High temperature biochar has a larger surface area and high pH as compared to biochar produced at low temperature (Cantrell *et al*., 2012).

The elemental composition of O-Gen biochar exhibits the same typical behaviour of plant biochar. It has high carbon content with low nitrogen and sulphur, similar was reported in literature for biochar derived from corn, switch grass and rice straw (Brewer *et al*., 2011; Lehmann *et al*., 2011; Ghani *et al*., 2013; Al-Wabel *et al*., 2013). Carbon, hydrogen and oxygen were the primary components of biochar but the elemental composition of biochar has changed from its original feedstock due to operating conditions and pyrolysis temperature (Calvelo Pereira *et al*., 2011). High temperature pyrolysis produced biochar with high carbon content but at the same time, it has loss of hydrogen due to weak bonds cleavage in biochar (Zhang *et al*., 2013).

It is clear from comparison of the three published standards that there is some variability in acceptable limits of potentially toxic elements (PTEs) within biochar. The BQM limits for PTEs are the most relaxed (but not always) and this may reflect the inclusion of waste materials within their remit rather than just virgin biomass. Demolition wood is a waste product and in order to use the resulting biochar as a soil improver it would need to be allocated end-of-waste status. Given the high concentrations of PTEs present, this biochar is unsuitable for use in agricultural or horticultural settings. Nevertheless, it may still be useful for application to contaminated sites to enable revegetation and rehabilitation.

It should be noted that the suggested biochar standards had not been developed/published at the time this PhD was established. It is nevertheless important to understand the effects of applying biochar derived from waste material to land since the published standards are not a legal requirement and any unintended consequences of such application should be quantified

3.8 Conclusion

Biochar is a very heterogeneous material with lots of variations in production method and materials from which it was generated. These variations results in variability of properties and behaviour of biochar when exposed to soil and environment. Some properties are absolute like structural stability high carbon and nutrient content which induce changes in soil and plant system when biochar was exposed for long times. In our study, biochar used was generated from demolished wood, which was highly contaminated and had high content of PTEs.On comparison with the biochar standards and safe limits for biochar application to soil, it was not recommended for agricultural purposes. However we can use our biochar for the restoration of forest land, coal mines or low carbon soils.

Chapter 4 Effects of wood biochar application on Strawberry Biomass production, nutrient concentration and ecosystem gas exchange

4.1 Overview

The data presented and discussed in this chapter was obtained from the growth room experiment where strawberry plants were grown for 370 days under controlled conditions. In this study, we aimed to evaluate the performance of biochar produced at high temperature (1100 °C) on strawberry plant growth and ecosystem gas exchange. Soil was amended with biochar at different rates (Control, 2.5, 5, 10 and 15 % biochar on w/w basis). Three treatments were created with biochar (Control, 10 and 15 %) without strawberry plants to compare the changes in (soil only and soil plus biochar) properties due to absence or presence of plant roots. Strawberry growth parameters, leaf conductance, leaf temperature, total nutrient profile of strawberry root and shoot were determined at harvest.

4.2 Introduction

Biochar , a solid and carbon-rich element, can enhance plant productivity and growth due to changes in soil biogeochemistry that might be attributed to the increase in soil fertility due to immobilization of metals in soil and stable metal-organic complexes formation (Lehmann *et al*., 2003; Chan *et al*., 2007; Van Zwieten *et al*., 2007; Glaser *et al*., 2015; Xu *et al*., 2016; Gonzaga *et al*., 2018; Kumar *et al*., 2018; Ahmad *et al*., 2018; Werner *et al*., 2018; Cornelissen *et al*., 2018; Chiomento *et al*., 2021). The mechanism behind this fertility increase can be improved soil water retention due to high pore volume of biochar (Bruun *et*

al., 2014; ; Koide *et al*., 2015; Melorose *et al*., 2015;Chacon *et al*., 2020), improved soil texture and structure (Atkinson *et al*., 2010; Obia *et al*., 2016; Obia *et al*., 2017), improved nutrient retention (Laird *et al*., 2010; ; Biederman and Harpole, 2013; Hale *et al*., 2013; Martinsen *et al*., 2014; Chacon *et al*., 2020), enhanced crop performances due to high carbon stock (Herath *et al*., 2013; Doan *et al*., 2015; Agegnehu *et al*., 2016; Mehmood *et al*., 2017; Dubey *et al*., 2020) or combinations of these mechanisms.

Effects of biochar application on plant growth, nutrient uptake, soil nutrient availability and biological activities as a result of specific interactions of chars and plants have been reported in various studies (Zhang *et al*., 2020; Increased crop growth (Reynolds *et al*., 2003; Marris, 2006) and yield of various crops such as cowpea (Yamato *et al*., 2006), soybean (Tagoe *et al*., 2008), maize (Yamato *et al*., 2006; Rodríguez *et al*., 2009), and rice (Haefele, 2007; Haefele *et al*., 2008; Asai *et al*., 2009) has been reported following biochar application. The authors argued that increasing levels of biochar were related to increased soil capillary water-holding capacity and nutrient supply, significantly correlating with seed germination rate and shoot dry weight.

The application of cherry wood biochar (@2 and 3%) promoted seed germination significantly by 28–30% as compared to the control. Bu *et al*., (2020) reported an increase in seed germination rate (*Robinia pseudoacacia* L.) by using rice husk and woodchip biochars. Similar findings confirming an enhanced germination rate of castor seeds were reported by Hilioti *et al*., (2017) after addition of 1 and 5% castor stalk biochar. Strawberries are high-value horticultural species, cultivated worldwide, in open agricultural land, high tunnels, greenhouses, and in all continents due to their tasty and healthy fruits (Nestby and Retamales, 2020; Chiomento *et al*., 2021). Strawberries are important in the human diet because of their

attractive and tasty fruit have antioxidant and anti-inflammatory potential, and anthocyanins (Smeriglio *et al*., 2016; Duarte *et al*., 2018).

4.3 Aims and Objectives/Hypothesis

The research questions addressed in this chapter were as follows:

1. Identify the best rate of biochar in terms of plant growth parameters
2. Identify the worst rate of biochar, potentially harmful for the plant growth (due to the release of toxic elements or due to the dissolution of toxic elements up to the levels where it hinders plant growth and instead of any improvement, it causes reduction in plant growth)
3. How biochar and time both influence ecosystem respiration and photosynthesis?

It was hypothesized that biochar is potentially helpful for plant growth and nutrient content and it affects ecosystem respiration and photosynthesis by improving plant growth.

4.4 Material and Methods

4.4.1 Growth parameters

All growth parameters were measured at final harvest. Please refer to the section 2.4.1 (Chapter 2) for detailed procedures and methods, used for the estimation of strawberry growth parameters.

4.4.2 Total carbon and nitrogen content of strawberry plants

All plant material (root and shoot) was analyzed for total carbon (C) and total N concentration using a CN analyzer (Section 2.4.2. Chapter 2).

4.4.3 Total nutrient profile of strawberry root and shoot

Total nutrient concentrations were measured at final harvest by ICPMS analysis. For detailed method refer to section 2.4.3 in chapter 2.

4.4.4 Leaf conductance

Stomatal conductance was measured using a leaf porometer, through which the rate of exchange of carbon dioxide (CO_2) or water vapour was quantified in between leaf stomata and air (Section 2.4.4. Chapter 2)

4.4.5 Leaf temperature

Leaf temperature was measured as temperature difference of dry and wet filter papers under the same conditions (Section 2.4.5. Chapter 2).

4.4.6 Ecosystem gas exchange

Net ecosystem exchange (NEE) and ecosystem respiration (R_e) were measured for each pot. Gross ecosystem photosynthesis (GEP) was estimated by subtracting the mean pot R_e by the mean pot NEE fluxes for each measurement period. To assess the effects of biochar over a period of time, three separate measurements were undertaken 5 weeks apart over a 1-week period (at 7, 13, 18 and 52 weeks) in addition to the first sampling at 7 days. For detailed procedures and methods, used for the estimation of NEE, R_e and GEP, please refer to the Section 2.6 (Chapter 2).

4.4.7 Statistical analysis

The data recorded for plant growth parameters, total nutrient profile, leaf conductance and leaf temperature were analysed by general ANOVA using biochar amendment and plant (±)

as the main factors and means were compared using a Tukey's test for significance. Ecosystem gas exchange data was analyzed by two way ANOVA using biochar, plant and time as factors and treatment means were compared for significance at the $P < 0.05$ level using the Fisher's Least Significant Difference (LSD).

4.5 Results

4.5.1 Strawberry Growth Parameters

The data obtained for number of leaves, number of stems, biomass (aboveground and below ground), root/shoot ratio, total root/shoot nutrient concentration were presented and described here.

4.5.1.1 Biomass

Among all strawberry growth parameters, only root dry weight and total biomass (root + shoot dry weight) were statistically significant. In all other parameters like shoot dry weight, no. of leaves, no of stems, root/shoot ratio, total N in shoot and root, and Total C in root and shoot, biochar failed to produce any significant difference in various biochar levels.

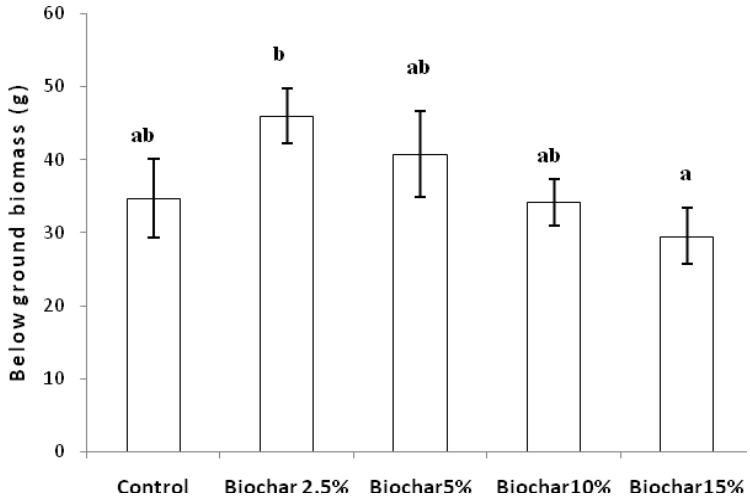


Figure 4.1 Effect of biochar on root biomass (g) in strawberry shoot
(Biochar: $F_{4,16} = 3.70$ P 0.026) .Columns similarly superscripted and not significantly different.

Data regarding the root dry weight showed that biochar treatments improved dry weight as compared with the control (Figure 4.1). The root dry weight enhanced sharply with biochar addition @ 2.5 %, which significantly increased as compared to control. In the next treatments with increasing levels of biochar, there was a decrease reported in root dry weight. The root dry weight reached a maximum at 2.5 % biochar treatment. Treatments with 5 and 10 % biochar decreased as compared to control. The lowest value for root dry weight was observed where biochar was applied at highest rate (15 %).

Data regarding total biomass showed that biochar had produced the same pattern, which was observed in root dry weight (Figure 4.2). All treatments were significantly different for all levels of biochar. Maximum biomass was recorded in 2.5 % biochar treatment, followed by 5, 10 and 15 % biochar. The total biomass increased sharply in first biochar treatment (2.5 %) as compared with the control. With the further increase in biochar, decrease in total biomass was reported. The lowest value was reported in the treatment where biochar was applied at highest rate 15 %.

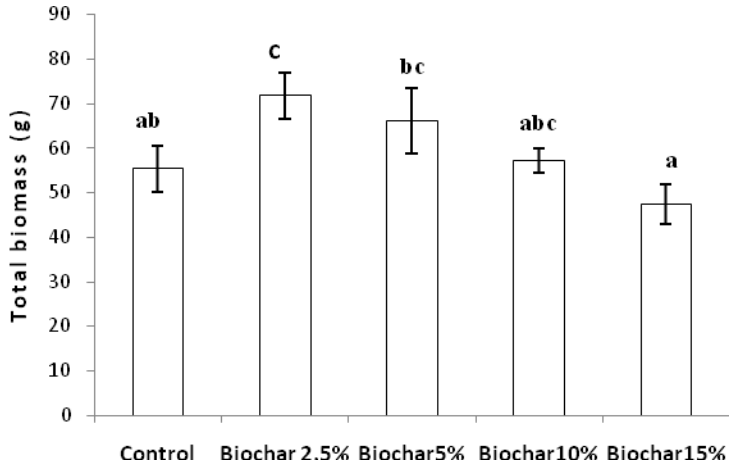


Figure 4.2 Effect of biochar on total biomass (g) in strawberry shoot
(Biochar: $F_{4,16} = 7.85$ $P < 0.001$)

Data presented in Table 4.1 clearly reflected that biochar failed to produce any significant difference in all other growth parameters in this experiment.

Table.4.1 p value for growth parameters of strawberry plants

Characteristic	p value	Grand mean
Total biomass (g)	**<.001**	59.6
Shoot dry weight (g)	0.278	22.6
Root dry weight (g)	**0.026**	37.0
Root/Shoot ratio	0.917	0.662
No. of leaves	0.107	36.0
No. of stems	0.428	12.04
Root N	0.965	1.33
Shoot N	0.070	2.23
Root C	0.512	42.58
Shoot C	0.349	43.78
Root P	0.172	1266
Shoot P	**<.001**	4638
Leaf resistance (gs)	0.755	289
Leaf temperature (IG)	0.184	1.28

4.5.2 Total Nutrient profile of strawberry root and shoot

The total macro and micronutrients present in Strawberry root and shoot are summarized in Table 4.2. The Na, K, Ca and Mg are considered as plant macronutrients while Fe, Al, Ti, V, Cr, Mn, Zn, Cu, Co, Ni, As, Sr, Cs, Ba, Pb and Mo are grouped as plant micronutrients. Effect of biochar was proved statistically significant for shoot Zn, K, P, Cu, As, and Pb. while for the rest of macro and micro nutrients, biochar failed to produce any difference in strawberry root and shoot.

In strawberry shoot, K, P , Zn, Cu, and As content increased with biochar, while Pb content decreased with increasing level of biochar rates as compared to control. Other than these, none of macro and micro nutrients exhibited a clear trend with or without biochar in

strawberry shoot. However in case of strawberry root, all macro and micro nutrients were exhibiting almost same trend having non-significant differences among all treatments where biochar applied at different rates including control.

Zinc content of strawberry shoot (Figure 4.3) significantly varied among different biochar treatments (Biochar: $F_{4, 16} = 3.00$ P 0.051). There was a sharp increase in shoot Zn in the first biochar treatment, where we applied BC @2.5 %. That's why lowest values were reported where no biochar was applied. This trend was followed by treatments with Biochar 10 and 15 % where increase was reported in Zn content in strawberry shoot as compared to Control with no BC. The highest Zn content was found in strawberry shoot grown in soil with 15 % biochar.

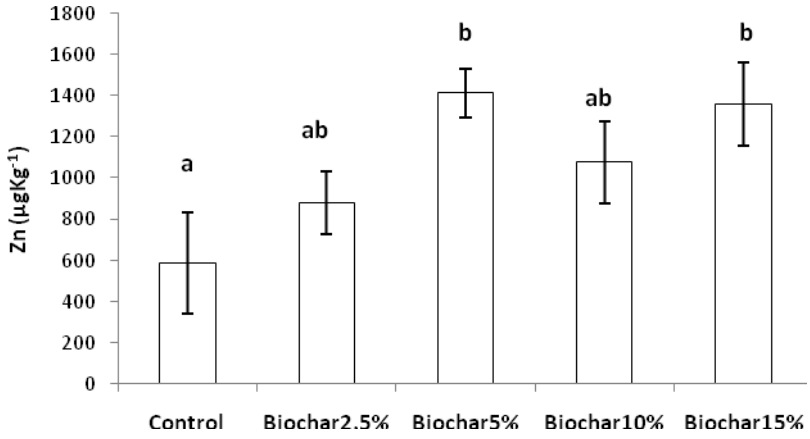


Figure 4.3 Effect of biochar on Zinc (µg kg^{-1}) in strawberry shoot
(Biochar: $F_{4,16} = 3.00$ P 0.051)

The potassium content of strawberry shoot quantified at the end of harvest, (Figure 4.4) significantly varied among different biochar treatments (Biochar: $F_{4,16} = 5.18$ P 0.007). There was a little increase in shoot K recorded in the first biochar treatment, where we applied BC @2.5 % as compared to control. That's why lowest values were reported in

control with no biochar followed by the treatment where biochar was applied @ 10 %. The increase in potassium shoot content was observed in treatments where biochar was applied @ 5 and 15 %. The highest K content was found in strawberry shoot grown in soil with the highest rates of biochar (15%).

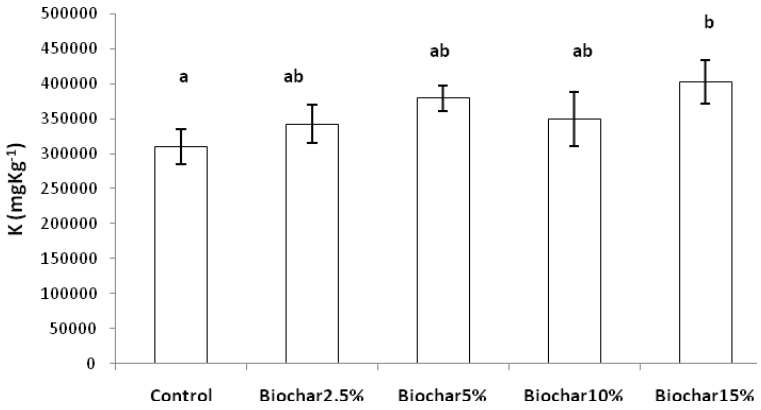


Figure 4.4 Effect of biochar on Potassium (mg kg^{-1}) in strawberry shoot
(Biochar: $F_{4,16} = 5.18$ P 0.007< 0.05)

The Phosphorous content of strawberry shoot, (Figure 4.5) significantly varied among different biochar treatments (Biochar: $F_{4,16} = 5.18$ P 0.007). The lowest values were reported where biochar was applied @ 2.5 %. Almost same P content was reported in control and 5 % BC. The increase in phosphorus shoot content was observed in high biochar treatments. The maximum P was recorded, where biochar was applied @ 10 % followed by the treatment with 15 % BC.

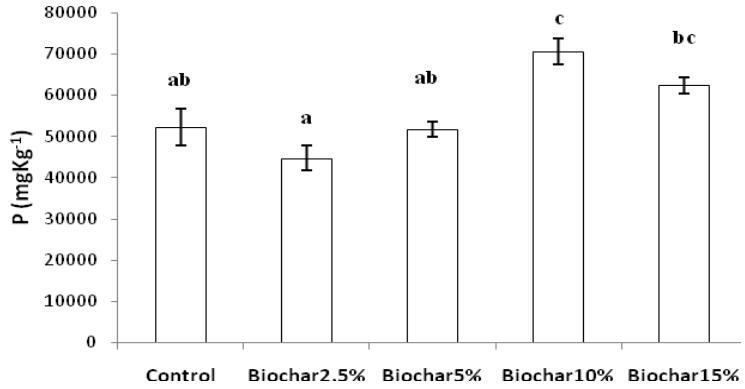


Figure 4.5 Effect of biochar on Phosphorus (mg Kg^{-1}) in strawberry shoot
(Biochar: $F_{4,16} = 12.58$ P<.001)

Cu content of strawberry shoot (Figure 4.6) significantly varied among different biochar treatments (Biochar: $F_{4,16} = 5.88$ P 0.004< 0.05). There was a slight increment in shoot Cu content was recorded in treatments, with increasing rates of biochar. The lowest values were reported in control treatment where biochar was not applied. The trend was followed by treatments with Biochar 2.5 and 10 % where slight increase was reported in Cu content as compared to Control with no BC. The highest Cu content was found in strawberry shoot grown in biochar amended soil with 5 and 15 % biochar.

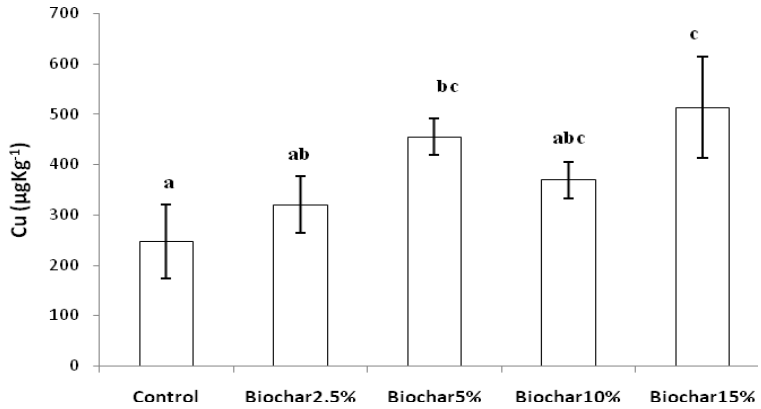


Figure 4.6 Effect of biochar on Copper (µg kg^{-1}) in strawberry shoot
(Biochar: $F_{4,16} = 5.88$ P 0.004< 0.05)

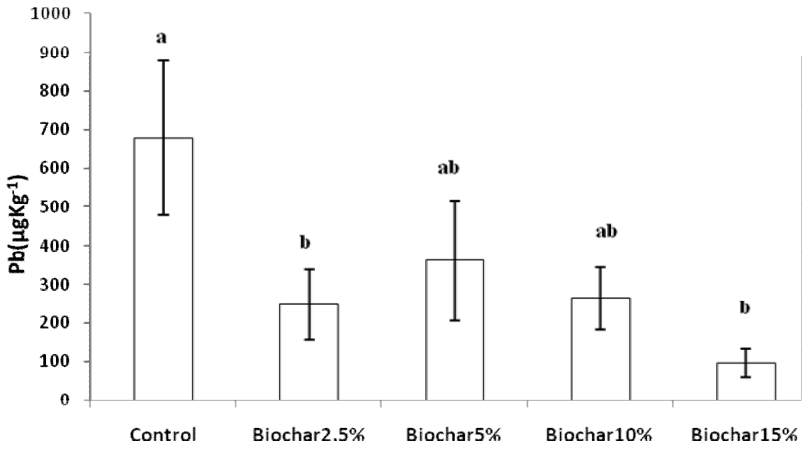


Figure 4.7 Effect of biochar on Lead (µg kg^{-1}) in strawberry shoot
(Biochar: $F_{4,16} = 5.04$ P 0.008< 0.05)

Pb content of strawberry shoot (Figure 4.7) significantly varied among different biochar treatments (Biochar: $F_{4,16} = 5.04$ P 0.008< 0.05). The notable drop in shoot Pb was recorded in the treatment, where we applied BC @2.5%. Slightly high values were recorded in BC 5% and BC 10% as compared to treatment where we applied BC @ 2.5%. The highest Pb content was found in strawberry shoot grown in control soil with no biochar unlike all other micronutrients. The trend was same as lowest value of shoot lead was reported in treatment where highest level of BC (15 %) was applied.

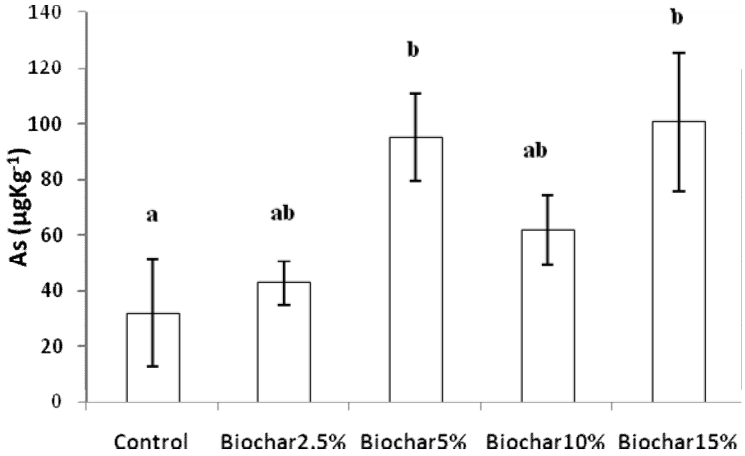


Figure 4.8 Effect of biochar on Arsenic (µg kg^{-1}) in strawberry shoot
(Biochar: $F_{4,16} = 3.82$ P 0.023< 0.05)

The trend noted for shoot As is same as reported for Zn. As content of strawberry shoot (Figure 4.8) significantly varied among different biochar treatments (Biochar: $F_{4,16} = 3.82$ P 0.023< 0.05). There was a increase in shoot As in the BC treatment, where we applied BC @2.5 %. Increase in As shoot content was noted in the following two treatments (BC 5 and 10 %) as compared to BC 2.5 %. The lowest As content was found in strawberry shoot grown in control soil with no biochar, where as the highest As content was recorded in BC 5 and 15 % treatment.

Table.4.2 p value for total nutrient content in strawberry root and shoot

Nutrient	Shoot p value	Grand Mean	Root p value	Grand Mean
Na (mg kg^{-1})	0.113	10474	0.533	13864
Mg (mg kg^{-1})	0.489	74320	0.654	62861
K (mg kg^{-1})	**0.007**	357687	0.117	112714
Ca (mg kg^{-1})	0.437	313641	0.142	383914
Al (µg kg^{-1})	0.067	13990	0.282	53812
Ti (µg kg^{-1})	**0.041**	160	0.421	847
V (µg kg^{-1})	0.856	1.9	0.356	1517
Cr (µg kg^{-1})	0.112	377	0.579	1211
Mn (µg kg^{-1})	0.146	1017	0.151	3164
Fe (µg kg^{-1})	0.118	23063	0.405	111305
Co (µg kg^{-1})	0.077	15.8	0.275	59.3
Ni (µg kg^{-1})	0.296	196	0.508	809
Cu (µg kg^{-1})	**0.004**	384	0.521	2277
Zn (µg kg^{-1})	**0.051**	1051	0.150	6259
As (µg kg^{-1})	**0.023**	67.0	0.860	361
Sr (µg kg^{-1})	0.296	1037	0.834	1371
Mo (µg kg^{-1})	0.240	-35	0.733	158
Cd (µg kg^{-1})	0.074	2.65	0.893	16.9
Cs (µg kg^{-1})	0.068	1.19	0.444	3.37
Ba (µg kg^{-1})	0.190	2178	0.546	4325
Pb (µg kg^{-1})	**0.008**	331	0.880	2400

P values and grand means were presented in Table 4.2 for all macro and micro nutrients contents reported in strawberry root and shoot over a period of one year grown in biochar amended soil. Statistically significant p values for nutrients in strawberry shoot are bold.

4.5.3 Leaf Temperature

Data regarding leaf temperature showed that biochar failed to produce any significant difference in leaf temperature among different biochar treatments (Table 4.1).

4.5.4 Leaf Conductance

Leaf conductance was proved statistically non-significant for all biochar treatments (Table 4.1).

4.5.5 Ecosystem Gas Exchange

Data regarding ecosystem respiration, net ecosystem exchange (NEE) and gross ecosystem exchange (GEE) was presented and described here:

4.5.5.1 Ecosystem Respiration (Re)

Biochar and time both were proved statistically significant in producing differences in ecosystem respiration for all biochar treatments. However, their interaction was failed to have any prominent effect. Data about ecosystem respiration (Re) (Biochar: $F_{4,16} = 26.67$ $P < 0.001$) showed that biochar treatment (5%) produced highest rate over all the treatments including control (Figure 4.9). The rate of respiration declined with the increasing rates of biochar application, with the lowest value reported in BC 15 %.

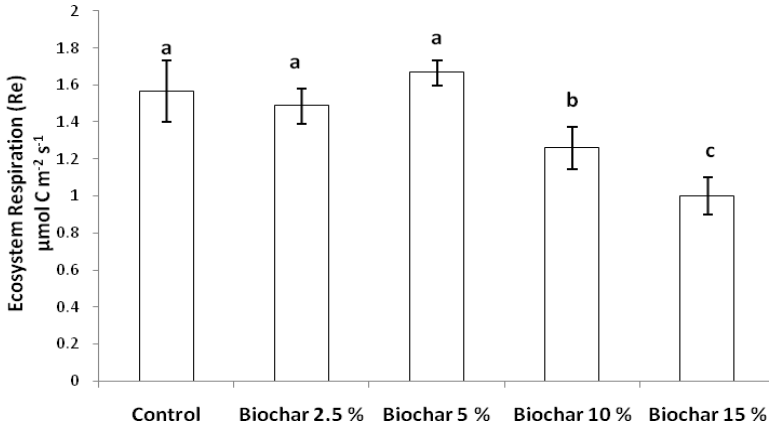


Figure 4.9 Effect of biochar on Ecosystem Respiration (Re) µ mol C m^{-2} s^{-1}
(Biochar: $F_{4,16} = 26.67$ $P < 0.001$)

Data about ecosystem respiration (Re) (Time: $F_{3,60} = 35.08$ $P < 0.001$) showed that time had significant effect and the value recorded after 52 weeks was highest as compared to all other timings (Figure 4.10). The lowest respiration rate was recorded at 7 weeks followed by 13, and 18 weeks.

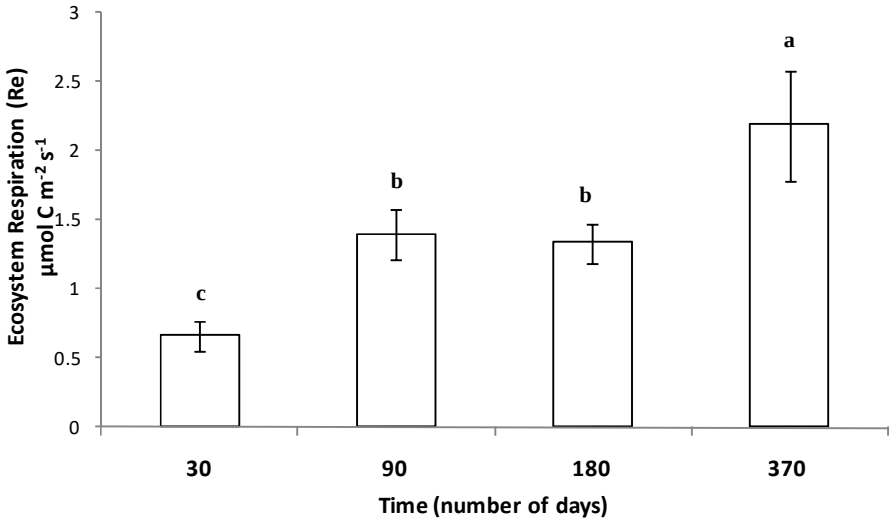


Figure 4.10 Effect of time on Ecosystem Respiration (Re) µ mol C m^{-2} s^{-1}
(Time: $F_{3,60} = 35.08$ $P < 0.001$)

4.5.5.2 Net ecosystem exchange (NEE)

Data about net ecosystem exchange (NEE) (Biochar: $F_{4,16} = 15.78$ $P < 0.001$) showed that control treatment produced highest NEE values over all the treatments (Figure 4.11). The value of NEE declined with the increasing rates of biochar application, with the lowest value reported in BC 15 %.

Data about net ecosystem exchange (NEE) (Time: $F_{3,60} = 39.03$ $P < 0.001$) showed that time had significant effect and the negative value recorded after 52 weeks was highest as compared to all other timings (Figure 4.12). The lowest respiration rate was recorded at 7 weeks followed by 18, and 13 weeks.

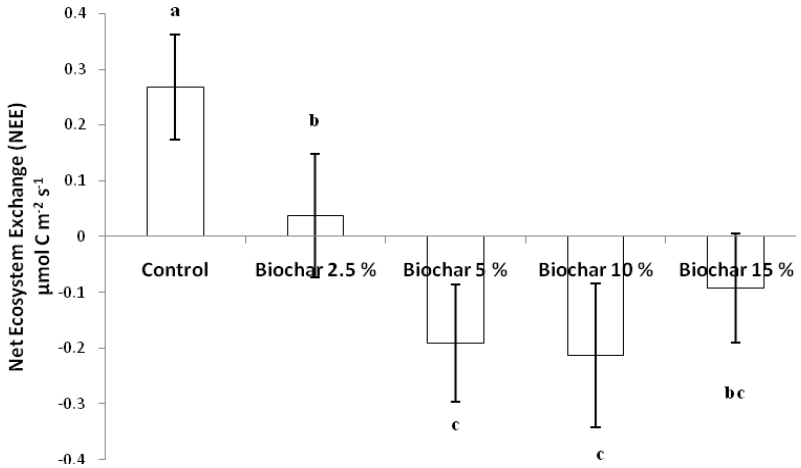


Figure 4.11 Effect of biochar on Net ecosystem exchange (NEE) µ mol C m^{-2} s^{-1}
(Biochar: $F_{4,16} = 15.78$ $P < 0.001$)

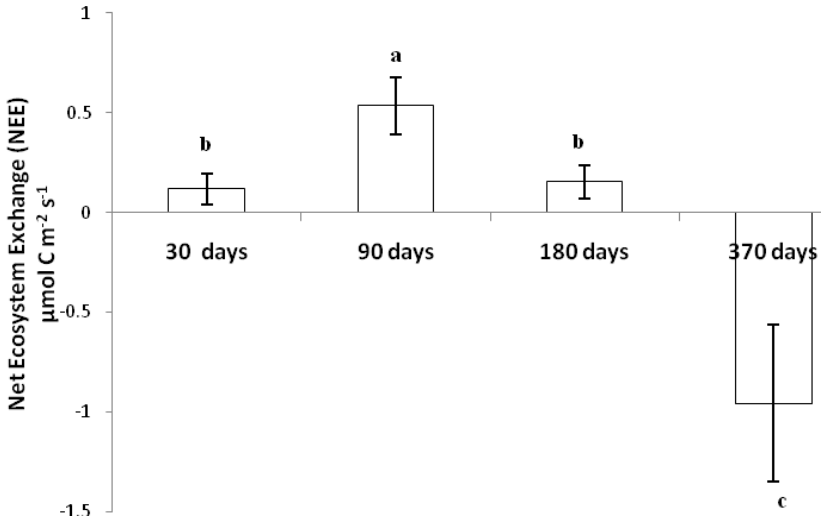


Figure 4.12 Effect of time on Net ecosystem exchange (NEE) µ mol C m^{-2} s^{-1}
(Time: $F_{3,60}$ = 39.03 P< 0.001)

4.5.5.3 Gross ecosystem exchange (GEE)

Data about gross ecosystem exchange (GEE) (Biochar: $F_{4,16}$ = 5.87 P 0.004) showed that treatment with BC 5 % produced highest GEE values over all the treatments (Figure 4.13). The value of GEE declined with the increasing rates of biochar application, with the lowest value reported in BC 15 %. However control and BC 10 % produced the same GEE values.

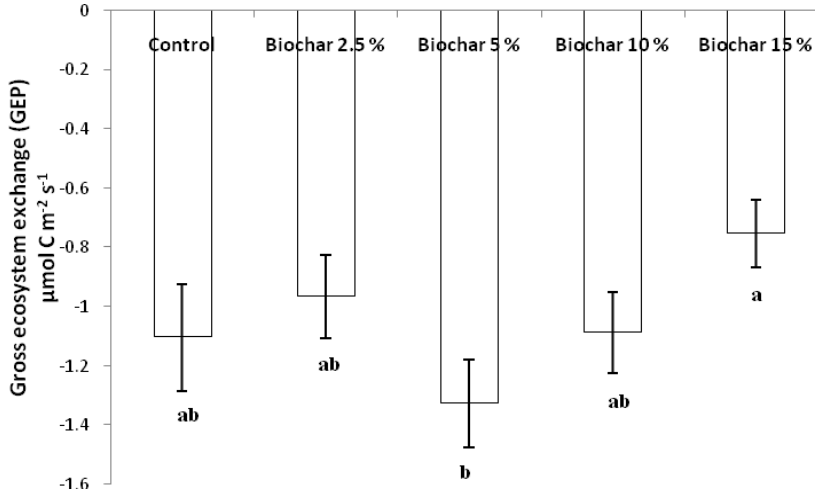


Figure 4.13 Effect of biochar on Gross ecosystem exchange (GEP) µ mol C m^{-2} s^{-1}
(Biochar: $F_{4,16}$ = 5.87 P 0.004)

Data about gross ecosystem exchange (GEE) (Time: $F_{3,60} = 11.75$ $P < 0.001$) showed that time had significant effect and the negative value recorded after 52 weeks was highest as compared to all other timings (Figure 4.14). The lowest respiration rate was recorded at 7 weeks followed by 13, 18 and 52 weeks.

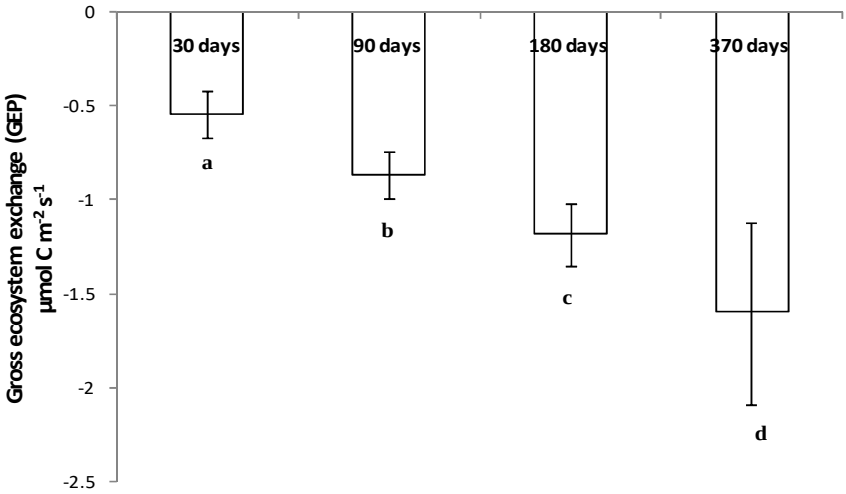


Figure 4.14 Effect of time on Gross ecosystem exchange (GEP) µ mol C m^{-2} s^{-1}
(Time: $F_{3,60} = 11.75$ $P < 0.001$)

Table.4.3 p values for ecosystem gas exchange

Experimental factors	d.f	Ecosystem Respiration (RE) µmol C m^{-2} s^{-1}	Net Ecosystem Exchange (NEE) µmol C m^{-2} s^{-1}	Gross Ecosystem Exchange (GEP) µmol C m^{-2} s^{-1}
Biochar	4	**<.001**	**<.001**	**0.004**
Time	3	**<.001**	**<.001**	**<.001**
Biochar*Time	12	0.609	0.768	0.995
Residuals	60			

4.6 Discussions

This study had investigated the effect of biochar on strawberry growth parameters, nutrient content of shoot and root and ecosystem gas exchange. We described and discussed the

obtained results and possible reasons for variations and changes due to biochar application in following paragraphs:

Effect of biochar on strawberry Growth parameters

Strawberry growth parameters were determined at harvesting after 370 days at the completion of experiment. Among all strawberry growth parameters, only root dry weight and total biomass (root + shoot dry weight) were statistically significant. In all other parameters like shoot dry weight, no. of leaves, no of stems, root/shoot ratio, total N in shoot and root, and Total C in root and shoot, biochar failed to produce any significant difference at various biochar levels. Our results regarding measurements of plant height, number of leaves and shoot dry weight are in contrast with the other studies who found that cherry wood biochar treatments of 2 and 3% significantly increased these plant growth-related parameters of basil (Jabborova *et al*., 2021). Several other researchers also reported that biochar induced increase in plant growth and yields in soybean, chickpeas, basil, lettuce, plantain, cotton and okra (Agboola *et al*., 2015; Głodowska *et al*., 2017; Sarma *et a*l., 2017; Ma *et al*., 2019; Hashem *et al*., 2019; Zhaoxiang *et al*., 2020 Qayyum *et al*., 2020; Nobile *et al*.,2020).

Data regarding the root showed that application of wood biochar improved root dry weight and total biomass as compared with the control. Root growth is of utmost importance to identify the ability of plant roots to uptake water and nutrient as well as to determine root longevity (Rewald and Meinen, 2013 ; Yue *et al*.,2019; Jabborova *et al*., 2021). Bryanin and Makoto, (2017) also reported the positive correlation of charcoal biochar with root growth. Our results obtained with strawberry and wood biochar are in agreement with the findings of Saxena *et al*., (2013); Carter *et al*., (2013), who found increase in root biomass and dry weight in French beans, lettuce and cabbage as compared to control. Further more significant

improve in root parameters and development of various arable crops were discussed in numerous studies (Uzoma *et al*., 2011; Mulcahy *et al*., 2013;Głodowska *et al*., 2017; Jabborova *et al*., 2020; Bu *et al*.,2020; Nobile *et al*.,2020).

In our study leaf conductance and leaf temperature were remained unaffected by high or low biochar application. It was supported by research conducted by Zhang *et al*., (2020) and Jabborova *et al*., (2021) with rice and reed, which used high temperature lignin rich biochar. This biochar exhibits plant inhibitory effects specifically caused by phenolic compounds, which blocks epidermal opening reducing the leaf gas exchange.

Effect of biochar on Total nutrient content of strawberry root and shoot

Effect of biochar was proved statistically significant for shoot Zn, K, P, Cu, As, and Pb. while for the rest of macro and micro nutrients, biochar failed to produce any difference in strawberry root and shoot. In strawberry shoot, K, P, Zn, Cu and As content increased with biochar, while except Pb content decreased with increasing level of biochar rates as compared to control. Other than these, none of macro and micro nutrients exhibited a clear trend with or without biochar in strawberry shoot. However in case of strawberry root, all macro and micro nutrients were exhibiting almost same trend having non-significant differences among all treatments where biochar applied at different rates including control.

Biochar can act as fertilizer even in a small amount thus it can easily influence the availability and uptake of nutrient in rhizosphere (Tender *et al*., 2020). Several studies reported the increase in P contents of strawberry after high temperature wood biochar application (Olmo *et al*., 2016; Amery *et al*., 2021), K contents in strawberry (Tender *et al*.,

2020; Amery *et al*., 2021), Mg and Mn (Glaser *et al*., 2015), and N and P in tomato and sweet pepper (Levesque *et al*., 2020).

Biochar contains large amounts of nutrients specially if the source is animal or deciduous plant, because of its porous structure, negative charge, high surface area and strong affinity sites and it can elevate the soil nutrient concentration (Asadabadi *et al*., 2021). However the release of nutrients from biochar depends on soil conditions, cation or anion immobilization and mineralization potential (Ahmad *et al*., 2022)

Yu *et al*., 2019 concluded that biochar helps retain plant nutrients, which helps maintaining water quality and reduce the loss of nutrients from system by runoff. This is possible mainly due to high adsorption potential of biochar, which sometimes make it difficult for plant to uptake nutrients specially cations. Small biochar particles strongly attached to soil or root surfaces, sometime form shell like structure which effectively reduce the uptake of cations. These shell like structures are responsible for various other interactions of soil and biochar particles with root exudates and mineral complexes in root regions. However the bulk biochar particles settle down quickly and have no chance to contact with plant roots. This strong affinity of small biochar particles help in reducing the phyto-toxicity of some elements (Lehmann *et al*., 2003; Clough and Condron, 2010; Laird *et al*., 2010).

Effect of biochar on Ecosystem gas exchange

Gross ecosystem photosynthesis (GEP) was estimated by subtracting the mean pot R_e by the mean pot NEE fluxes for each measurement period. Positive flux values indicate a release of CO_2 to the atmosphere while negative CO_2 flux values indicate an uptake of CO_2. Biochar and time both were proved statistically significant in producing differences in ecosystem

respiration, NEE and GEP for all biochar treatments. He *et al*., (2020) reported increase in photosynthesis, transipiration rate, and cholorophyll content in tomato, lettuce (Agegnehu *et al*., 2015; Petruccelli *et al*., 2015; Speratti *et al*., 2018), maize (Jabborova *et al*., 2021) , basil (Ding *et al*., 2020), and okra (Batool *et al*., 2015; Sarma *et al*., 2017). This increased photosynthesis rate is possible due to improved essential nutrients absorption after biochar application (Jabborova *et al*., 2021; Li and Cai , 2021; Ahmad *et al*., 2022).

4.7 Conclusion

Biochar meant to maximize plant growth and soil productivity by different mechanisms. Plant growth can also be affected by biochar-induced changes in soil nutrient conditions; their solubility and presence in soil exchange complex. The findings of the present study concluded that biochar apparently has no significant effect on plant growth parameters. Among all strawberry growth parameters, only root dry weight and total biomass were statistically significant. In all other parameters like shoot dry weight, no. of leaves, no of stems, root/shoot ratio, total N in shoot and root, and Total C in root and shoot, biochar failed to produce any significant difference in various biochar levels. In strawberry shoot, K, P , Zn, Cu, and As content increased with biochar, while Pb content decreased with increasing level of biochar rates as compared to control. Other than these, none of macro and micro nutrients exhibited a clear trend with or without biochar in strawberry shoot. However in case of strawberry root, all macro and micro nutrients were exhibiting almost same trend having non-significant differences among all treatments where biochar applied at different rates including control. Biochar and time both were proved statistically significant in producing differences in ecosystem respiration, net ecosystem exchange (NEE), gross ecosystem exchange (GEE) for all biochar treatments. However, their interaction was failed to have any prominent effect.

Chapter 5 Effects of wood biochar application on Soil microbial, biochemical properties and soil respiration

5.1 Overview

In this chapter biochar effects and influences on soil biochemical and microbial properties were discussed. Microbial biomass carbon, nitrogen, Dehydrogenase, beta glucosidase and phosphatase were chosen as indicators of biological activity. In the presence or absence of strawberry plant soil respiration (CO_2 and CH_4 flux) was also recorded.

5.2 Introduction

Biochar ,a stable carbon material, occasionally identified as black carbon or charcoal made by the thermal combustion of organic or inorganic residues under complete absence of oxygen with the purpose of its application to agricultural fields (Banik *et al*., 2021; Jiang *et al*., 2021 ; Liao *et al*., 2022). Biochar is known as a tool to sequester carbon, support the reclamation of contaminated soils and restoration of soil fertility and health (Khan *et al*., 2020). Soil fertility is more commonly indicated by physical, chemical and microbial properties but biochemical indicators are more popular due to the quick response in any change in soil and environment (Huang *et al*., 2013; Al-Wabel *et al*., 2018; Marousek *et al*., 2019; Liao *et al*., 2022).

Soil organic matter is a composite and stable soil quality indicator, but it is not sensitive to temporal changes in soil management practices. It took years to notice the potential difference in total carbon and nitrogen contents of soil (Li *et al*., 2020; Amoakwah *et al*.,

2022). Biochar is rich in persistent carbon, almost unavailable for chemical and microbial degradation and that's why regulatory effects on soil microorganism and enzymes have been noted (Sakin *et al*., 2021). Enzymes are the substances secreted by soil microorganisms during cell destruction, intracellularly or extracellularly.

The enzymes can be free in soil solution or form a complex bound with organic and inorganic components of the soil, without losing their potential activity (Tang *et al*., 2019; Sakin *et al*., 2021; Liao *et al*., 2022). These extra and intracellular enzymes are the main drivers of all soil organic matter decomposition.

The extracellular enzymes are short lived and produced by soil microorganisms at the expense of cell growth with heavy input of energy, either due to low essential nutrients or presence of complex molecules (Sakin *et al*., 2021). They have high sorption affinity for clay particles and humic substances, which improves their stabilization and prolonged activities in soil solution. On the other hand intracellular enzymes do not stay out of microbial cells and are considered best measures for microbial activity and response to the external factors (Liao *et al*., 2022; Ahmad *et al*., 2022). The main intracellular enzyme is dehydrogenase, which can extensively catalyze oxidation-reduction reactions, and its activity reflects the respiration or functional potential of whole soil microbial community (Jiang *et al*., 2021; Liao *et al*., 2022).

Extracellular enzymes are involved in carbon, nitrogen and phosphorous metabolism. The main carbon acquisition enzyme is beta glucosidase, which are hydrolytic enzyme responsible for the breakdown of simple to complex organic molecules (Jian *et al*., 2016; Luo *et al*., 2017). Phosphatase, the most prominent phosphorus acquisition enzyme, catalyzes

phosphorous availability to plants by degrading esters and anhydrides of phosphoric acid (Khalid *et al*., 2020; Ahmad *et al*., 2022; Liao *et al*., 2022).

5.3 Aims and Objectives

The research questions addressed in this chapter were as follows:

4. Study was planned to quantify the effects of biochar and plant on enzyme activities
5. How biochar influences microbial biomass carbon and nitrogen and how it was affected by presence or absence of strawberry plant
6. How different biochar application rates affected CO_2 and CH_4 flux

It was hypothesized that biochar on addition to soil ameliorate soil microbial activities and effect enzyme functionality and CO_2 and CH_4 exchange,

5.4 Material and Methods

5.4.1 Soil Enzymes

Soil enzymes activities were measured throughout the experimental period.

5.4.1.1 Dehydrogenase

Dehydrogenase activity was measured after 30, 90, 180 and 370 days. For detailed method refer to section 2.3.3.2 in chapter 2.

5.4.1.2 β-glucosidase

β-glucosidase activity was measured after 30, 90, 180 and 370 days. For detailed method refer to section 2.3.3.3 in chapter 2.

5.4.1.3 Phosphatase

Phosphatase activity was measured after 30 and 370 days. For detailed method refer to section 2.3.3.4 in chapter 2.

5.4.2 Microbial biomass

5.4.2.1 Microbial biomass carbon

For detailed method for microbial biomass carbon, refer to section 2.3.3.1 in chapter 2.

5.4.2.2 Microbial biomass nitrogen

Microbial biomass nitrogen detailed method was described in section 2.3.3.1 in chapter 2.

5.4.3 Soil respiration

5.4.3.1 CO_2 flux

CO_2 flux was measured at 30, 60, 120, 180, 210, 240, 300 days. Detailed method for measuring CO_2 flux was described in section 2.5 in chapter 2.

5.4.3.2 CH_4 flux

CH_4 flux was measured at 30, 60, 120, 180, 210, 240, 300 days. Detailed method for measuring CH_4 flux was described in section 2.5 in chapter 2.

5.4.4 Statistical analysis

All the collected data for enzyme activities, microbial biomass and CO_2 and CH_4 fluxes were analysed by statistical software Genstat (17th Edition, VSN International Ltd, UK) using biochar, plant and time as factors. Data collected at four different time intervals during the

experiment, was analyzed by repeated measures analysis of variance (ANOVA). The treatment means were compared for significance at the $P < 0.05$ level using the Fisher's Least Significant Difference (LSD). Standard errors of means were calculated and presented with respective data in figures.

5.5 Results

Soil enzymes (Dehydrogenase, beta glucosidase and Phosphatase) and microbial biomass (carbon and nitrogen) and soil respiration (CO_2 and CH_4 fluxes) were discussed and presented in the following paragraphs;

5.5.1 Soil enzymes

5.5.1.1 Dehydrogenase

Data obtained for dehydrogenase activity in soil showed that biochar (planted: Biochar: $F_{4,16}$ = 58.51, P <0.001; unplanted :Biochar: $F_{2,8}$ = 65.85, P <0.001) and time (planted :Time: $F_{3,60}$ = 24.04, P <0.001; unplanted: Time: $F_{3,36}$= 16.28, P <0.001) were proved statistically significant as individual factor for both planted and unplanted treatments. Presence of strawberry plant (Plant: $F_{1, 20}$ = 4.83, P 0.040) was also proved significant for dehydrogenase activity in all treatment with or without biochar application.

In planted (Figure 5.1: a) the highest dehydrogenase activity was recorded in control treatment with no biochar, with the decreasing activity reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. There was a sharp decrease in first biochar treatment where 2.5 % biochar was applied than control. This was followed by a smooth decreasing trend in dehydrogenase activity with the increasing rate of biochar. The lowest activity was reported in the treatment where the highest rate of biochar 15% was applied.

In unplanted (Figure 5.1: b) the highest dehydrogenase activity was reported in control treatment where no biochar was applied. There was a decrease in dehydrogenase activity in treatments with BC 10 and 15%.

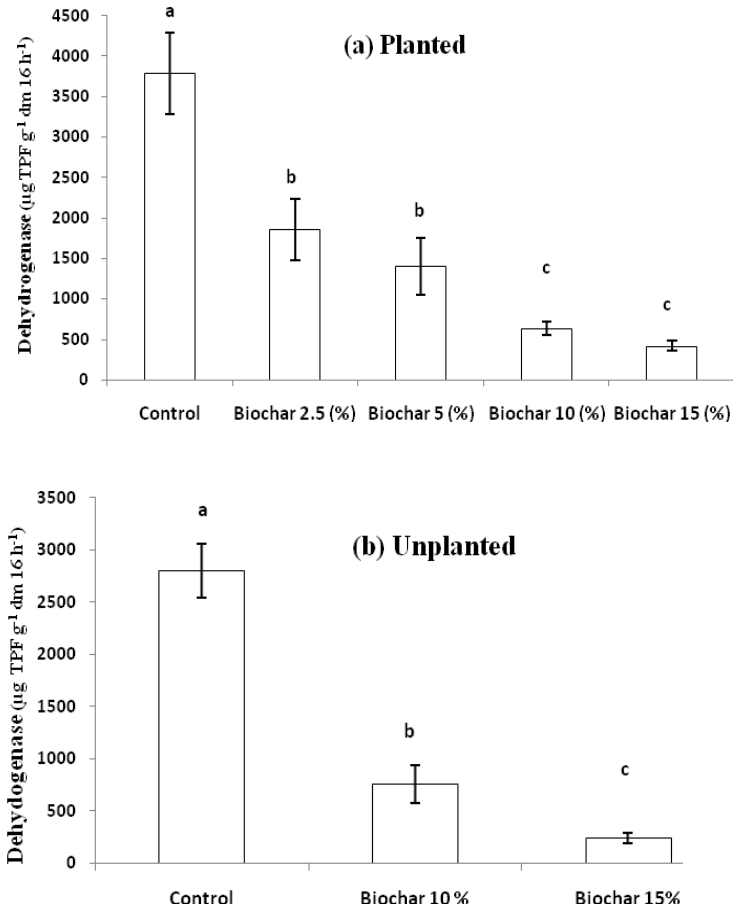


Figure 5.1 Effect of biochar on Dehydrogenase activity (µg TPF g^{-1}dm 16 h^{-1}) with plants (Bio char:$F_{4,16}$ = 58.51, *P* <0.001) (b) without plants (Biochar: $F_{2,8}$ = 65.85, *P* <0.001).Columns similarly superscripted and not significantly different.

Figure 5.2 presented the effect of Time x Biochar interaction (planted: Time x Biochar: $F_{12,60}$ = 2.93, *P* 0.013; unplanted : Time x Biochar: $F_{3,36}$ = 4.28, *P* 0.006) in producing statistically significant changes in dehydrogenase activity measured over the period of one year in all treatments with or without strawberry plants. In planted (Figure 5.1: a) the highest dehydrogenase activity was recorded in control treatment with no biochar at the end of

experimental period (370 days), followed by the treatments where biochar was applied @ 2.5, 5, 10 and 15%.

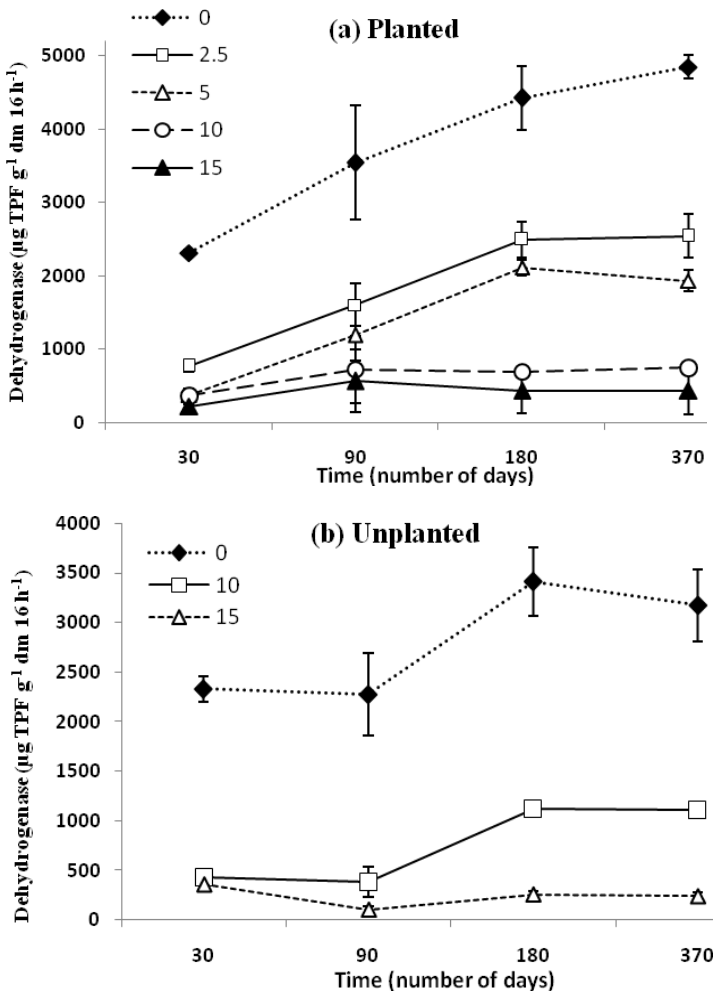


Figure 5.2 Effect of Time x Biochar interaction on Dehydrogenase activity (µg TPF g^{-1}dm 16 h^{-1}) (a) with plants (Time x Biochar: $F_{12,60}$ = 2.93, *P* 0.013), (b) without plants (Time x Biochar: $F_{3,36}$ = 4.28, *P* 0.006)

The lowest activity was reported in the treatment with highest rate of biochar 15%. There was increasing trend in dehydrogenase activity in all treatments (control, BC 2.5%, and BC 5%) from day 30 , 90, 180 with highest value at 370 days except the treatments where biochar was applied at high rate (BC 10 and 15%). There was a slight or no difference observed in dehydrogenase activity in biochar treatments with 10 and 15% BC over the time.

In unplanted (Figure 5.2: b) the highest dehydrogenase activity was reported in control treatment where no biochar was applied at 180 days in 370 days experimental period. There was a sharp increase in dehydrogenase activity at 90 days but then activity was declined at 370 days in control treatment. In unplanted treatment with biochar 10% dehydrogenase activity was increased over the time with minimum at 30 days and highest at 370 days. However in treatment where biochar was applied @15%, there was a slight or no difference in dehydrogenase activity over the time.

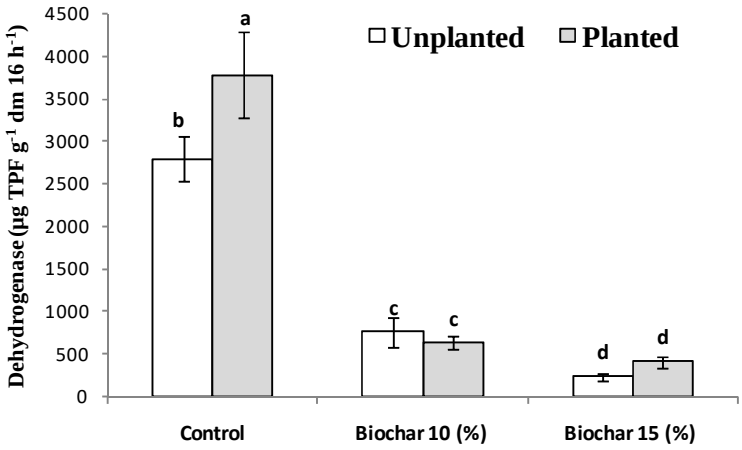


Figure 5.3 Effect of plant x biochar interaction on Dehydrogenase activity (µg TPF g^{-1}dm 16 h^{-1})
(Biochar x Plant: $F_{2, 20}$ = 4.46, P 0.025). Columns similarly superscripted and not significantly different.

Figure 5.3 represented the effect of plant x biochar (Biochar x Plant: $F_{2, 20}$ = 4.46, P 0.025) interaction on dehydrogenase activity over a period of one year. The highest activity was reported in control with maximum value in planted treatment than unplanted followed by biochar treatment with 10% BC (maximum dehydrogenase activity in unplanted than planted treatment).

The lowest activity of dehydrogenase enzyme was reported in treatment where biochar was applied at high rate 15% without strawberry plants, followed by control 15% BC.

The interaction of time x biochar was also proved significant for dehydrogenase activity, however time x plant and time x BC x plant interactions were proved statistically non-significant in producing any difference in dehydrogenase activity for all the treatments.

5.5.1.2 β-glucosidase

Data obtained for beta-glucosidase activity in soil showed that biochar (planted: Biochar: $F_{4,16}$ = 54.47, P <0.001; unplanted : Biochar: $F_{2,8}$ = 28.41, P <0.001) and time (planted : Time: $F_{3,60}$ = 21.88, P <0.001; unplanted: Time: $F_{3,36}$= 5.11, P 0.013) were proved statistically significant as individual factor for both planted and unplanted treatments. Presence of strawberry plant was proved non-significant as a single factor for beta-glucosidase activity in all treatment with or without biochar application.

The interaction of time x biochar was also proved significant for beta-glucosidase activity, however time x plant, plant x biochar and time x BC x plant interactions were proved statistically non-significant in producing any difference in beta-glucosidase activity for all the treatments.

Figure 5.4 presented the effect of Biochar in producing statistically significant changes in beta-glucosidase activity measured over the period of one year in all treatments with or without strawberry plants. In planted (Figure 5.4: a) the highest activity was recorded in control treatment with no biochar, with the decreasing activity reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The lowest activity was reported in the treatment where the highest rate of biochar 15% was applied. There was a smooth decreasing trend in beta-glucosidase activity with the increasing rate of biochar. In unplanted

(Figure 5.4: b) the highest phosphatase activity was reported in control treatment with no biochar followed by treatments with BC 10 and 15%.

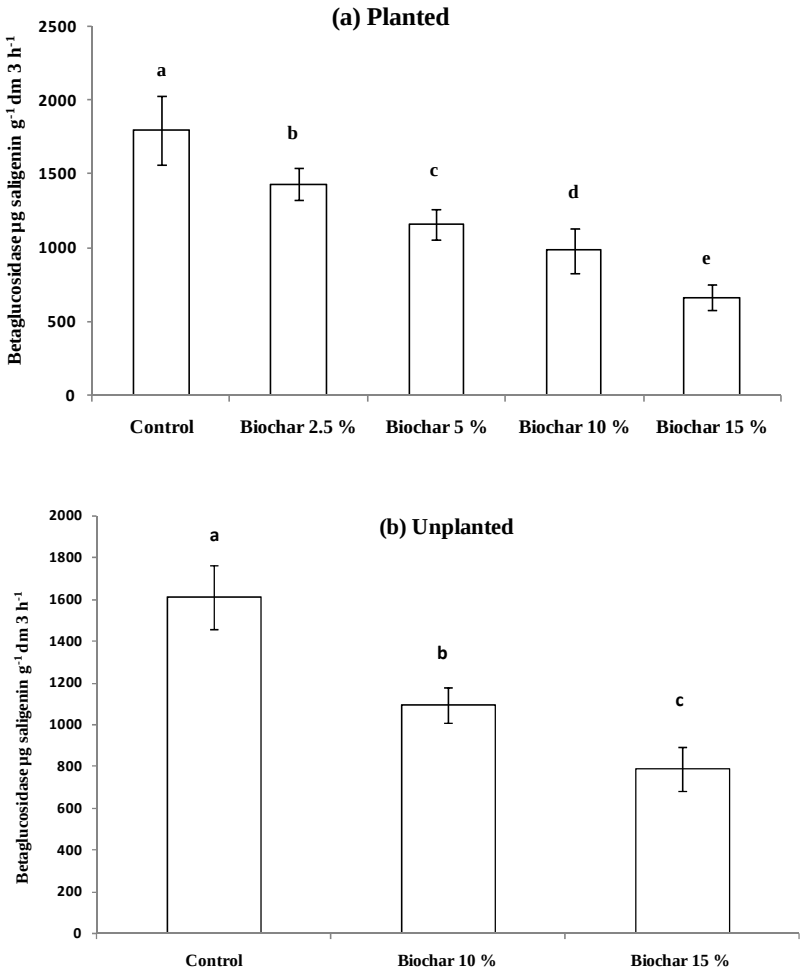


Figure 5.4 Effect of biochar on β-glucosidase activity (µg saligenin g^{-1} dm $3h^{-1}$) (a) with plants (Biochar: $F_{4,16} = 54.47$, $P < 0.001$) (b) without plants (Biochar: $F_{2,8} = 28.41$, $P < 0.001$). Columns similarly superscripted and not significantly different.

Figure 5.5 presented the effect of Time x Biochar interaction (planted: Time x Biochar: $F_{12,60} = 7.18$, P <0.001; unplanted : Time x Biochar: $F_{6,36} = 5.99$, P 0.002) in producing statistically significant changes in beta-glucosidase activity measured over the period of one year in all treatments with or without strawberry plants. In planted (Figure 5.5: a) the highest beta-glucosidase activity was recorded in control treatment with no biochar at the end of

experimental period (370 days), followed by the treatments where biochar was applied @ 2.5, 5, 10 and 15%.

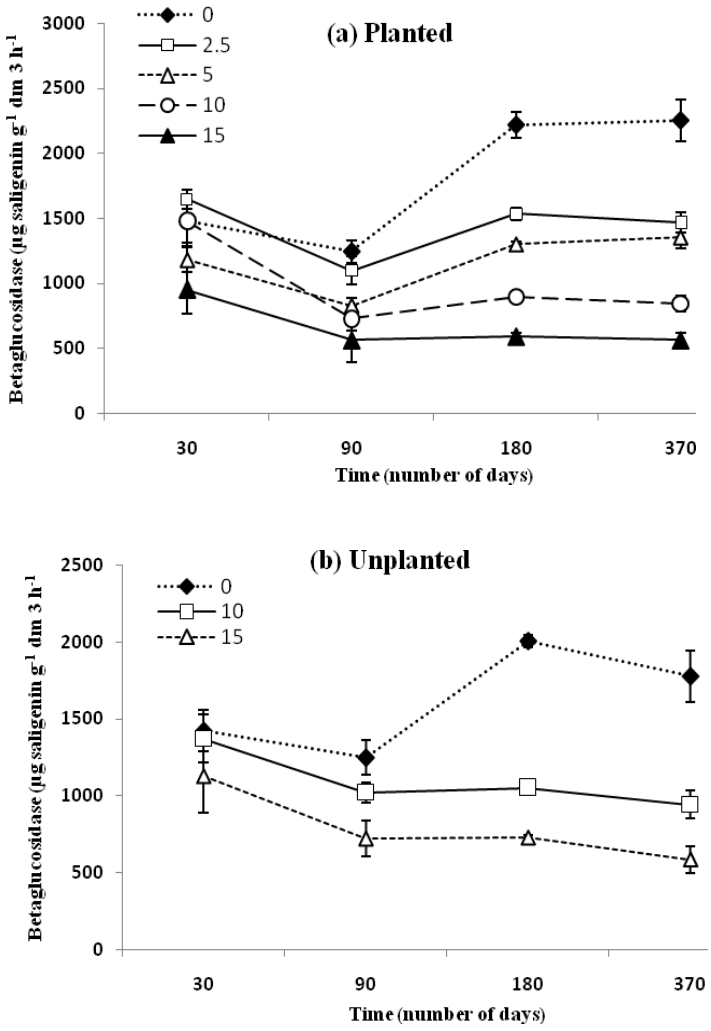


Figure 5.5 Effect of Time x Biochar interaction on β-glucosidase activity (µg saligenin g^{-1} dm $3h^{-1}$) (a) with plants (Time x Biochar: $F_{12,60}$ = 7.18, P <0.001) (b) without plants (Time x Biochar: $F_{6,36}$ = 5.99, P 0.002)

The lowest activity was reported in the treatment with highest rate of biochar 15% at day 370. There was a sharp decrease observed in beta-glucosidase activity at day 90 from day 30 in all treatments with or without biochar. After that, the increasing trend in beta-glucosidase activity was recorded in all treatments (control, BC 2.5%, and BC 5%) from day 30 , 90, 180 with highest value at 370 days except the treatments where biochar was applied at high rate (BC 10 and 15%).

There was a slight or no difference observed in beta-glucosidase activity in biochar treatments with 10 and 15% BC over the time. In unplanted (Figure 5.5: b), again the same trend of enzyme activity was reported. There was an initial decline in beta-glucosidase activity at day 90 from the values reported at day 30 in all treatments, followed by a record boost in enzyme activity recorded at day 180 with same or slightly increased values at day 370.

The highest beta-glucosidase activity was reported in control treatment where no biochar was applied at 180 days in 370 days experimental period. However in treatments, where biochar was applied @10 or 15%, there was no difference or slight decrease in beta-glucosidase activity at the end of experiment.

5.5.1.3 Phosphatase

Data obtained for phosphatase activity in soil showed that biochar (planted: Biochar: $F_{4, 16} = 10.64$, $P < 0.001$; unplanted: Biochar: $F_{2,8} = 42.20$, $P < 0.001$) was proved statistically significant as an individual factor for both planted and unplanted treatments. Figure 5.6 presented the changes in phosphatase activity measured over the period of one year in all treatments with or without strawberry plants.

In planted (Figure 5.6: a) the highest phosphatase activity was recorded in control treatment with no biochar, with the decreasing activity reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The lowest activity was reported in the treatment where the highest rate of biochar 15% was applied.

There was a smooth decreasing trend in phosphatase activity with the increasing rate of biochar. In unplanted (Figure 5.6: b) the highest phosphatase activity was reported in control treatment where no biochar was applied. There was a decrease in phosphatase activity was reported in treatments with BC 10 and 15%.

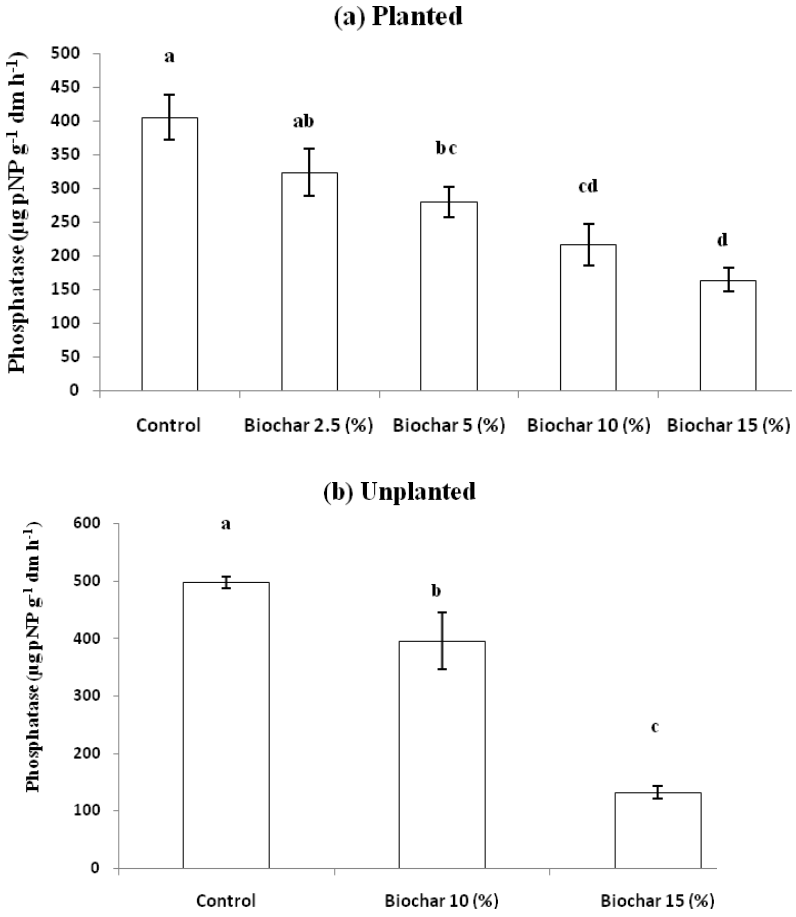


Figure 5.6 Effect of biochar on Phosphatase activity (µg pNP g^{-1} dm h^{-1})
(a) with plants (Biochar:$F_{4,16}$ = 10.64, *P* <0.001) (b) without plants (Biochar: $F_{2,8}$ = 42.20, *P* <0.001). Columns similarly superscripted and not significantly different.

Figure 5.7 represented the effect of time (planted: Time: $F_{1, 20}$ = 5.07, *P* 0.036; unplanted: Time: $F_{1, 12}$ = 0.84, *P* 0.378) on phosphatase activity over a period of one year. Time was statistically proved significant in producing difference in phosphatase activity only in planted treatments (Figure 5.7: a), however the effect of time was non-significant in unplanted treatments (Figure 5.7: b). The data was presented for just comparison. In planted treatments

(Figure 5.6: a) the maximum phosphatase activity was reported at day 30 with the minimum activity at day 370.

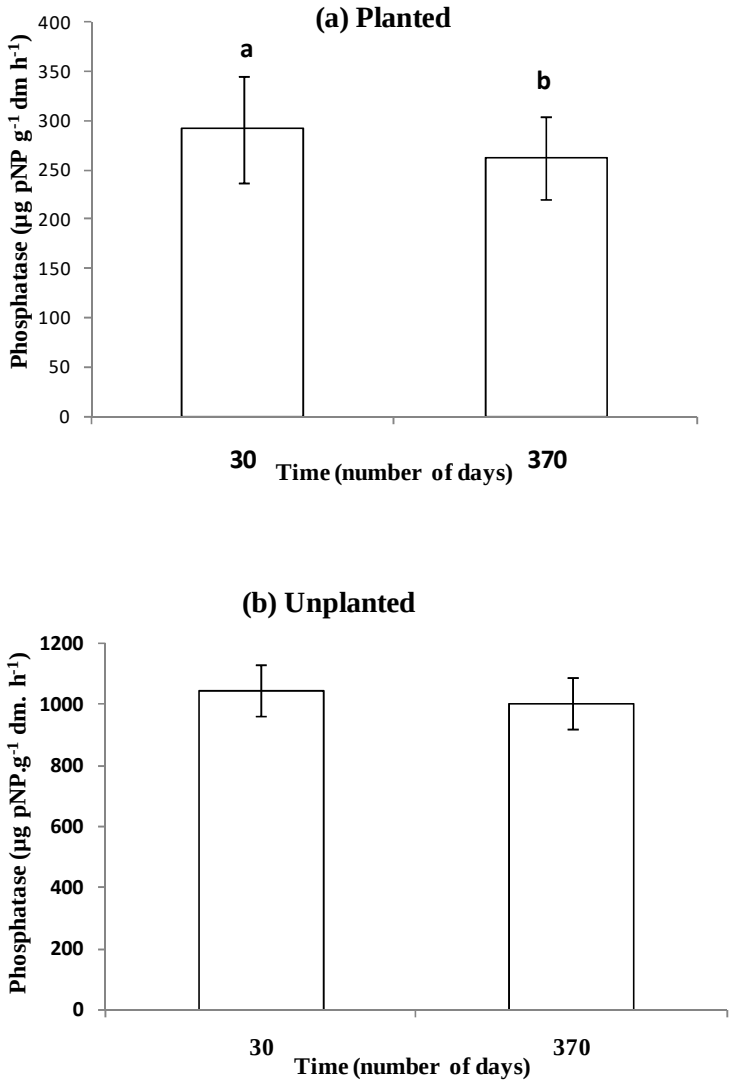


Figure 5.7 Effect of time on Phosphatase activity (µg pNP g^{-1} dm h^{-1})
(a) with plants (Time:$F_{1,20}$ = 5.07, *P* 0.036) (b) without plants (Time: $F_{1,12}$ = 0.84, *P* 0.378). Columns similarly superscripted and not significantly different.

Presence of strawberry plant (Plant: $F_{1,\ 20}$ = 9.87, *P* 0.005) was also proved significant for phosphatase activity in all treatment with or without biochar application (Figure 5.8). The maximum activity was noted in unplanted than planted treatments.

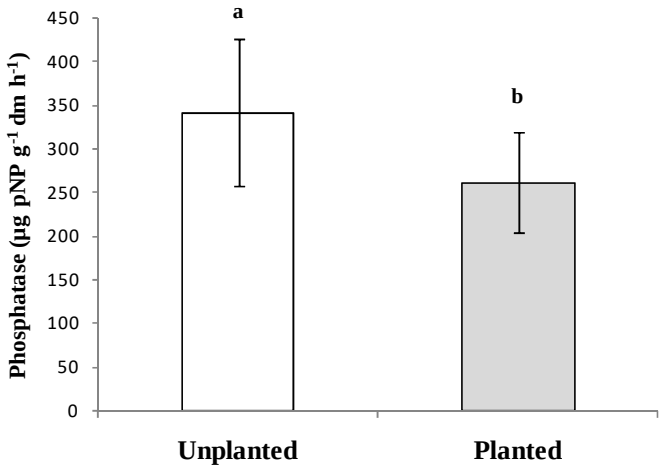


Figure 5.8 Effect of strawberry plant on Phosphatase activity (µg pNP g^{-1} dm h^{-1})
(Plant: $F_{1, 20} = 9.87$, *P* 0.005). Columns similarly superscripted and not significantly different.

Figure 5.9 presented the effect of Biochar x plant interaction (Biochar x Plant: $F_{2, 20} = 5.81$, *P* 0.010) in producing statistically significant changes in phosphatase activity measured over the period of one year in all treatments with or without strawberry plants. The highest phosphatase activity was recorded in control treatment with no biochar at the end of experimental period (370 days), in unplanted as compared to planted treatment.

The same pattern was reported in treatment where biochar was applied @10%, with the maximum activity in unplanted than planted treatment. In treatment with biochar 15% a slight difference in phosphatase activity was reported in planted treatment as compared to unplanted with the lowest activity recorded at the end of experiment.

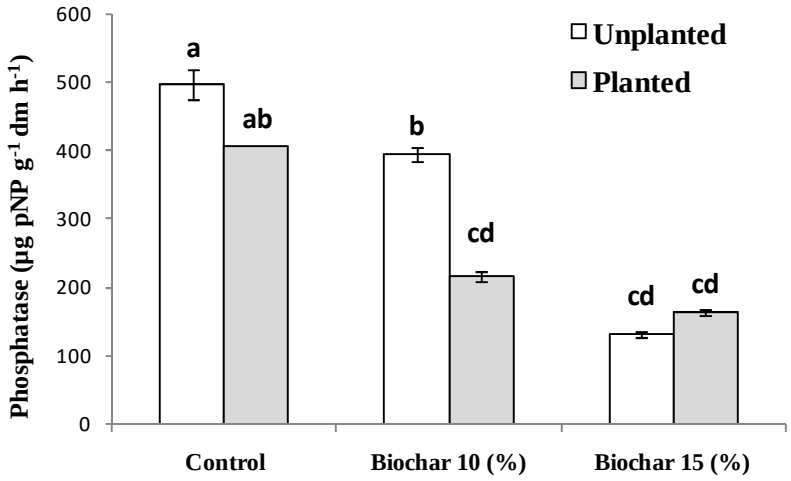


Figure 5.9 Effect of biochar x plant interaction on Phosphatase activity (µg pNP g^{-1} dm h^{-1}) (Biochar x Plant: $F_{2, 20} = 5.81$, P 0.010). Columns similarly superscripted and not significantly different.

Table 5.1 p value of biochar amended soils for soil enzymes in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Dehydrogenase activity (µg TPF.g^{-1}.dm.16 h^{-1})	Beta-glucosidase (µg saligenin.g^{-1} dm.3 h^{-1})	Phosphatase (µg pNP.g^{-1} dm. h^{-1})
Planted	Biochar	4	**<.001**	**<.001**	**<.001**
	Time	3	**<.001**	**<.001**	**0.036**
	Time *Biochar	12	**0.013**	**<.001**	0.553
	Residuals	60			
Unplanted	Biochar	2	**<.001**	**<.001**	**<.001**
	Time	3	**<.001**	**0.013**	0.378
	Time *Biochar	6	**0.006**	**0.002**	0.871
	Residuals	36			
Planted x Unplanted	Biochar	2	**<.001**	**<.001**	**<.001**
	Time	3	**<.001**	**<.001**	0.056
	Plant	1	**0.04**	0.815	**0.005**
	Time *Biochar	6	**0.001**	**<.001**	0.759
	Time *Plant	3	0.082	0.255	0.352
	Plant *Biochar	2	**0.025**	0.118	**0.01**
	Time*Biochar*Plant	6	0.203	0.53	0.483
	Residuals	72			

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

The interaction of time x biochar was non-significant for phosphatase activity in both planted and unplanted treatments. However, time x plant, time x plant and time x BC x plant interactions, were proved statistically non-significant in producing any difference in phosphatase activity for all the treatments.

5.5.4 Microbial biomass carbon

Data obtained for microbial biomass carbon in soil showed that biochar was proved statistically non-significant as a single factor. The effect of time (planted: Time: $F_{3,60} = 22.3$ $P < 0.001$; unplanted: Time: $F_{3,36} = 15.29$ $P < 0.001$) was proved statistically significant as an individual factor affecting microbial biomass carbon for both planted and unplanted treatments. Presence of strawberry plant was also proved non-significant for microbial biomass carbon in all treatment with or without biochar application.

Figure 5.10 presented the effect of time on microbial biomass carbon measured over the period of one year in all treatments with or without strawberry plants. In planted (Figure 5.10: a) the maximum value for microbial biomass carbon at the end of experimental period (370 days), followed by the values recorded at 30 and 180 days. The lowest microbial biomass carbon was determined at day 90. In unplanted (Figure 5.10:b), the pattern was same for the maximum microbial biomass carbon, which was recorded at day 370 at the end of experiment, followed by the values of microbial biomass carbon reported at day 180 and 30. The lowest microbial biomass carbon was recorded at day 90.

The interactions of time x biochar, time x plant, plant x biochar and time x BC x plant, were proved statistically non-significant in producing any difference in microbial biomass carbon for all the treatments.

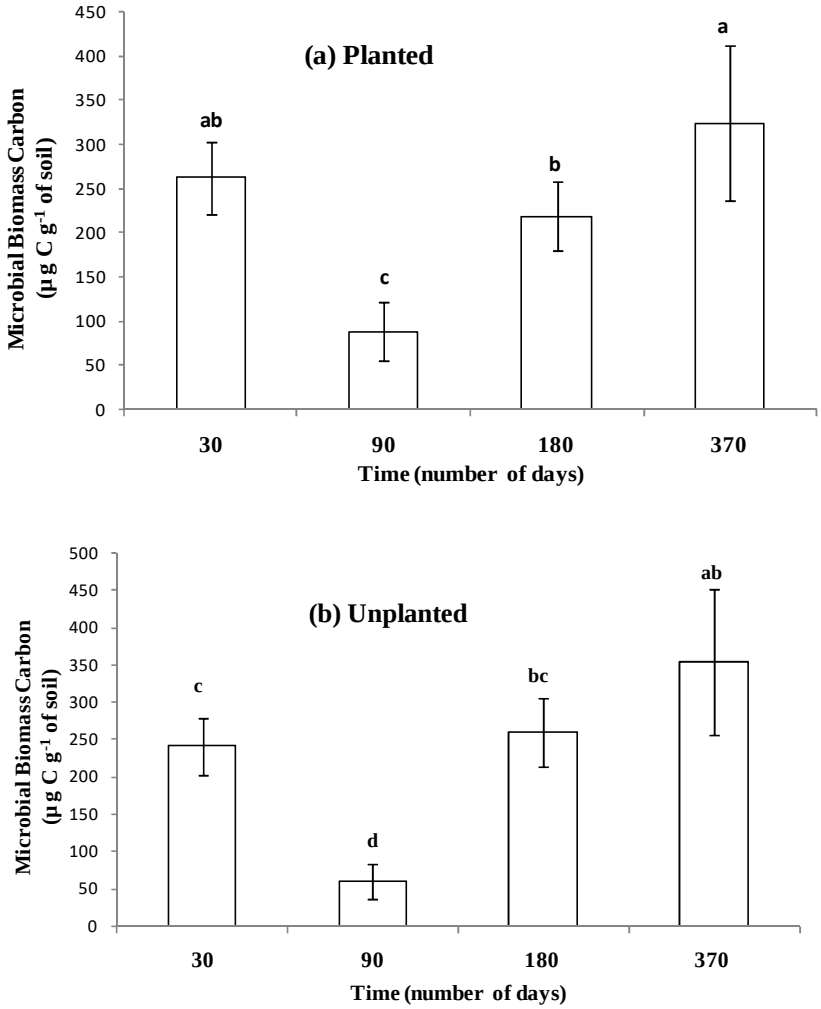


Figure 5.10 Effect of time on Microbial biomass Carbon (µ g C g^{-1} of soil)
(a) With plants (Time: $F_{3,60}$ = 22.3 *P* <0.001);(b) Without plants (Time: $F_{3,36}$ = 15.29 *P* <0.001). Columns similarly superscripted and not significantly different.

5.5.5 Microbial biomass nitrogen

Data obtained for microbial biomass nitrogen in soil showed that biochar was proved statistically non-significant as a single factor. The effect of time was proved statistically significant as an individual factor affecting microbial biomass nitrogen for only unplanted treatments. Presence of strawberry plant (Plant: $F_{1, 20}$ = 5.23 *P* 0.033) was also proved significant for microbial biomass nitrogen in all treatment with or without biochar application.

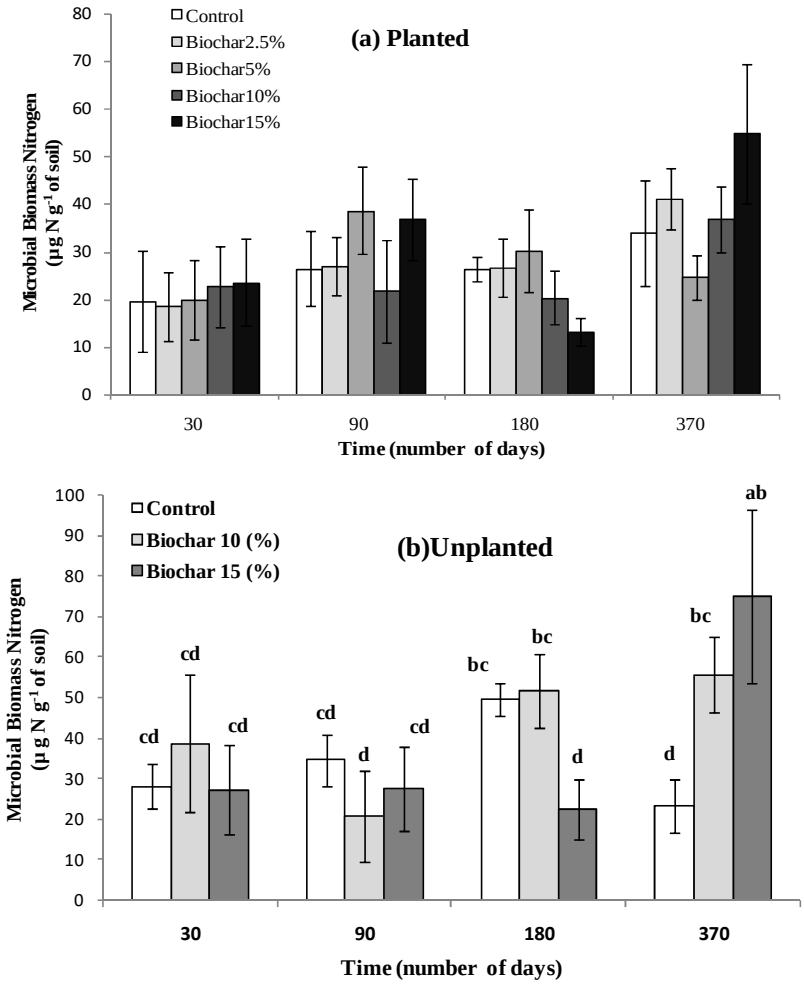


Figure 5.11 Effect of time x biochar interaction on Microbial biomass Nitrogen (µ g N g^{-1} of soil)
(a) With plants (Time x Biochar: $F_{12, 60}$ = 0.9 *P* 0.535); (b) Without plants (Time x Biochar: $F_{6, 36}$ = 3.18 *P* 0.032). Columns similarly superscripted and not significantly different.

Figure 5.11 presented the effect of Time x Biochar interaction (planted: Time x Biochar: $F_{12, 60}$ = 0.9 *P* 0.535; unplanted : Time x Biochar: $F_{6, 36}$ = 3.18 *P* 0.032) in producing significant changes in microbial biomass nitrogen measured over the period of one year in all treatments without strawberry plants, however the effect was non-significant for planted treatment. The data was presented here for comparison only.

In unplanted (Figure 5.11: b) the highest value for microbial biomass nitrogen was recorded in treatment with maximum biochar (15%) at the end of experimental period (370 days),

followed by the treatments where biochar was applied @ 10 and 0%. The pattern was reversed for the values recorded at 180, 90 and 30 days, where the maximum values of microbial biomass nitrogen was noted in treatment with BC 10%, followed by control and BC 15%.

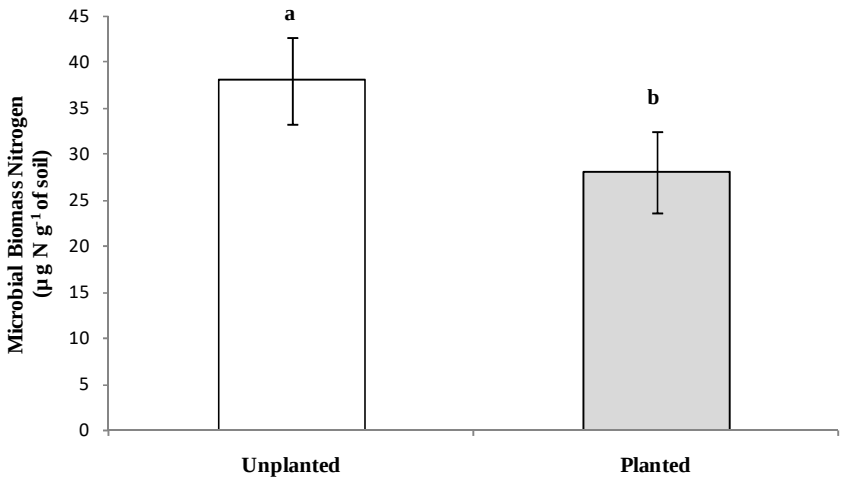


Figure 5.12 Effect of plant on Microbial biomass Nitrogen (µ g N g^{-1} of soil)
(Plant: $F_{1, 20}$ = 5.23 *P* 0.033). Columns similarly superscripted and not significantly different.

Figure 5.12 represented the effect of plant (Plant: $F_{1, 20}$ = 5.23 *P* 0.033) on microbial biomass nitrogen over a period of one year. The highest value was reported in unplanted treatment. The lowest value of microbial biomass nitrogen was reported in treatment with strawberry plants.

The interaction of time x plant, plant x biochar and time x BC x plant, were proved statistically non-significant in producing any difference in soil microbial biomass carbon for all the treatments.

Table 5.2 p value of biochar amended soils for soil microbial biomass in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Microbial Biomass Carbon (µ g C g^{-1} of soil)	Microbial Biomass Nitrogen (µ g N g^{-1} of soil)
Planted	Biochar	4	0.763	0.798
	Time	3	**<.001**	**0.016**
	Time *Biochar	12	0.094	0.535
	Residuals	60		
Unplanted	Biochar	2	0.576	0.646
	Time	3	**<.001**	0.053
	Time *Biochar	6	0.351	**0.032**
	Residuals	36		
Planted x Unplanted	Biochar	2	0.521	0.652
	Time	3	**<.001**	**0.006**
	Plant	1	0.861	**0.033**
	Time *Biochar	6	0.112	**0.013**
	Time *Plant	3	0.506	0.287
	Plant *Biochar	2	0.492	0.579
	Time*Biochar*Plant	6	0.967	0.592
	Residuals	72		

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

5.5.6 Soil respiration

Soil respiration data was collected for CO_2 and CH_4 flux presented and explained in following paragraphs:

5.5.6.1 CO_2 Flux

Data obtained for CO_2 flux in soil showed that biochar (planted: Biochar: $F_{4,16}$ = 12.52, P <0.001; unplanted : Biochar: $F_{2,8}$ = 40.79, P <0.001) and time (planted :Time: $F_{6,120}$ = 28.32, P <0.001; unplanted: Time: $F_{6,72}$= 27.5, P <0.001) were proved statistically significant as individual factor for both planted and unplanted treatments. Presence of strawberry plant was also non-significant for CO_2 flux in all treatment with or without biochar application.

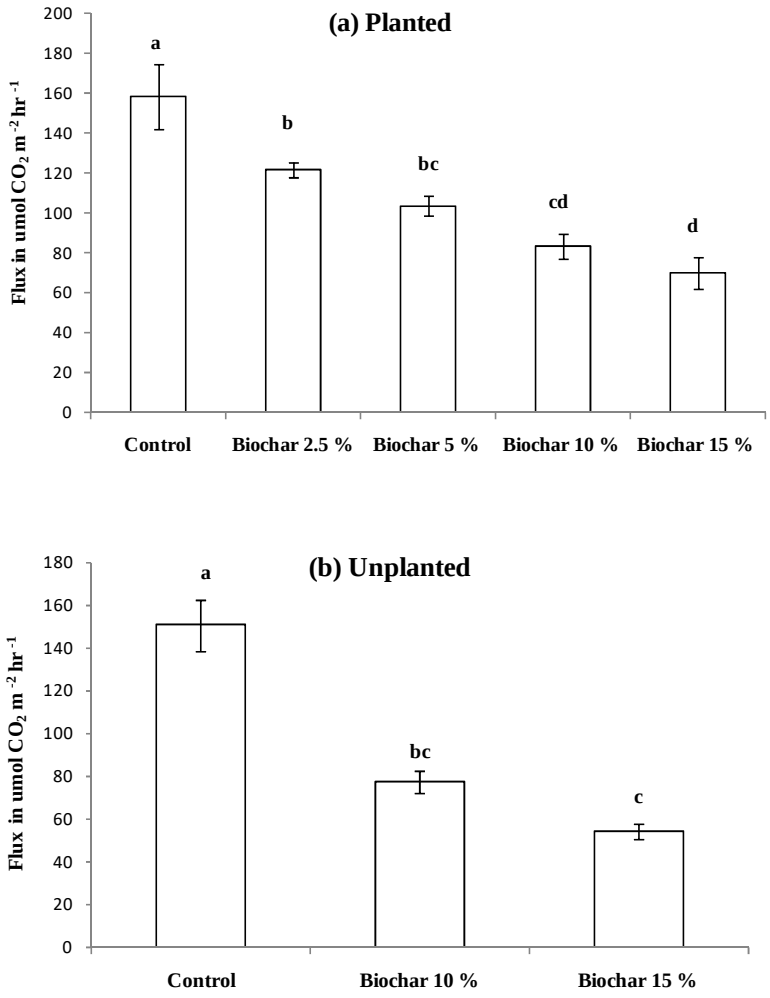


Figure 5.13 Effect of biochar on CO_2 Flux (µ mol CO_2 m^{-2} hr^{-1})
(a) With plants (Biochar:$F_{4,16}$ = 12.52, *P* <0.001) (b) without plants (Biochar: $F_{2,8}$ = 40.79, *P* <0.001). Columns similarly superscripted and not significantly different.

Figure 5.13 presented the effect of Biochar application (planted: Biochar:$F_{4,16}$ = 12.52, *P* <0.001; unplanted : Biochar: $F_{2,8}$ = 40.79, *P* <0.001) in producing statistically significant changes in CO_2 flux measured over the period of one year in all treatments with or without strawberry plants. In planted (Figure 5.13: a) the highest value was recorded in control treatment with no biochar, followed by the treatments where biochar was applied @ 2.5, 5, 10 and 15%. The lowest activity was reported in the treatment with highest rate of biochar 15%. There was overall decreasing trend in CO_2 flux in all treatments (control, BC 2.5%, BC 5%, BC 10% and BC 15%). In unplanted (Figure 5.13: b) the highest CO_2 flux was reported in

control treatment where no biochar was applied. With the application of biochar @10% a big decrease in CO_2 flux was reported. The slight difference was reported between two biochar treatments (10 and 15%) in absence of strawberry plants.

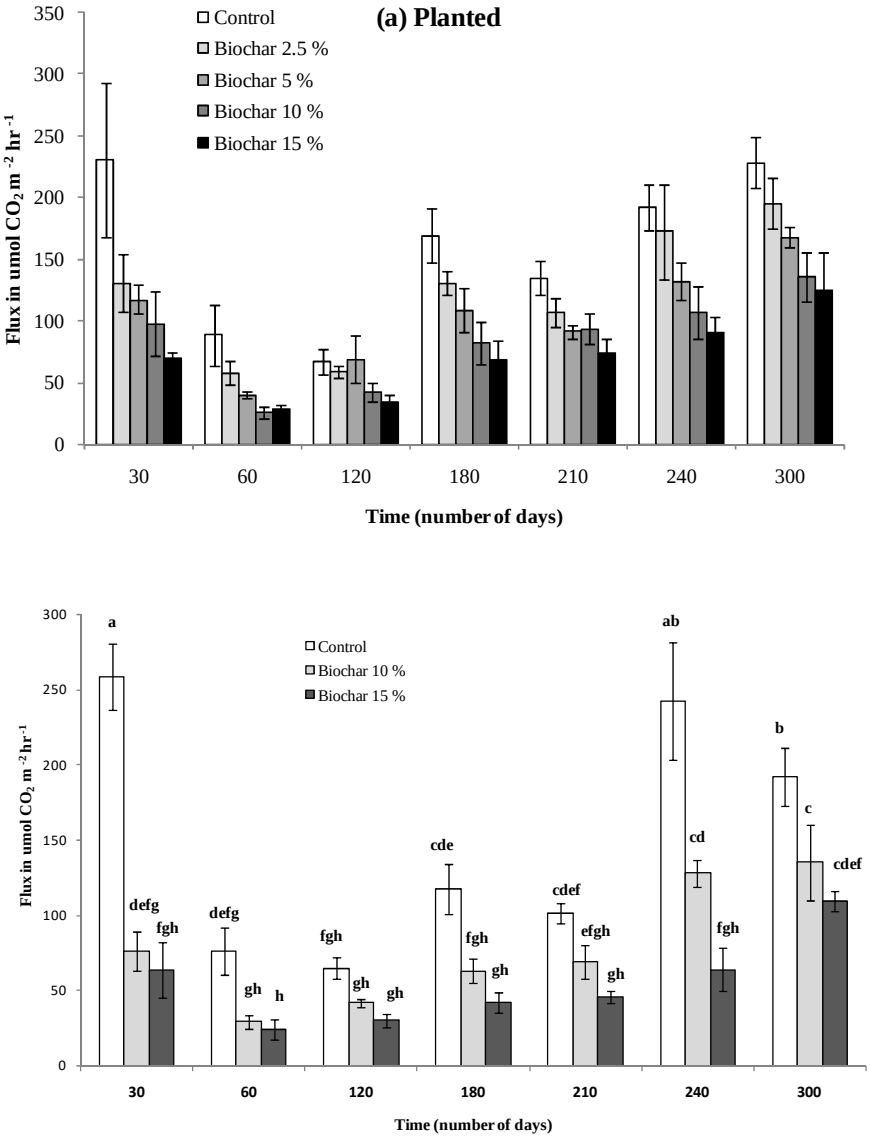


Figure.5.14 Effect of Time x Biochar interaction in CO_2 Flux (µ mol CO_2 m^{-2} hr^{-1})
(a) **With plants** (Time x Biochar: $F_{24, 120}$ = 0.96, P 0.495) (b) **without plants** (Time x Biochar: $F_{12, 72}$= 5.59, P <0.001). Columns similarly superscripted and not significantly different.

Figure 5.14 presented the effect of Time x Biochar interaction (planted: Time x Biochar: $F_{24, 120}$ = 0.96, *P* 0.495; unplanted : Time x Biochar: $F_{12, 72}$= 5.59, *P* <0.001) in producing

significant changes in CO_2 flux measured over the period of one year in all treatments with or without strawberry plants. In planted (Figure 5.143: a) the effect of time x biochar interaction was statistically non-significant but the data was presented here for comparison.

In unplanted (Figure 5.14: b) the highest CO_2 flux values were reported in control treatments, where no biochar was applied at every sampling interval in 370 days experimental period as compared to biochar amended treatments (10 and 15%). CO_2 fluxes were high at day 30, 240 and 300 as compared to 60, 120, 180 and 210 days. The interaction of time x plant, plant x biochar and time x BC x plant interactions, were proved statistically non-significant in producing any difference in CO_2 flux for all the treatments.

5.5.6.2 CH_4 Flux

Data obtained for CH_4 flux in soil showed that time (planted: Time: $F_{3,60}$ = 24.04, *P* <0.001; unplanted: Time: $F_{3,36}$= 16.28, *P* <0.001) was proved statistically significant as an individual factor for both planted and unplanted treatments. The effect of biochar as a factor proved to be significant only in unplanted treatments (Biochar: $F_{2, 8}$ = 7, *P* 0.018); however it has failed to produce any difference in CH_4 flux in presence of strawberry plants. Plant (Plant: $F_{1, 20}$ = 4.83, *P* 0.040) as a factor was also proved non-significant for CH_4 flux in all treatment with or without biochar application.

Figure 5.15 presented the effect of Biochar (planted: Time x Biochar: $F_{12,60}$ = 2.93, *P* 0.013; unplanted : Time x Biochar: $F_{3,36}$ = 4.28, *P* 0.006) in producing statistically significant changes in CH_4 flux measured over the period of one year in all treatments with or without strawberry plants. In planted (Figure 5.15: a) the highest CH_4 flux was recorded at day 30,

followed by the values determined at day 300 at the end of experiment. At day 60,180, 210 and 240, the lowest values of CH_4 flux were noted.

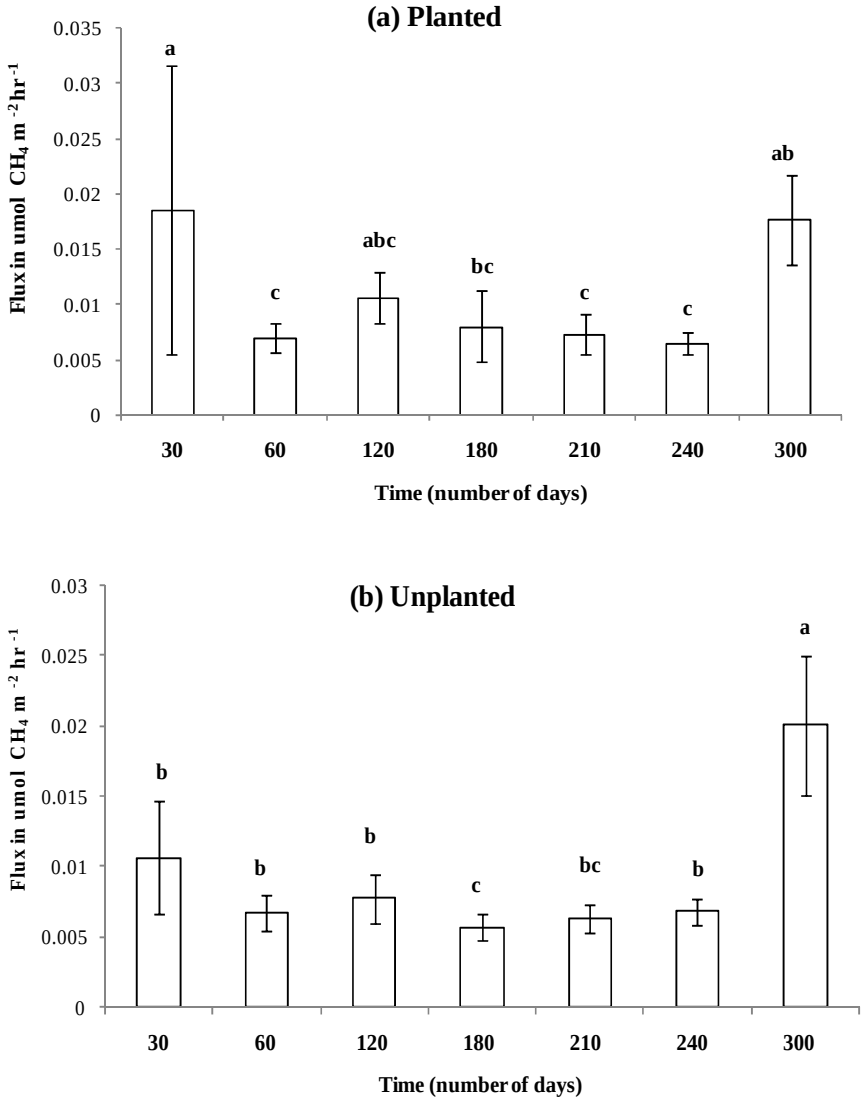


Figure.5.15 Changes in CH_4 Flux (µ mol CH_4 m^{-2} hr^{-1}) (a) with plants (Time: $F_{6,120}$ = 3.59, P= 0.056) **(b) without plants** (Time: $F_{6,72}$= 9.92, *P* <0.001). Columns similarly superscripted and not significantly different.

In unplanted (Figure 5.15: b) the pattern of CH_4 flux was same as reported for planted treatments. The highest flux of CH_4 was reported at day 300 at the end of experiment, followed by the fluxes reported at 30 day and 120 days. The lowest fluxes of CH_4 were reported for the measurements made at 180, 210 and 240 days.

The interaction of time x biochar, biochar x plant, time x plant and time x BC x plant, were proved statistically non-significant in producing any difference in CH_4 flux for all the treatments.

Table.5.3 p value for ecosystem gas exchange in biochar amended soils in presence or absence of strawberry plants

Group	Experimental factors	d.f	CH_4 Flux in µmol CH_4 m^{-2} hr^{-1}	CO_2 Flux in µmol CO_2 m^{-2} hr^{-1}
Planted	Biochar	4	0.95	**<.001**
	Time	6	**0.056**	**<.001**
	Time *Biochar	24	0743	0.495
	Residuals	120		
Unplanted	Biochar	2	**0.018**	**<.001**
	Time	6	**<.001**	**<.001**
	Time *Biochar	12	0.272	**<.001**
	Residuals	72		
Planted x Unplanted	Biochar	2	0.532	**<.001**
	Time	6	**0.003**	**<.001**
	Plant	1	0.247	0.214
	Time *Biochar	12	0.305	**<.001**
	Time *Plant	6	0.393	0.227
	Plant *Biochar	2	0.813	0.856
	Time*Biochar*Plant	12	0.797	0.632
	Residuals	144		

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

5.6 Discussions

Enzymes are important ecological drivers of biogeochemical cycle and have irreplaceable role in soil organic matter conversion, nutrient release, fertility management through microbial degradation (Aponte *et al*., 2020; Ibrahim *et al*., 2020; Jiang *et al*., 2021). Soil enzyme activities are sensitive quality indicators and used as proxies for determining the result of various natural and anthropogenic interventions on soils and microbial communities

(Liao *et al*., 2022; Wojewódzki *et al*., 2022). The response of intracellular or extracellular microbial enzymes to biochar addition in soil directly or indirectly linked to following aspects:

- Feedstock of biochar (Ouyang *et al*., 2014),
- Pyrolysis temperature (Gasco *et al*., 2016),
- Level of application (Oleszczuk *et al*., 2014; Saffari *et al*., 2020),
- Soil characteristics (Awad *et al*., 2013; Ameloot *et al*., 2013), and
- Nature and types of enzymes (Bailey *et al*., 2011; Biederman and Harpole, 2013; Wang *et al*., 2016; Gao *et al*., 2019; Li *et al*., 2020).

In the present study we investigated the changes in the activities of three key soil enzymes Dehydrogenase, Betaglucosidase and Phosphatase, because these key enzymes regulate functions related to nutrient cycling and soil health. Soil dehydrogenase, is an intracellular enzyme mainly catalyze oxidation-reduction reactions, and its activity can reflect the respiration or functional potential of soil microorganisms (Khalid *et al*., 2020 Amoakwah *et al*., 2022 Liao *et al*., 2022; Ahmad *et al*., 2022). Phosphatase, an extracellular enzyme, can originate from both plants and soil microorganisms (Janes-Bassett *et al*., 2022; Nannipieri *et al*., 2011; George *et al*., 2011), catalyse the hydrolysis of ester phosphate bonds, leading to the release of phosphate (P), which can be taken up by plants or microorganisms (Janes-Bassett *et al*., 2022). Betaglucosidase is involved in the degradation of soil organic matter in soils and has potential for monitoring biological soil quality (Tang *et al*., 2020; Liu *et al*., 2022). In our study, the negative effect of biochar was reported on dehydrogenase, betaglucosidase and phosphatase activities. There was a declining trend reported in enzyme activities with increasing amount of biochar applied to soil. This was supported by various recent studies (Paz-Ferreiro *et al*., 2012; Feng *et al*., 2018; Guan *et al*., 2019 ; Li *et al*., 2021),

who reported the negative correlation between biochar and enzyme activities. This concept was further explained by the fact that when organic substances of anthropogenic origin specifically biochar added to the soil, it failed to act like a substrate for enzymes that's why highest activities were reported in control soil treatment, where the natural organic matter of soil exist (Amoakwah *et al*., 2022).

In biochar amended soils, the lack of relationship between the introduced organic carbon and the enzymatic activity of soils may be related to the low contribution of humic substances from biochar in the total content of soil organic matter, which limits the availability of easily digestible carbon, which determines the growth of soil enzyme-producing bacteria. Enzymes can be bound in humic complexes present at biochar surfaces, which can protect enzyme proteins, but high molecular weight substrates deactivated enzymes (Wojewódzki *et al*., 2022).

In contrast to our findings, the positive effect of biochar on enzyme activities was reported in number of research studies (Herath *et al*., 2013; Ameloot *et al*., 2013; Oleszczuk *et al*., 2014; Saffari *et al*., 2020; Khalid *et al*., 2020; Jabborova *et al*., 2021; Ahmad *et al*., 2022 ; Amoakwah *et al*., 2022). The possible reasons for boost in enzyme activities after biochar addition could be; biochar can act as a source of food, carbon and mineral nutrients for microorganisms; biochar ameliorate soil environment by modifying soil physicochemical properties, which results in high microbial growth and enzyme production (Jiang *et al*., 2021). However at the same time, negligible or no effect of biochar addition on soil microbial enzymes and microorganisms was also reported (Elzobair *et al*., 2016).

The effect of time was proved statistically significant but failed to produce any consistent effect in all three enzymes (dehydrogenase, phosphatase and betaglucosidase) in both planted and unplanted treatments. In some treatments, initially high enzyme activity was noted but with the time, the rate of activity dropped. The initial high enzyme activity accelerate degradation rate of soil organic matter, leading to the depletion of easily digestible organic carbon (Wojewódzki *et al*., 2022). When soil organic carbon content was low, the activity of enzymes may be inhibited due to lack of substrates and energy (Zhang *et al*., 2019; Pokharel *et al*., 2020). Li *et al*., 2020 also reported initial increase and thereafter decrease in enzyme activities after biochar addition. Biochar tends to bind the active sites of extracellulaer enzymes that interferes the substrate diffusion which ultimately lowers down the rate of enzyme kinetics (Lehmann *et al*., 2011; Nannipieri *et al*., 2012; Ameloot *et al*., 2013; Gul *et al*., 2015).

Soil microbial biomasses are an important microbiological indicator for reflecting the environmental changes in soil and soil quality. It has been recognized as an important source of nutrients to plants because of its fast and significant turnover. The results of microbial biomass carbon and microbial biomass nitrogen indicated the possible increase or decrease in microbial carbon use efficiency and carbon turnover in response to biochar addition (Jiang *et al*., 2021; Ahmad *et al*., 2022).

In our study effect of biochar was proved non-significant in producing any difference in microbial biomass carbon and nitrogen. The possible reason for negligible or no effect of biochar was because: sometimes soil microbes failed to respond to increased decomposable substrate (Sugihara *et al*., 2014), or due to the absence of enough moisture to stimulate the microbial activity (Ahmad *et al*., 2022). However biochar application significantly increase

the surface soil organic carbon content, that overall creates a positive carbon budget (Agegnehu *et al*., 2015; El-Naggar *et al*., 2018)

Time has significant effect on both microbial biomass carbon and nitrogen which shows that biochar exposure in soil for long period of time increase the overall carbon content of soil. The change in total carbon content of soil is positively correlated with soluble organic matter content, clay content in soil humus, number of microorganisms and their activities (Wang *et al*., 2015). The addition of biochar increases carbon content of soil and resulted in improved C/N ratio and in turn increased microbial and enzyme activities (Tu *et al*., 2020; Jabborova *et al*., 2021 ; Jiang *et al*., 2021).

CO_2 emission and accumulation in soil and atmosphere are most important environmental threats of today's world and the major reason of global climate change. Biochar is a well known strategy to reduce carbon dioxide emission and global warming (Zhang *et al*., 2018; He *et al*., 2021; Khan *et al*., 2021). In our study biochar has significant effect on CO_2 and CH_4 flux. The rate of CO_2 flux was showing decrease with increasing biochar amount in both planted and unplanted treatments. These results are in line with the results reported by Sakin *et al*., 2021; Liao *et al*., 2022; Seki *et al*., 2021; Benbi and Brar, 2022 and Ahmad *et al*., 2022.The possible reasons behind this decline in carbon dioxide emission is because of high resistance of biochar to microbial decomposition.

Biochar application in soil increases soil C pool due to the difference in C/N ratio which likely affect and reduce the microbial activities (Lehmann *et al*., 2011; Al-Wabel *et al*., 2018; Li *et al*., 2018). Lot of factors are held responsible for affecting CO2 flux such as soil moisture and temperature (Zhou et al. (2017)) and microbial factors such as microbial

biomass carbon and metabolic rate (Schmidt et al., 2011). Many studies have been conducted under controlled conditions like our research on the impact of biochar addition on soil respiration (Gul *et al*., 2015; El-Naggar *et al*., 2018; Senbayram *et al*., 2019. They all concluded that biochar not only reduces gas emission from soil but also help in reducing global warming by sequestering carbon in soil.

5.7 Conclusions

It was concluded that biochar application would a sustainable and effective option to prevent or recover the soil health by increasing total soil carbon content. Biochar application in soil not only lowers down the possible degradation of organic matter but also induce changes in enzyme activity and microbial community composition, which could possibly be involved in degradation and mineralization. Biochar application could be a successful strategy to develop effective C management.

Chapter 6 Effects of wood biochar application on Soil physical and chemical properties

6.1 Overview

The results discussed in this chapter were obtained from the main experiment conducted to assess the potential of Biochar as soil amendment in terms of influencing soil physical and chemical properties in presence or absence of strawberry plants as regulator of soil-plant system.

6.2 Introduction

Biochar, a product of organic material pyrolysis, has been credited with many desirable properties, when applied as a soil amendment after the realization of high levels of sustained fertility in the Terra Preta soils that contain 70 percent more black carbon than the surrounding soils in the humid tropics of the Amazon Basin (O'Neill *et al.,* 2009). Biochar is perceived as being similar to activated carbon which is known to react or adsorb reactive molecules such as organic compounds (Hilber *et al.,* 2009), potentially modifying their ultimate bioavailability consequently enhancing crop productivity (Yang *et al.,* 2009).

Soil functions depend on three key properties, physical, chemical, and biological, and biochar has been applied as an amendment because of its strong manipulating impacts on soil properties. Positive effects of biochar have been reported on soil nutrient status and C sequestration (Glaser *et al*., 2002; Lehmann *et al.,* 2008), As a soil conditioner (Novotny *et*

al., 2009); Fertility enhancer (Van Zwieten *et al.,* 2010); reducing green house gases emission (Lehmann *et al*., 2006); boosting effects on soil biota (Lehmann *et al*., 2011); excellent sorbent of pollutants (Yu *et al.,* 2009) ; facilitate supply of hormones (Kim *et al.,* 2007).

6.3 Research Questions/Hypothesis

The research questions addressed in this chapter were as follows:

1. How Biochar got a status of soil improver/amendment, when it was mainly produced as waste product from demolished wood containing lots of impurities and high molecular organic compounds?

2. How high pyrolysis biochar affects physicochemical premises of soil either positively or negatively?

We hypothesized that biochar as a soil amendment improves physical and chemical properties of soil.

6.4 Material and Methods

6.4.1 Soil physical properties

Soil was sampled after 30, 90, 180 and 370 days and analyzed for soil physical properties. For detailed method refer to section 2.3.2 in chapter 2.

6.4.1.1 Soil moisture

For detailed method refer to section 2.3.2.1 in chapter 2.

6.4.1.2 Soil bulk density

For detailed method refer to section 2.3.2.2 in chapter 2.

6.4.1.3 Water filled pore space

For detailed method refer to section 2.3.2.3 in chapter 2.

6.4.2 Soil chemical properties

Soil chemical properties were measured at four different time intervals during the whole experimental period which were; 30, 90, 180 and 370 days. For detailed method refer to section 2.3.1 in chapter 2.

6.4.2.1 Soil pH

For detailed method refer to section 2.3.1.1 in chapter 2.

6.4.2.2 Loss on ignition

For detailed method refer to section 2.3.1.2 in chapter 2.

6.4.2.3 Ammonium nitrogen

For detailed method refer to section 2.3.1.3 in chapter 2.

6.4.2.4 Nitrate nitrogen

For detailed method refer to section 2.3.1.3 in chapter 2.

6.4.2.5 Total carbon

Total carbon was measured by CNS analyzer. For detailed method refer to section 2.3.1.7 in chapter 2.

6.4.2.6 Total nitrogen

Total nitrogen was measured by CNS analyzer. For detailed method refer to section 2.3.1.7 in chapter 2.

6.4.2.7 Total sulphur

Total sulphur was measured by CNS analyzer. For detailed method refer to section 2.3.1.7 in chapter 2.

6.4.2.8 Total soil nutrients

Total sulphur was measured by CNS analyzer. For detailed method refer to section 2.3.1.7 in chapter 2.

6.4.2.9 Available soil nutrients

Total sulphur was measured by CNS analyzer. For detailed method refer to section 2.3.1.7 in chapter 2.

6.4.3 Statistical analysis

Data were subjected to two way analysis of variance (ANOVA) and treatment means separated by the Tukey's test, at 5 % probability error, using the Genstat 17th edition.

6.5 Results

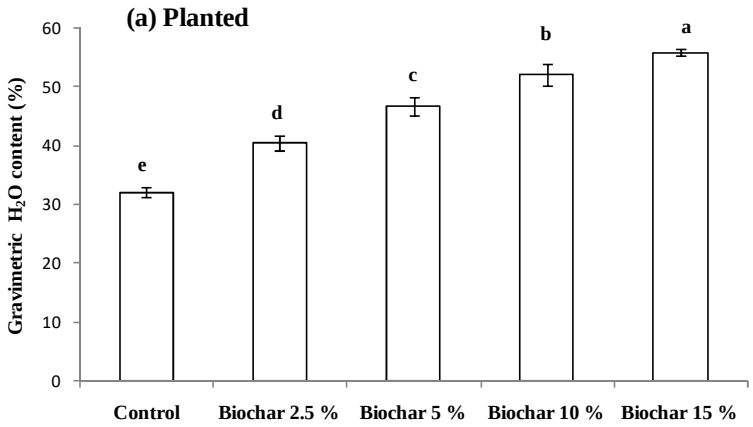
The recorded observations for soil properties were interpreted and presented in following paragraphs:

6.5.1 Changes in Soil Physical Properties

Soil physical properties (gravimetric water content, soil bulk density, and water filled pore space and saturation percentage) were discussed and presented in following paragraphs:

Gravimetric H_2O content

Data obtained for gravimetric H_2O content in soil showed that biochar (planted: Biochar: $F_{4,16}$ = 61.33, P <0.001; unplanted : Biochar: $F_{2,8}$ = 90.63, P <0.001) and time (planted : Time: $F_{3,60}$ = 127.82, P <0.001; unplanted: Time: $F_{3,36}$= 121.58, P <0.001) were proved statistically significant as individual factor for both planted and unplanted treatments. Presence of strawberry plant (Plant: $F_{1, 20}$ = 8.05, P 0.01) was also proved significant for gravimetric H_2O content in all treatment with or without biochar application.



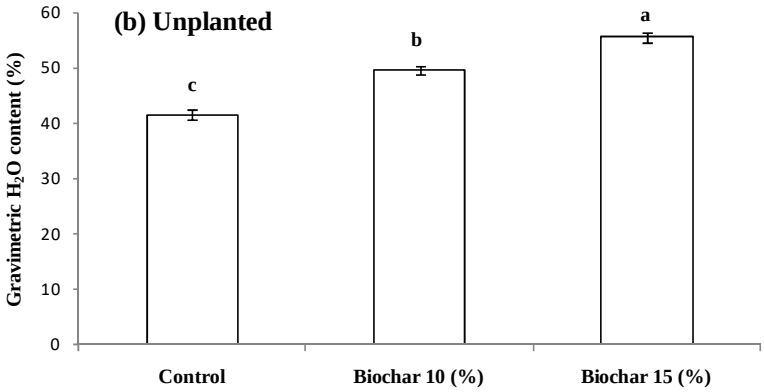
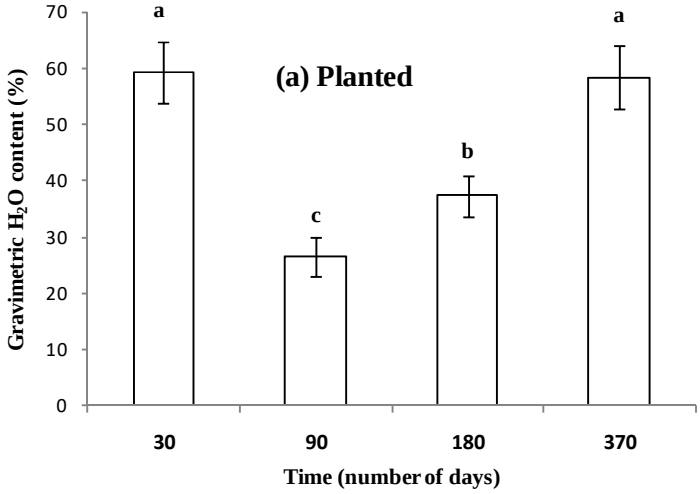


Figure 6.1 Effect of biochar on changes in gravimetric soil H_2O content (%)
(a) with plants (Biochar: $F_{4,16} = 61.33$, $P < 0.001$) (b) without plants (Biochar: $F_{2,8} = 90.63$, $P < 0.001$). Columns similarly superscripted and not significantly different.

Figure 6.1 presented the effect of Biochar (planted: Biochar: $F_{4,16} = 61.33$, $P < 0.001$; unplanted : Biochar: $F_{2,8} = 90.63$, $P < 0.001$) in producing statistically significant changes in gravimetric H_2O content measured over the period of one year in all treatments with or without strawberry plants. In planted (Figure 6.1: a) the highest gravimetric H_2O content was recorded in treatment with biochar@ 15%, followed by the treatments where biochar was applied @ 10, 5, and 2.5%.



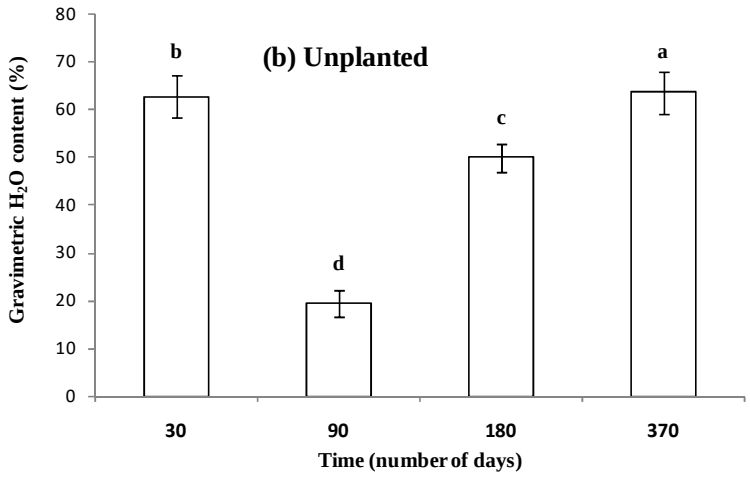


Figure 6.2 Effect of time on changes in gravimetric soil H_2O content (%)
(a) with plants (Time: $F_{3,60}$ = 127.82, P <0.001) (b) without plants (Time: $F_{3,36}$= 121.58, P <0.001). Columns similarly superscripted and not significantly different.

The lowest value was reported in the control treatment with no biochar. There was increasing trend in water content in all treatments with increase in biochar level. In unplanted (Figure 6.1:b) the highest gravimetric H_2O content was reported in treatment where biochar was applied @15% in 370 days experimental period followed by treatments with biochar 10% and control. There was a slight statistically significant difference in water content of these treatments over the time.

Figure 6.2; a, represented the effect of time (planted: Time: $F_{3,60}$ = 127.82, P <0.001; unplanted: Time: $F_{3,36}$= 121.58, P <0.001) on gravimetric H_2O content over a period of one year. The highest value was reported at day 30, which was almost equal to the water contents recorded at 370 days at the end of experimental period. After 30 days, there was a significant decrease in gravimetric contents reported at 90 days and 180 days.

Figure 6.2; b, the same pattern was observed for gravimetric contents. As the highest contents were reported at day 30 and day 370 followed by the values recorded at day 180 and day 90. The lowest water content at day 90 was after the sharp decrease in values noted at day 30.

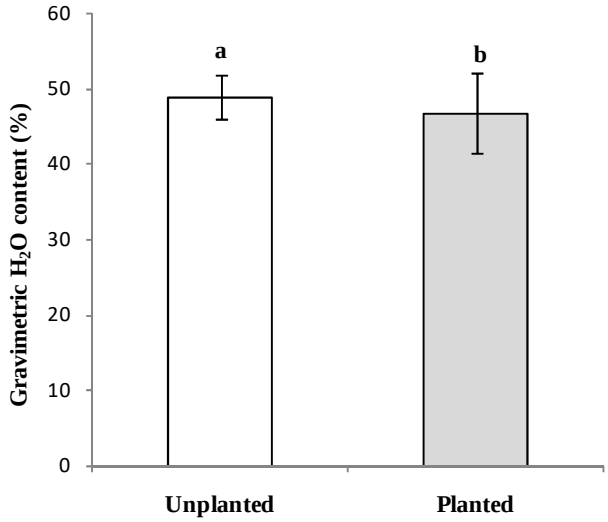


Figure 6.3 Effect of plant on changes in gravimetric soil H_2O content (%)
(Plant: $F_{1, 20} = 8.05$, *P* 0.01). Columns similarly superscripted and not significantly different.

Figure 6.3 showed the effect of plant on gravimetric contents of treatments with or without biochar. The higher water contents were reported in unplanted treatments. The interaction of time x plant and plant x biochar was also proved significant for gravimetric water contents, however time x biochar and time x BC x plant interactions were proved statistically non-significant in producing any difference in water contents for all the treatments.

Water filled pore space

Data obtained for water filled pore space in soil showed that biochar (planted: Biochar: $F_{4,16} = 2.38$, *P* 0.095; unplanted : Biochar: $F_{2,8} = 11.08$, *P* 0.005) was proved statistically significant as individual factor for only unplanted treatments. The data for planted treatment was presented for comparison.

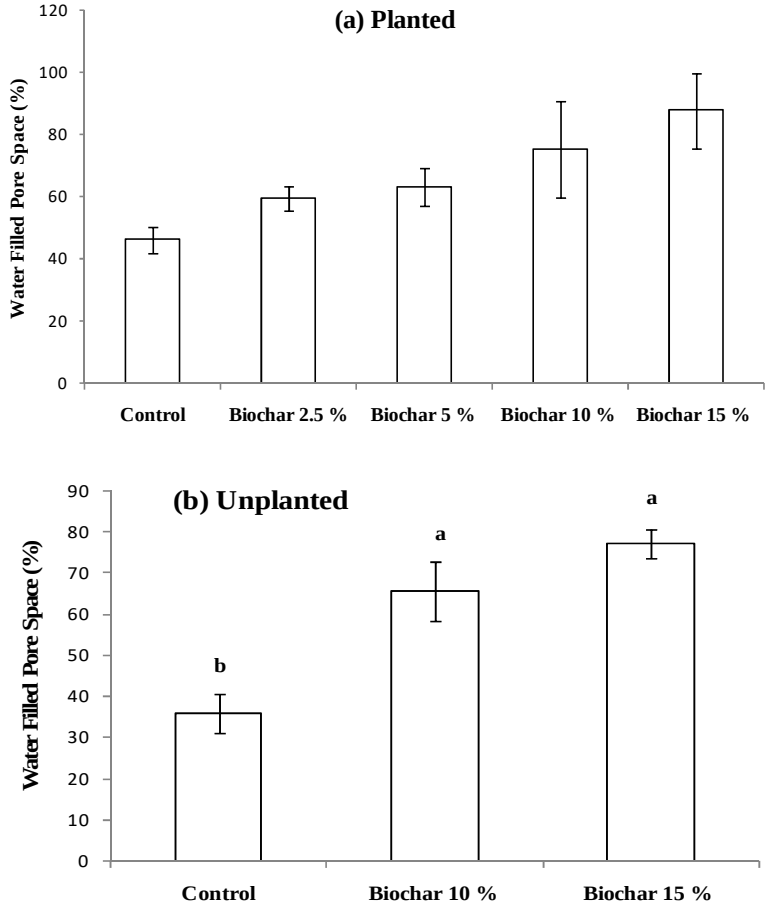


Figure 6.4 Effect of biochar on changes in water filled pore space (%)
(a)with plants (Biochar: $F_{4,16} = 2.38$, P 0.095) (b) without plants (Biochar: $F_{2,8} = 11.08$, P 0.005). Columns similarly superscripted and not significantly different.

Presence of strawberry plant and plant x biochar interaction was proved non-significant for water filled pore space in all treatment with or without biochar application.

Figure 6.4 presented the effect of biochar on changes in water filled pore space with varying levels of biochar applied with or without strawberry plants. In planted (Figure 6.4: a) the highest value was recorded in treatment with 15% biochar, followed by the treatments where biochar was applied @ 10, 5 and 2.5%. The lowest value for WFPS was reported in the control treatment with no biochar. The same trend was reported for unplanted (Figure 6.4:b),

the lowest value of WFPS was in control and a gradual increase was observed with biochar treatments 10 and 15%.

Soil bulk density

Data obtained for soil bulk density showed that biochar (planted: Biochar: $F_{4, 16} = 8.13$, $P < 0.001$; unplanted: Biochar: $F_{2, 8} = 8.13$, P 0.012) was proved statistically significant as individual factor for both planted and unplanted treatments. Presence of strawberry plant (Plant: $F_{1, 20} = 12.58$, P 0.002) was also proved significant for soil bulk density in all treatments with or without biochar application. In planted (Figure 6.5: a) the highest value for bulk density was recorded in control treatment with no biochar at the end of experimental period (370 days), followed by the treatments where biochar was applied @ 2.5, 5, 10 and 15%. The lowest bulk density was reported in the treatment with highest rate of biochar 15%. There was decreasing trend in bulk density in all treatments with increasing rates of biochar.

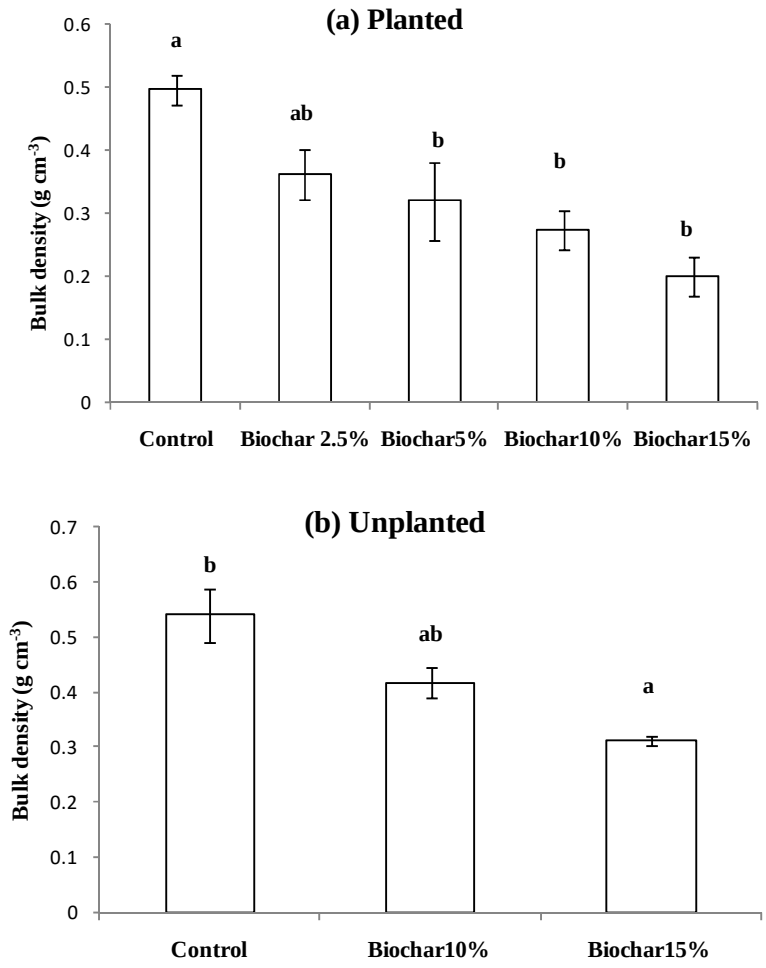


Figure 6.5 Effect of biochar on changes in Soil Bulk Density (g cm^{-3})
(a) with plants (Biochar: $F_{4, 16} = 8.13$, $P < 0.001$) (b) without plants (Biochar: $F_{2, 8} = 8.13$, P 0.012). Columns similarly superscripted and not significantly different.

In unplanted (Figure 6.5: b) the highest bulk density was reported in control treatment where no biochar was applied. There was a decrease in soil bulk density over the period of 370 days with increase in biochar. There was a slight difference in treatments with BC 10 and 15%.

Figure 6.6 represented the effect of presence or absence of strawberry plant on change in soil bulk density in biochar amended soils. The high values of bulk density were reported in the treatment where strawberry plants were absent as compared to the planted ones.

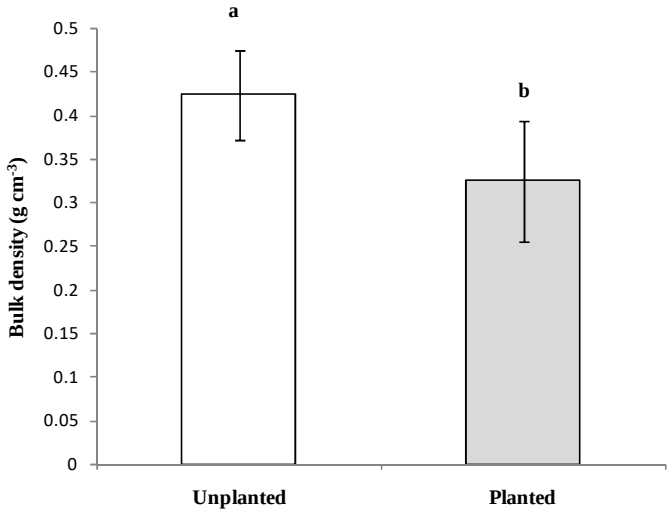


Figure 6.6 Effect of plant on changes in Soil Bulk Density (g cm^{-3})
(Plant: $F_{1, 20} = 12.58$, *P* 0.002). Columns similarly superscripted and not significantly different.

Table 6.1 p value of biochar amended soils for soil physical properties in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Soil moisture (%)	Bulk density (gcm^{-3})	Water Filled Pore Spaces (%)
Planted	Biochar	4	**<.001**	**<.001**	0.095
	Time	3	**<.001**	NA	NA
	Time *Biochar	12	0.074	NA	NA
	Residuals	60		**16**	**16**
Unplanted	Biochar	2	**<.001**	**0.012**	**0.005**
	Time	3	**<.001**	NA	NA
	Time *Biochar	6	0.214	NA	NA
	Residuals	36		8	8
Planted x Unplanted	Biochar	2	**<.001**	**<.001**	**0.002**
	Time	3	**<.001**	NA	NA
	Plant	1	**0.01**	**0.002**	0.241
	Time *Biochar	6	**<.005**	NA	NA
	Time *Plant	3	**<.001**	NA	NA
	Plant *Biochar	2	**<.001**	0.35	0.999
	Time*Biochar*Plant	6	0.591	NA	NA
	Residuals				

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

6.5.2 Changes in Soil Chemical Properties

Soil chemical properties were discussed and presented in following paragraphs:

Soil pH

Data obtained for soil pH showed that biochar (planted: Biochar: $F_{4, 16} = 50.54$, $P < 0.001$; unplanted: Biochar: $F_{2, 8} = 79.02$, $P < 0.001$) was proved statistically significant as individual factor for both planted and unplanted treatments. Presence of strawberry plant (Plant: $F_{1, 20} = 12.58$, P 0.002) was also proved significant for soil bulk density in all treatments with or without biochar application.

In planted (Figure 6.7: a) the lowest pH was recorded in control treatment with no biochar, with the increasing values reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The highest value for Soil pH was reported in the treatment where the highest rate of biochar 15% was applied. There was a smooth increasing trend in soil pH with the increasing rate of biochar.

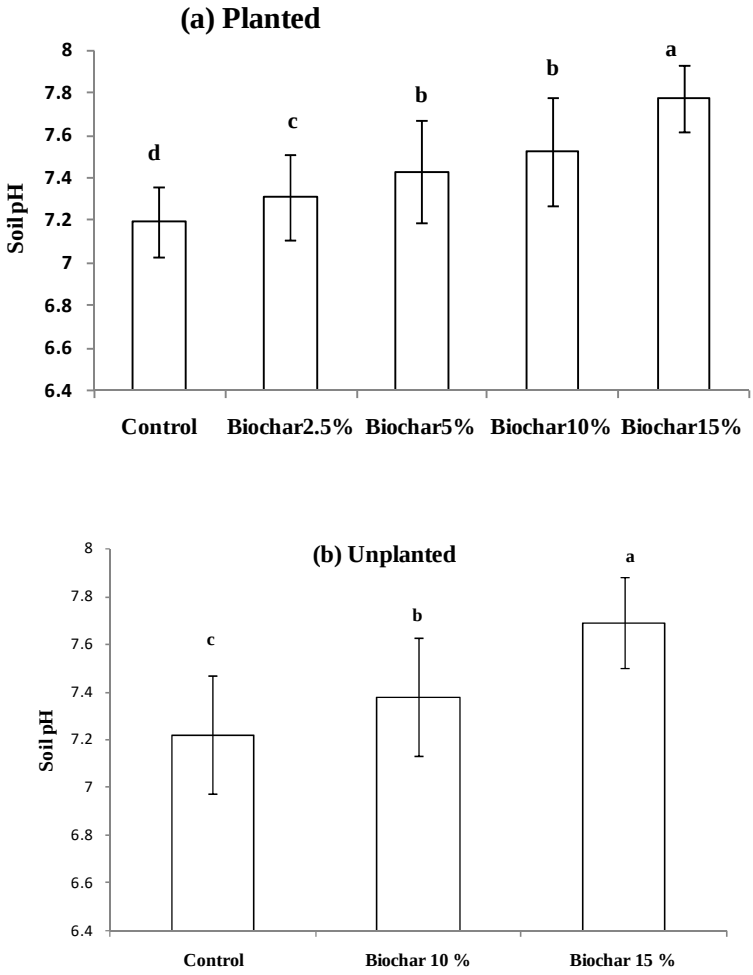


Figure 6.7 Effect of biochar on changes in Soil pH
(a)with plants (Biochar: $F_{4,16} = 50.54$, $P < 0.001$) (b) without plants (Biochar: $F_{2,8} = 79.02$, $P < 0.001$). Columns similarly superscripted and not significantly different.

In unplanted (Figure 6.7:b) the lowest pH was reported in control treatment where no biochar was applied. There was a gradual increase in soil pH in treatments with BC 10 and 15%.

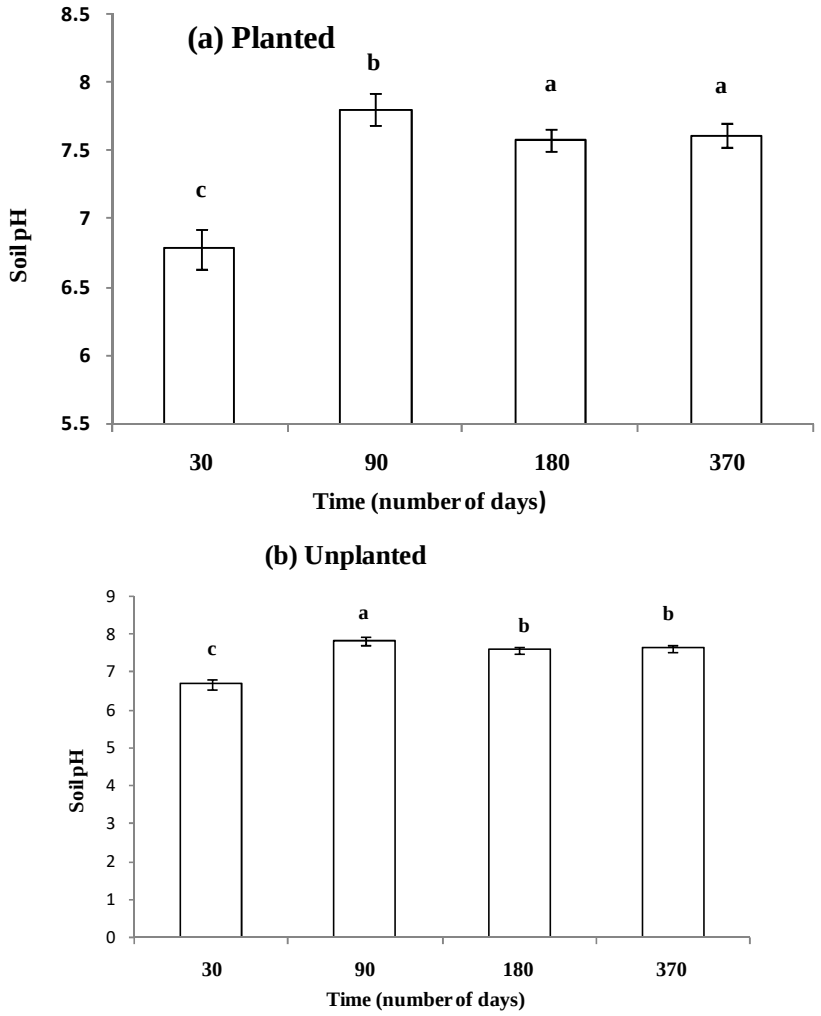


Figure 6.8 Effect of time on changes in soil pH (a) with plants (Time: $F_{3, 60} = 338.78$, $P < 0.001$) and (b) without plants (Time: $F_{3, 36} = 223.28$, $P < 0.001$). Columns similarly superscripted and not significantly different.

Results obtained showed that time was proved effective in changing soil pH over a year time in both planted and unplanted treatments. The response varied with biochar application rate. A major increase was observed in initial pH values recorded at 30 days (6.7 to 7.8 in planted ($F_{3, 60} = 338.78$, $P < 0.001$) Figure 6.8,a. 6.6 to 7.8 in unplanted ($F_{3, 36} = 223.28$, $P < 0.001$) Figure 6.8,b after 90 days but after that a stable pH value around 7.6 was stayed for rest of the experiment.

Organic matter content

Data obtained for organic matter content in soil showed that biochar (planted: Biochar: $F_{4,16}$ = 87.87, P <0.001; unplanted : Biochar: $F_{2,8}$ = 61.55, P <0.001) and time (planted : Time: $F_{3,60}$ = 13.11, P <0.001; unplanted: Time: $F_{3,36}$= 47.18, P <0.001) were proved statistically significant as individual factor for both planted and unplanted treatments. Presence of strawberry plant (Plant: $F_{1, 20}$ = 21, P< 0.001) was proved significant as a single factor for organic matter content in all treatment with or without biochar application.

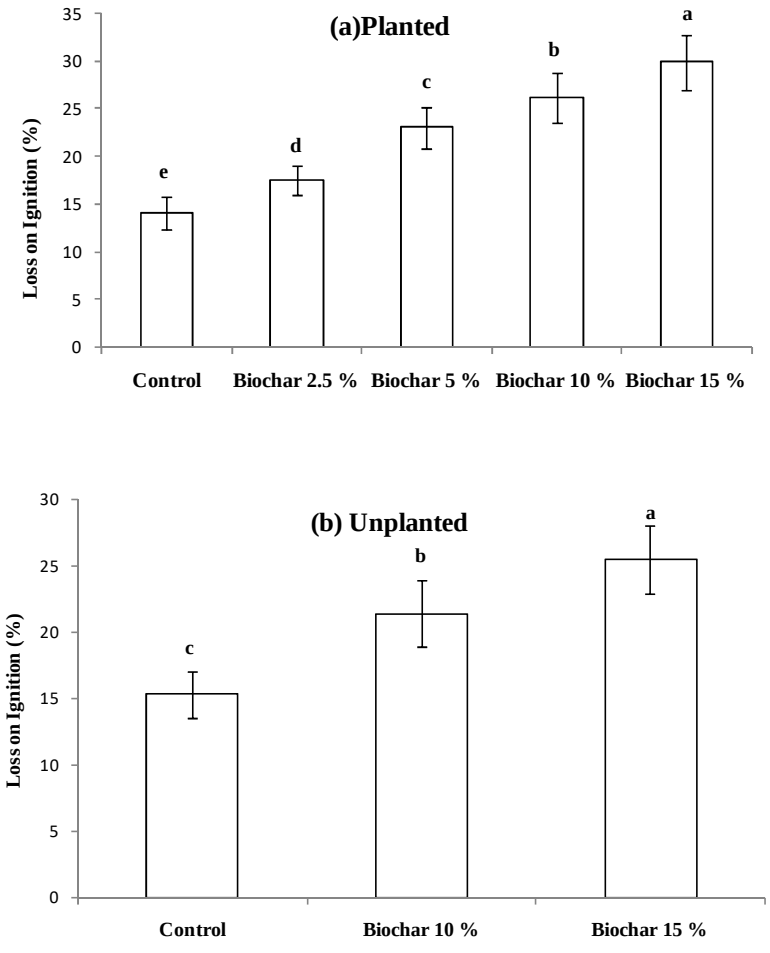


Figure 6.9 Effect of biochar applied at different rates on loss on ignition (%) (a) with plants (Biochar: $F_{4,16}$ = 87.87, P <0.001) and (b) without plants (Biochar: $F_{2,8}$ = 61.55, P <0.001) . Columns similarly superscripted and not significantly different.

The interaction of time x biochar and plant x biochar was also proved significant for soil organic matter content, however time x plant and time x BC x plant interactions were proved statistically non-significant in producing any difference in soil organic matter for all the treatments.

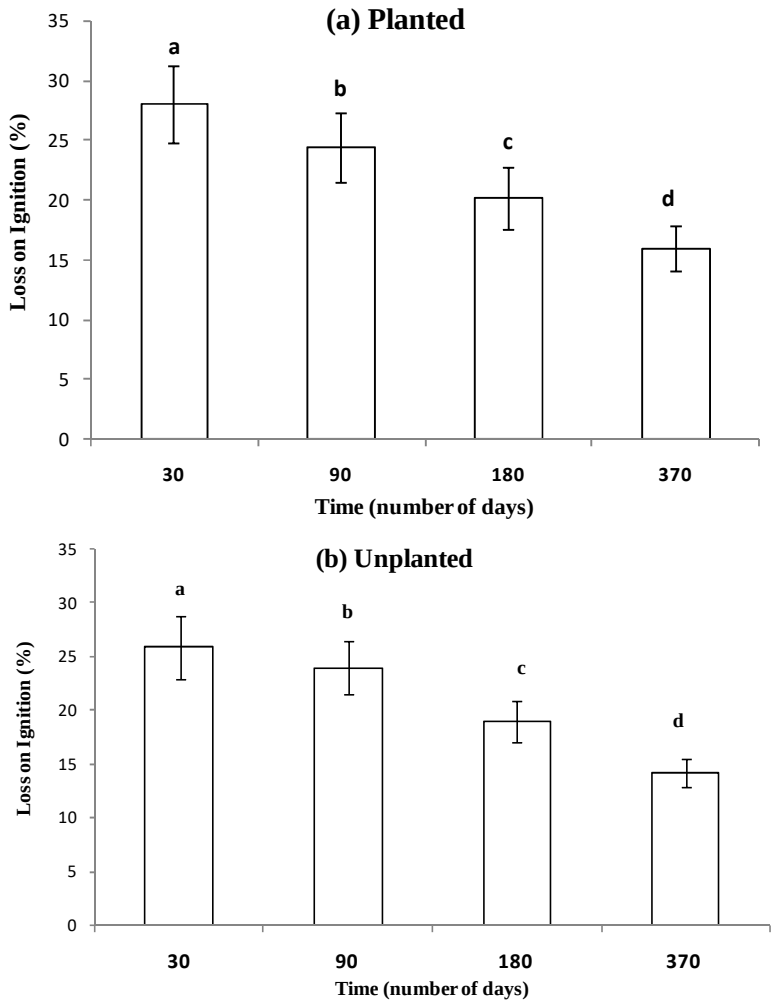


Figure 6.10 Effect of time on changes in loss on ignition (%)
(a) with plants (Time: $F_{3, 60} = 131.1$, $P < 0.001$) and (b) without plants (Time: $F_{3, 36} = 47.18$, $P < 0.001$) . Columns similarly superscripted and not significantly different.

In presence of plant biochar increased amount of organic matter with the percentage of biochar mixed in soil. All the treatments analyzed contain more OM than control. In 2.5 (%) biochar amended soil reported increase was 24 %, 51 % in 5 (%) biochar and 86 % in 10 (%) biochar amended soil. The highest value 30 was found in 15 (%) biochar which was 112 %

increase from control value 14.1 (Table 6.9, a). The similar trend was found in the treatments where organic matter was increased with increasing level of biochar: control < 10 % BC <15 % BC (Table 6.9, b).

Time has a very distinct and consistent effect on organic matter content of biochar enriched soils regardless of presence or absence of plant (Figure 6.10, a & b). High OM content was recorded in first soil sampling (30 days) in both planted and unplanted soil.

Percent decrease after 90 days observed in presence of plants was 15 %, 39 % after 180 days followed by 75 % decrease in initial OM after the completion of experiment (370 days). In unplanted 8% initial OM was decreased after 90 days accompanied with 36 % (180 DAS) and 81 % loss at the end of study. Presence of strawberry plant was also proved significant as the higher organic matter percentage (23.45 %) was reported in treatment where strawberry plants were present as compared to unplanted (20.76 %), although the percent increase was only 12.61 % (Figure 6.11).

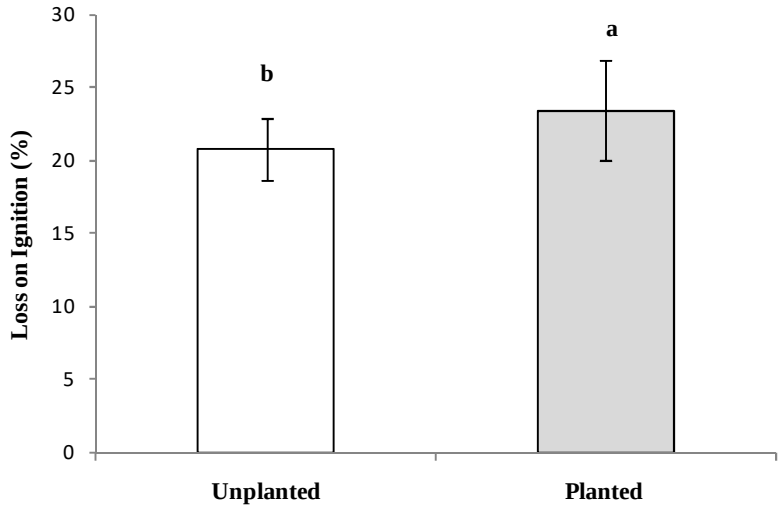


Figure 6.11 Effect of plant on Changes in organic matter (loss on ignition %)
(Plant: $F_{1, 20} = 21$, $P < 0.001$) . Columns similarly superscripted and not significantly different.

Nitrate nitrogen

Data obtained for nitrate nitrogen in soil showed that biochar (planted: Biochar: $F_{4,16}$ = 77.68, *P* <0.001; unplanted : Biochar: $F_{2,8}$ = 60.99, *P* <0.001) and time (planted : Time: $F_{3,60}$ = 436.68, *P* <0.001; unplanted: Time: $F_{3,36}$= 45.11, *P* 0.013) were proved statistically significant as individual factor for both planted and unplanted treatments.

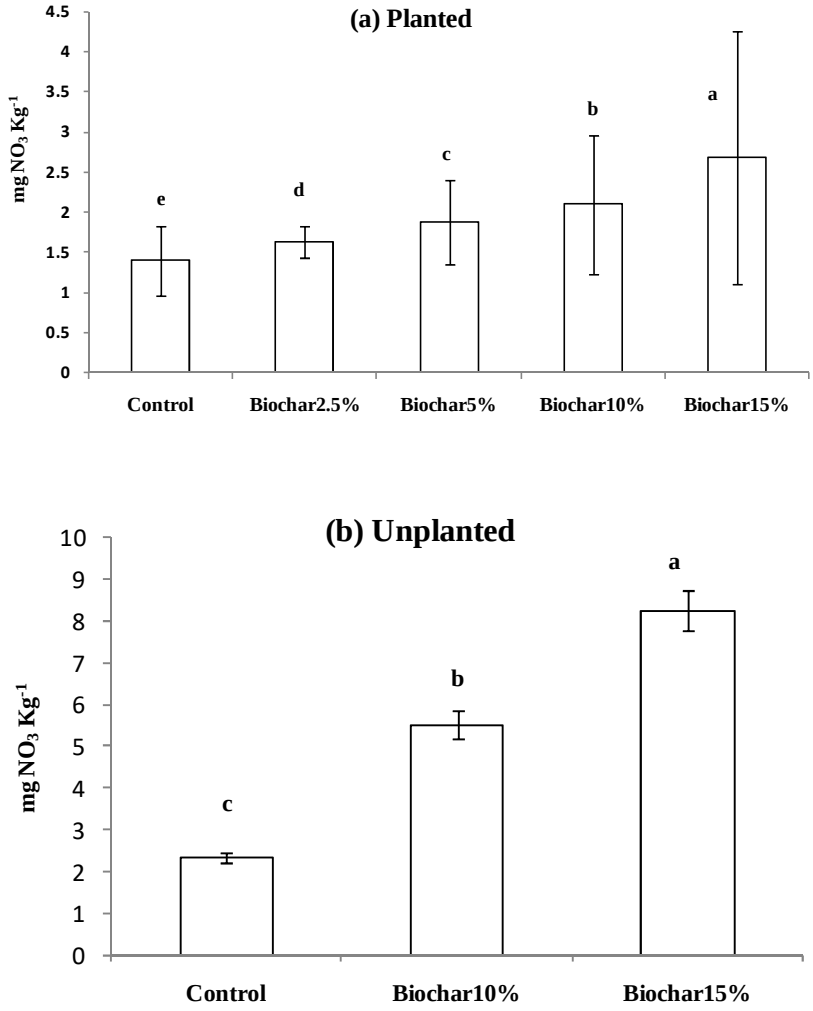


Figure 6.12 Effect of biochar amendment on Nitrate-N (mg NO_3 kg^{-1}) (a) with plants (Biochar: $F_{4,16}$ = 77.68, *P* <0.001) (b) without plants (Biochar: $F_{2,8}$ = 60.99, *P* <0.001) . Columns similarly superscripted and not significantly different.

Presence of strawberry plant (Plant: $F_{1, 20}$ = 247.74, P <0.001) was proved significant as a single factor for beta-glucosidase activity in all treatment with or without biochar application.

The interaction of time x biochar, time x plant, plant x biochar and time x BC x plant interactions were proved statistically significant in nitrate nitrogen for all the treatments.

In planted (Figure 6.12: a) the lowest NO_3-N was recorded in control treatment with no biochar, with the increasing values reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The highest amount was noted in the treatment where the highest rate of biochar 15% was applied.

In unplanted (Figure 6.12: b) the same increasing trend was noted like the planted treatments. The highest NO_3-N value was recorded in treatment where biochar was applied @15%. This was followed by treatment with BC 10% and control with 0 biochar.

Figure 6.13 represented the effect of time on nitrate nitrogen content over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted treatments (Figure 6.13: a) the maximum value was reported at the end of experiment (370 days) with the minimum observed at the start of experiment (30 days). There was a gradual increase in nitrate nitrogen with time.

In unplanted (Figure 6.13: b) the trend was inconsistent and the maximum value for nitrate nitrogen at 180 days with the minimum value for 90 days.

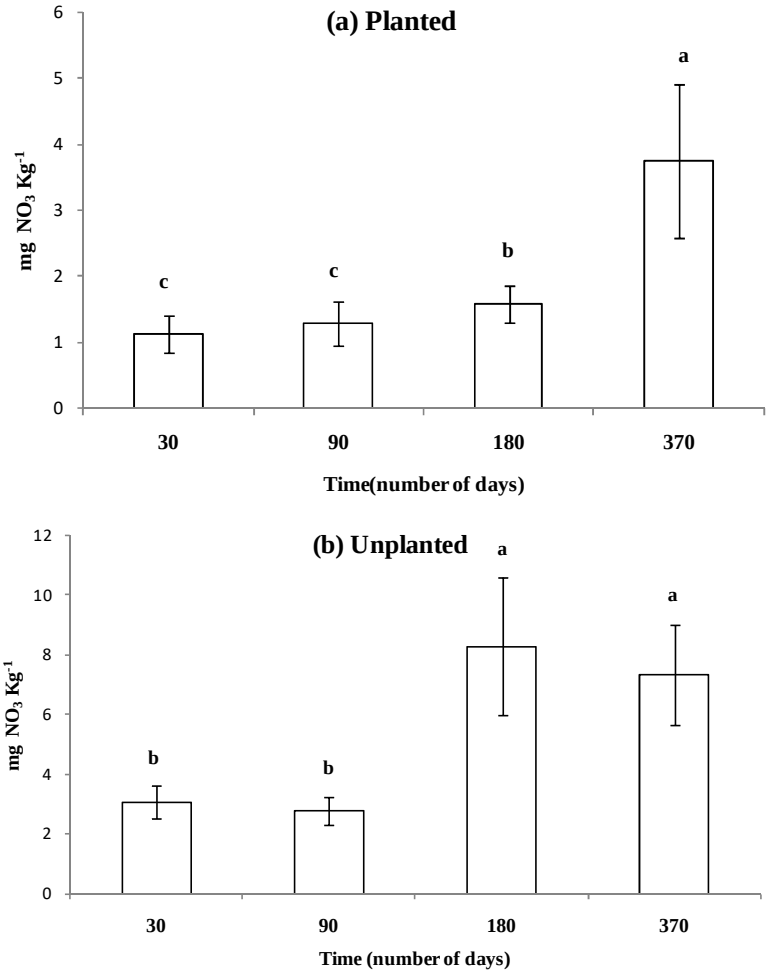


Figure 6.13 Effect of time on Nitrate-N (mg NO_3 kg^{-1}) (a) with plants (Time: $F_{3,60}$ = 436.68, *P* <0.001) (b) without plants (Time: $F_{3,36}$ = 45.11, *P* <0.001) . Columns similarly superscripted and not significantly different.

Figure 6.14 presented the effect of Time x Biochar interaction (planted: Time x Biochar: $F_{12,60}$ = 162.13, P <0.001; unplanted : Time x Biochar: $F_{6,36}$ = 8.14, P 0.003) in producing statistically significant changes in nitrate nitrogen measured over the period of one year in all treatments with or without strawberry plants.

In planted (Figure 6.14: a) the highest value was recorded in biochar 15% treatment at the end of experimental period (370 days), followed by the treatments where biochar was applied @ 10, 5, 2.5 %. The lowest value was reported in the control treatment where no biochar was applied at day 370.

The same trend was observed at day 180 for all the treatments with highest in 15% biochar followed by 10, 5 and 2.5 % biochar and control. The reverse trend was recorded at 30 days and 90 days. The highest nitrate nitrogen was recorded in control followed by 2.5, 5, 10 and 15 % biochar.In unplanted (Figure 6.14: b), again the same trend was reported in all values recorded throughout the experimental period. The lowest value was reported in control followed by 10 and 15 % biochar treatments.

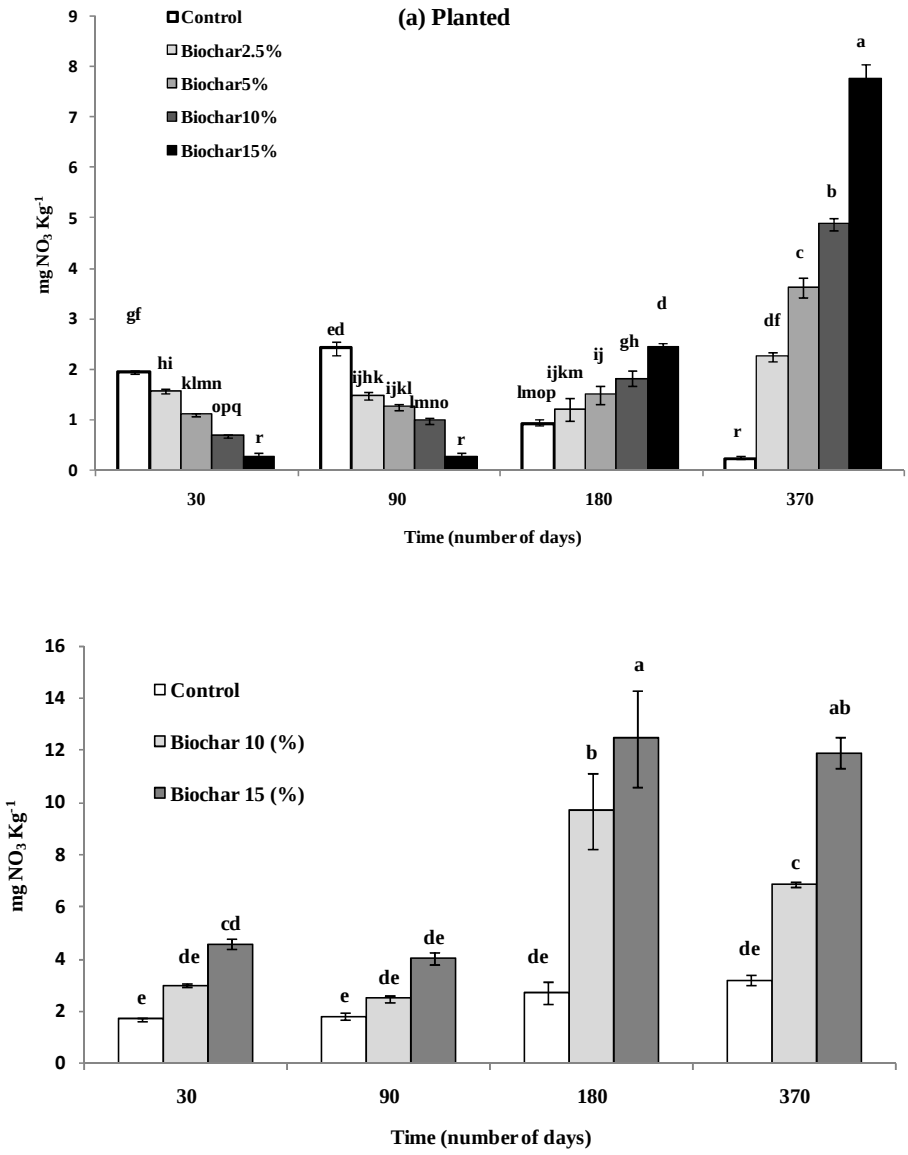


Figure 6.14 Effect of time x biochar interaction on Nitrate-N (mg NO_3 kg^{-1})
(a) with plants (Time x Biochar: $F_{12,60}$ = 162.13, *P* <0.001) (b) without plants (Time x Biochar: $F_{6,36}$ = 8.14, *P* 0.003) . Columns similarly superscripted and not significantly different.

Presence of strawberry plant (Plant: $F_{1, 20}$ = 247.74, P <0.001) was also proved significant for nitrate nitrogen in all treatment with or without biochar application (Figure 6.15). The maximum activity was noted in unplanted than planted treatments.

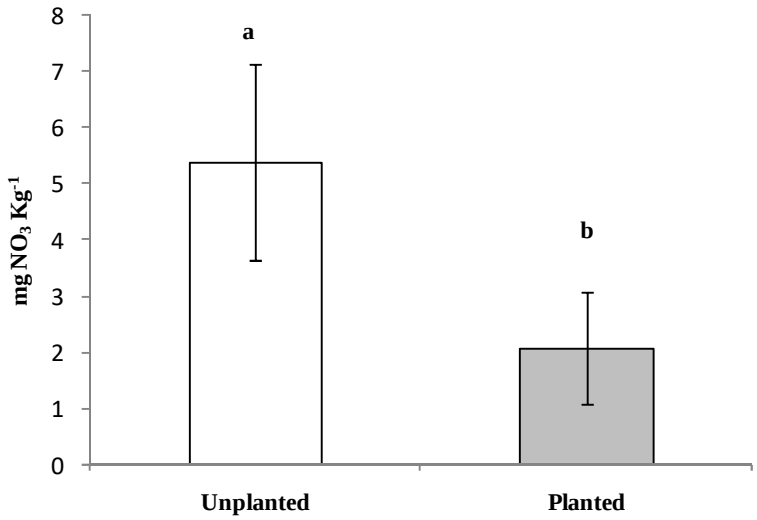


Figure 6.15 Effect of plant on Nitrate-N (mg NO_3 kg^{-1})
(Plant: $F_{1, 20}$ = 247.74, *P* <0.001) . Columns similarly superscripted and not significantly different.

Ammonium nitrogen

Data obtained for ammonium nitrogen in soil showed that time (planted: Time: $F_{3,60}$ = 5.45, *P* 0.025; unplanted: Time: $F_{3,36}$= 31.05, *P* <0.001) was proved statistically significant as individual factor for both planted and unplanted treatments. The effect of biochar was proved statistically non-significant for both planted and unplanted treatments. Presence of strawberry plant (Plant: $F_{1, 20}$ = 26.78, *P* <0.001) was also proved significant for ammonium nitrogen in all treatment with or without biochar application.

Figure 6.16 showed the effect of time on soil ammonium nitrate concentration. There was no actual difference in NH_4-N among control and biochar amended soil. However with time, the values were higher towards the end of experimental period.

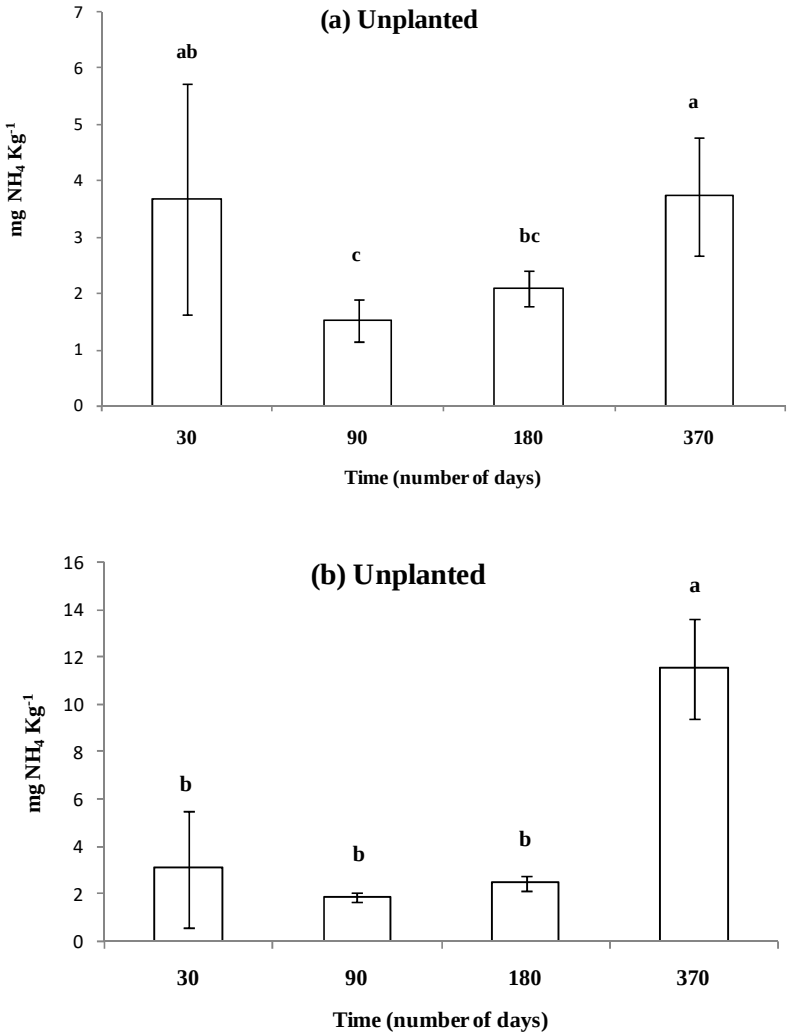


Figure 6.16 Effect of time on Ammonium-N (mg NH_4 kg^{-1})
(a) with plants (Time: $F_{3,60}$ = 5.45, *P* 0.025) (b) without plants (Time: $F_{3,36}$ = 31.05, *P* <0.001) . Columns similarly superscripted and not significantly different.

Presence of strawberry plant (Plant: $F_{1, 20}$ = 26.78, P <0.001) was also proved significant for ammonium nitrogen in all treatment with or without biochar application (Figure 6.17). The maximum activity was noted in unplanted than planted treatments.

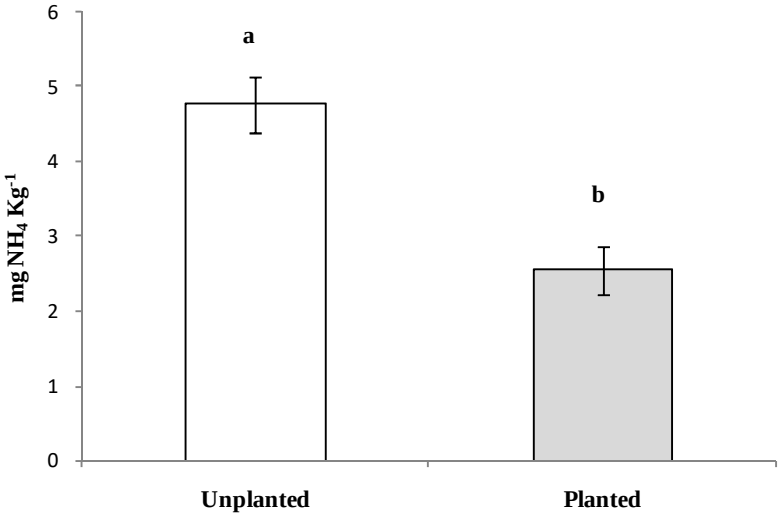


Figure 6.17 Effect of plant on Ammonium-N (mg NH_4 kg^{-1})
(Plant: $F_{1,20}$ = 26.78, *P* <0.001) . Columns similarly superscripted and not significantly different.

Olsen P

Data obtained for Olsen P in soil showed that time (planted: Time: $F_{3,60}$ = 29.16, *P* <0.001; unplanted: Time: $F_{3,36}$= 24.75, *P* <0.001) was proved statistically significant as individual factor for both planted and unplanted treatments.

In planted (Figure 6.18: a) the lowest Olsen P was recorded in control treatment with no biochar, with the increasing values reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The highest amount was noted in the treatment where the highest rate of biochar 15% was applied. In unplanted (Figure 6.18: b) the same increasing trend was noted like the planted treatments. The highest P value value was recorded in treatment where biochar was applied @15%. This was followed by treatment with BC 10% and control with 0 biochar.

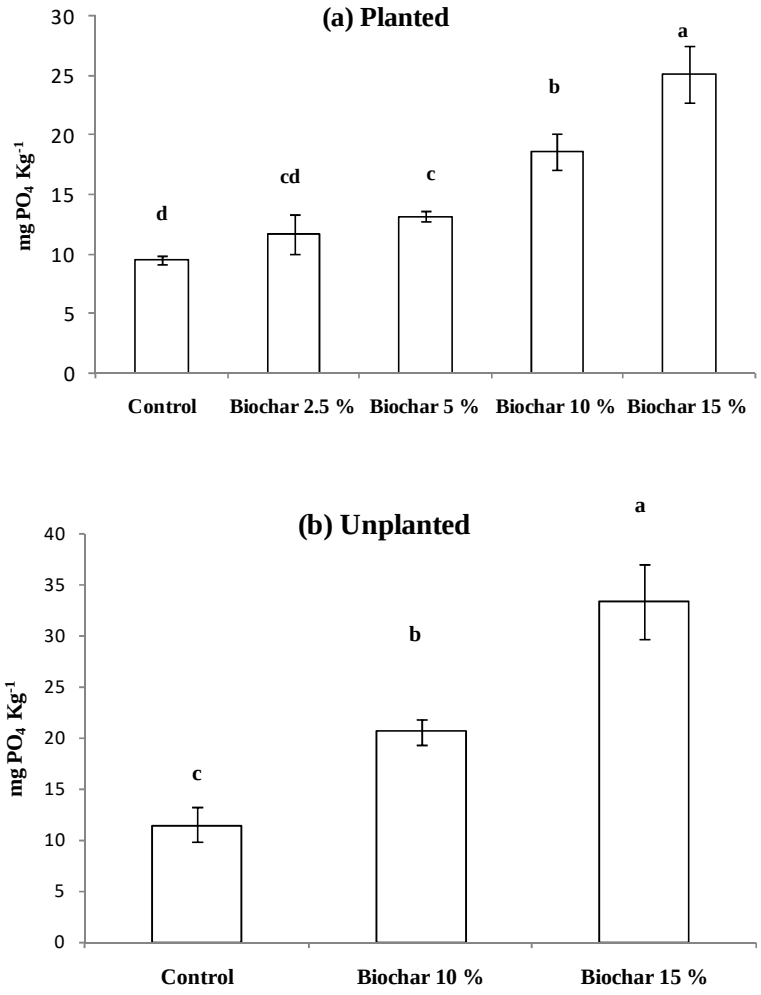


Figure 6.18 Effect of biochar on Soil Olsen-P (mg kg^{-1}) (a) with plants (Biochar: $F_{4,16} = 29.16$, $P < 0.001$) (b) without plants (Biochar: $F_{2,8} = 24.75$, $P < 0.001$) . Columns similarly superscripted and not significantly different.

Figure 6.19 represented the effect of time on Olsen P content over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted treatments (Figure 6.19: a) the maximum value was reported at the end of experiment (370 days) with the minimum observed at the start of experiment (30 days). There was a gradual increase in P with time.

In unplanted (Figure 6.19: b) the trend was inconsistent and the maximum value for Olsen P at 180 days with the minimum value for 90 days.

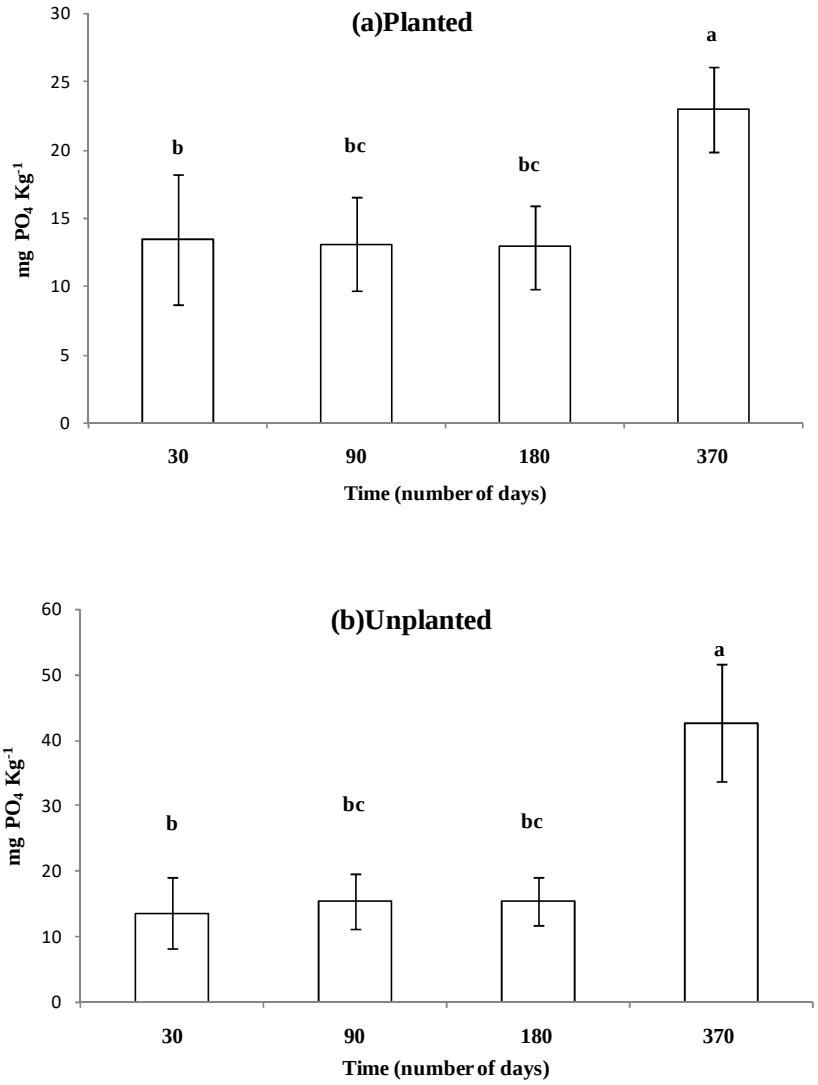


Figure 6.19 Effect of time on Soil Olsen-P (mg kg^{-1})
(a) with plants (Time: $F_{3,60}$ = 16.45, *P* <0.001) (b) without plants (Time: $F_{3,36}$= 32.76, *P* <0.001) . Columns similarly superscripted and not significantly different.

Presence of strawberry plant (Plant: $F_{1, 20}$ = 7.92, *P* 0.011) was also proved significant for nitrate nitrogen in all treatment with or without biochar application (Figure 6.20). The maximum activity was noted in unplanted than planted treatments.

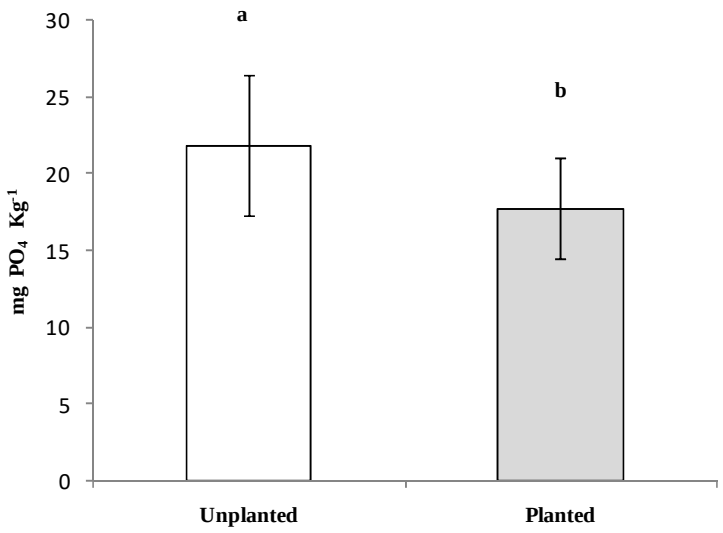


Figure 6.20 Effect of Strawberry plant on changes in soil Olsen-P (mg kg^{-1})
(Plant: $F_{1, 20} = 7.92$, *P* 0.011) . Columns similarly superscripted and not significantly different.

Table 6.2 p value of biochar amended soils for soil chemical properties in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Soil pH	Loss on Ignition (%)	NH_4-N	NO_3-N	Olsen-P
Planted	Biochar	4	**<0.001**	**<0.001**	0.334	**<0.001**	**<0.001**
	Time	3	**<0.001**	**<0.001**	**0.025**	**<0.001**	**<0.001**
	Time *Biochar	12	**0.001**	**0.031**	0.06	**<0.001**	0.892
	Residuals						
Unplanted	Biochar	2	**<0.001**	**<0.001**	0.06	**<0.001**	**<0.001**
	Time	3	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Time *Biochar	6	0.108	0.071	**0.02**	**<0.001**	0.621
	Residuals						
Planted x Unplanted	Biochar	2	**<0.001**	**<0.001**	0.063	**<0.001**	**<0.001**
	Time	3	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Plant	1	**0.009**	**<0.001**	**<0.001**	**<0.001**	**0.011**
	Time *Biochar	6	**<0.001**	**0.005**	**0.001**	**0.003**	0.915
	Time *Plant	3	**0.031**	0.299	**<0.001**	**<0.001**	**<0.001**
	Plant *Biochar	2	**0.018**	**<0.001**	0.292	**<0.001**	0.154
	Time*Biochar*Plant	6	0.086	0.652	0.258	**0.009**	0.456
	Residuals						

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

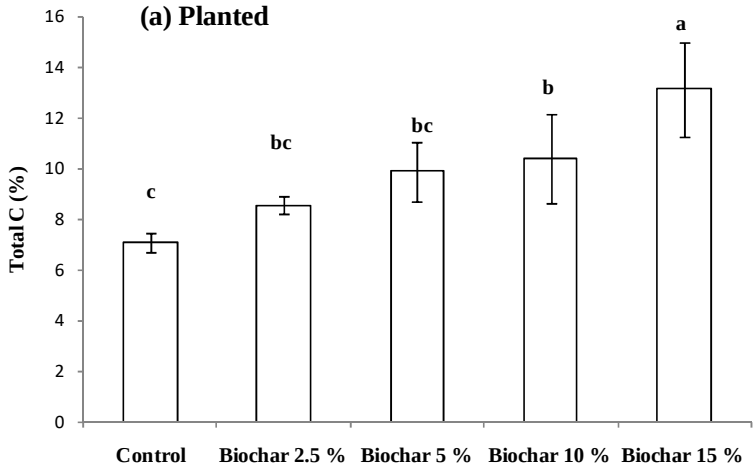
Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

Total Carbon

Data obtained for Total C in soil showed that biochar (planted: $F_{4,16}$ = 10.41, P <0.001) (unplanted: $F_{2,8}$ = 5.81, P 0.028) and time (planted: Time: $F_{3,60}$ = 20.73, P <0.001; unplanted: Time: $F_{3,36}$= 22.76, P <0.001) were proved statistically significant. The effect of biochar was proved statistically non-significant for both planted and unplanted treatments. Presence of strawberry plant was proved non-significant for Total C content in all treatment with or without biochar application

In planted (Figure 6.21: a) the lowest total carbon was recorded in control treatment with no biochar, with the increasing values reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The highest amount was noted in the treatment where the highest rate of biochar 15% was applied.

In unplanted (Figure 6.21: b) the same increasing trend was noted like the planted treatments. The highest carbon content was recorded in treatment where biochar was applied @15%. This was followed by treatment with BC 10% and control with 0 biochar.



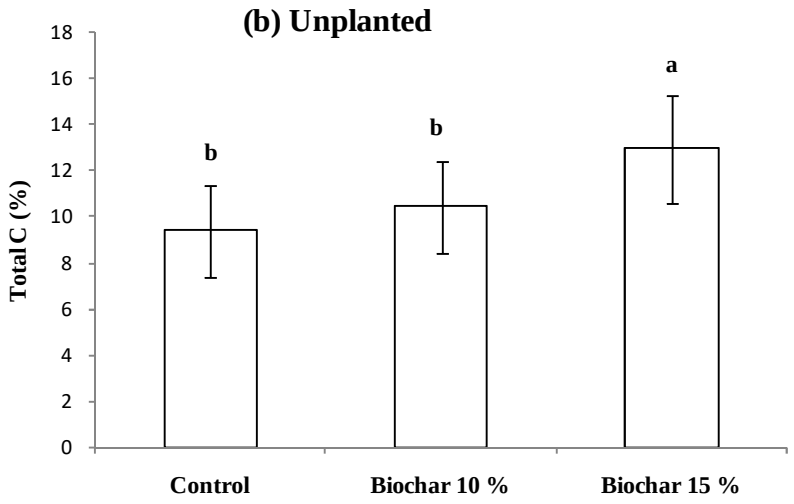


Figure 6.21 Effect of Biochar on changes in soil Total Carbon content (%)
(a) with plants (Biochar: $F_{4,16}$ = 10.41, *P* <0.001) (b) without plants (Biochar: $F_{2,8}$ = 5.81, *P* 0.028) . Columns similarly superscripted and not significantly different.

Figure 6.22 represented the effect of time on carbon content over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted treatments (Figure 6.22: a) the maximum value was reported at 90 days with the minimum observed at the end of experiment (370 days). There was a gradual decrease in carbon content with time.

In unplanted (Figure 6.13: b) the trend was inconsistent and the maximum value for total C was noted at 180 days with the minimum value for 30 days.

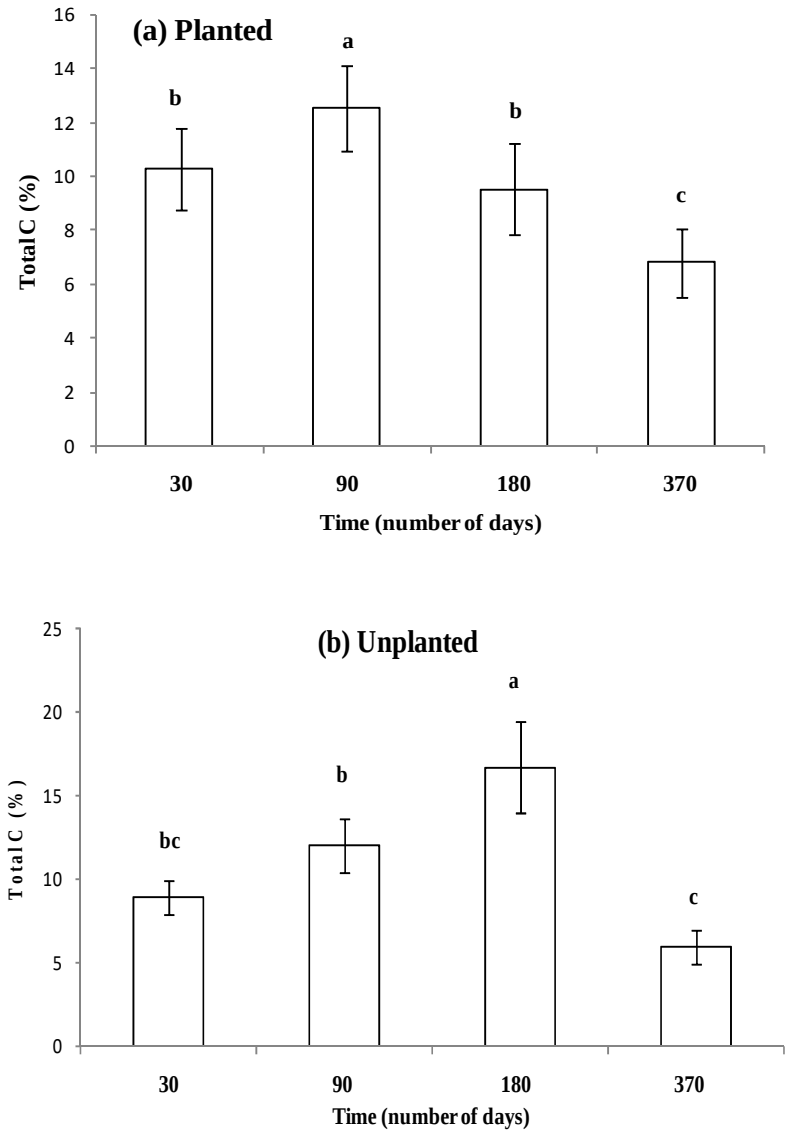


Figure 6.22 Effect of time on changes in soil Total Carbon content (%)
(a) with plants (Time: $F_{3,60}$ = 20.73, *P* <0.001) (b) without plants (Time: $F_{3,36}$= 22.76, *P* <0.001) . Columns similarly superscripted and not significantly different.

Total Nitrogen

Data obtained for total nitrogen in soil showed that biochar (planted: $F_{4,16}$ = 3.42, *P* 0.033) and (unplanted: $F_{2,8}$ = 1.61, *P* 0.259) and time (planted: Time: $F_{3,60}$ = 5.45, *P* 0.025; unplanted: Time: $F_{3,36}$= 31.05, *P* <0.001) was proved statistically significant as individual factor for both planted and unplanted treatments. Presence of strawberry plant (Plant: $F_{1, 20}$ =

28.75, *P* <0.001) was also proved significant for total nitrogen in all treatment with or without biochar application.

In planted (Figure 6.23: a) the trend was very inconsistent among different biochar treatments. In unplanted (Figure 6.23: b) the same increasing trend was noted like the planted treatments.

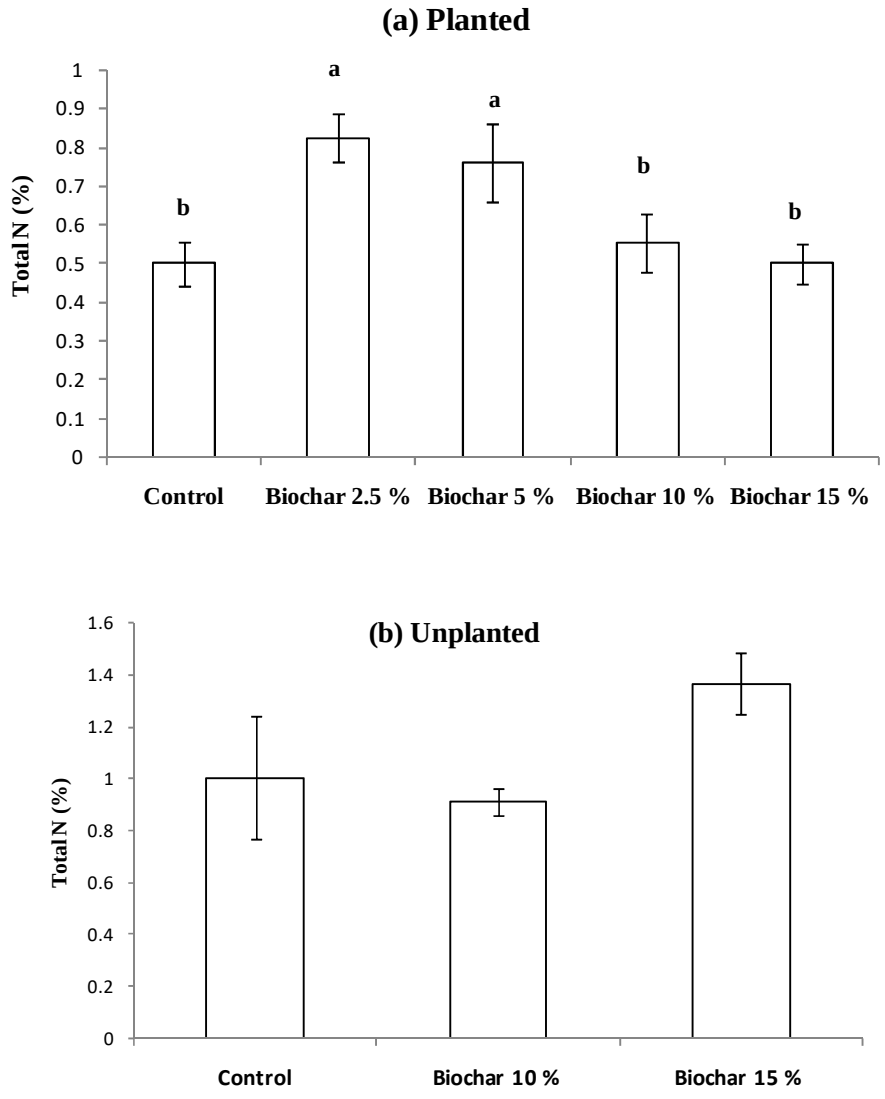


Figure 6.23 Effect of biochar on changes in soil Total Nitrogen (%)
(a) with plants (Biochar: $F_{4,16}$ = 3.42, *P* 0.033) and (b) without plants (Biochar: $F_{2,8}$ = 1.61, *P* 0.259) . Columns similarly superscripted and not significantly different.

Figure 6.24 represented the effect of time on total nitrogen content over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted treatments (Figure 6.24: a) the maximum value was reported at the end of experiment (370 days) with the minimum observed at the start of experiment (30 days). There was a gradual increase in total nitrogen with time.

In unplanted (Figure 6.13: b) the trend was inconsistent and the maximum value for total nitrogen at 180 days with the minimum value for 90 days.

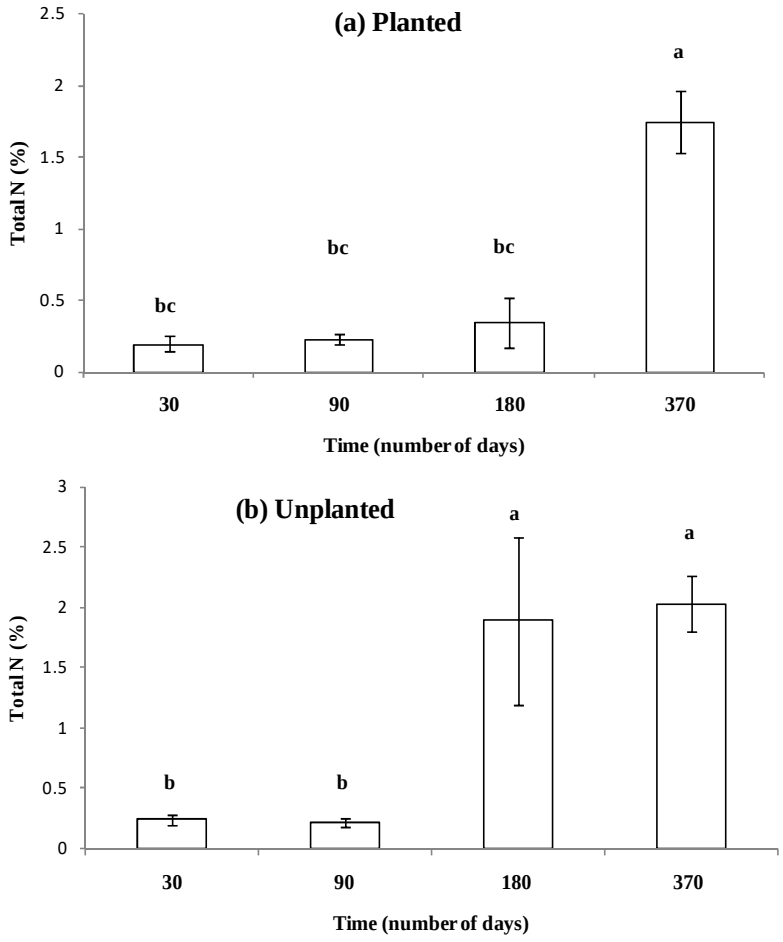


Figure 6.24 Effect of time on changes in soil Total Nitrogen content (%)
(a) With plants (Time: $F_{3,60}$ = 169.70, *P* <0.001) (b) without plants (Time: $F_{3,36}$= 16.97, *P* 0.001) . Columns similarly superscripted and not significantly different.

Presence of strawberry plant (Plant: $F_{1, 20}$ = 28.75, P <0.001) was also proved significant for total nitrogen in all treatment with or without biochar application (Figure 6.25). The maximum value was noted in unplanted than planted treatments.

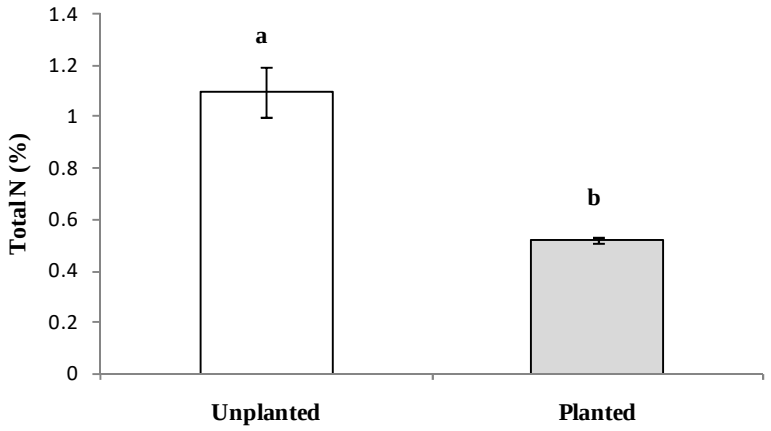


Figure 6.25 Effect of plant on soil Total Nitrogen content (%)
(Plant: $F_{1, 20}$ = 28.75, *P* <0.001) . Columns similarly superscripted and not significantly different.

Total Sulphur

Data obtained for total sulphur content in soil showed that time (planted: Time: $F_{3,60}$ = 12.84, *P* 0.002; unplanted: Time: $F_{3,36}$= 30.1, *P* <0.001) was proved statistically significant as individual factor for both planted and unplanted treatments. The effect of biochar was proved statistically non-significant for both planted and unplanted treatments. Presence of strawberry plant was also proved non-significant for total sulphur in all treatment with or without biochar application

Figure 6.26 represented the effect of time on total sulphur content over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted

treatments (Figure 6.26: a) the maximum value was reported at the end of experiment (370 days) with the minimum observed at the start of experiment (30 days). In unplanted (Figure 6.26: b) the trend was same and the maximum value was noted at 370 days with the minimum value at the start of experiment.

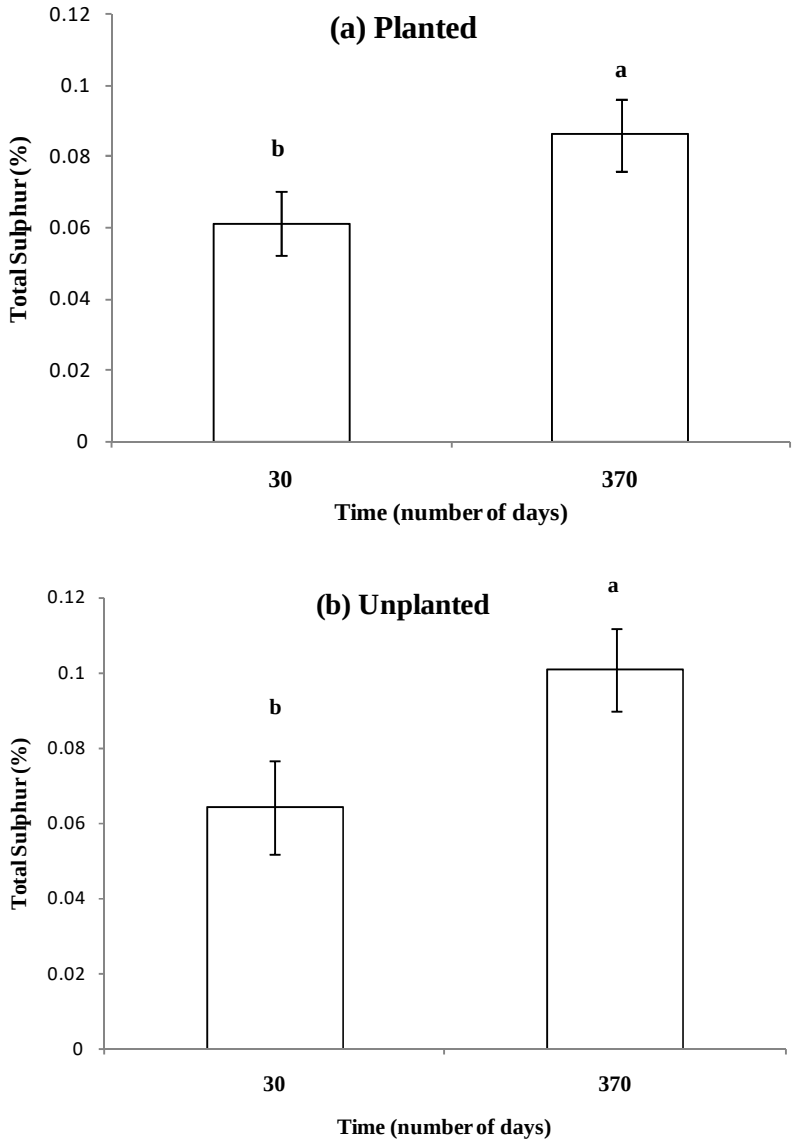


Figure 6.26 Effect of Time on changes in Total sulphur content (a) with plants (Time: $F_{1,20}$ = 12.84, P 0.002) ; (b) without plants (Time: $F_{1,12}$ = 30.1, *P* <0.001) . Columns similarly superscripted and not significantly different.

Table 6.3 p value of biochar amended soils for Total Carbon, Nitrogen and Sulphur in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Total C	Total N	Total S
Planted	Biochar	4	**<0.001**	**0.033**	0.348
	Time	3	**<0.001**	**<0.001**	**0.002**
	Time *Biochar	12	**0.004**	0.056	0.802
	Residuals				
Unplanted	Biochar	2	**0.028**	0.259	**0.016**
	Time	3	**<0.001**	**0.001**	**<0.001**
	Time *Biochar	6	0.145	0.672	0.232
	Residuals				
Planted x Unplanted	Biochar	2	**<0.001**	0.268	**<0.001**
	Time	3	**<0.001**	**<0.001**	**<0.001**
	Plant	1	0.238	**0.011**	0.132
	Time *Biochar	6	**0.002**	0.654	0.459
	Time *Plant	3	**<0.001**	**0.002**	0.525
	Plant *Biochar	2	0.174	0.167	0.321
	Time*Biochar*Plant	6	0.858	0.72	0.675
	Residuals				

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

6.5.3 Soil Available Nutrients

Data obtained for soil available macronutrients, micronutrients and heavy metals showed the following results:

Effect of biochar was significantly proven to affect the availability of Calcium ((planted: Biochar: $F_{4,16} = 4.52$, $P < 0.012$; unplanted : Biochar: $F_{2,8} = 0.14$, $P < 0.874$) and Cation exchange capacity in soil (planted: Biochar: $F_{4,16} = 3.89$, $P < 0.022$; unplanted : Biochar: $F_{2,8} = 0.5$, $P < 0.622$).

Effect of time was proved significant for the following available nutrients: Some of the soil available nutrients contents increase in soil with time; Calcium (planted: Time: $F_{3,60}$ = 89.77, *P* <0.001; unplanted: Time: $F_{3,36}$= 58.25, *P* <0.001); Potassium (planted: Time: $F_{3,60}$ = 14.69, *P* <0.001; unplanted: Time: $F_{3,36}$= 19.19, *P* <0.001).

Table 6.4 p value of biochar amended soils for Available macronutrients in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Available macronutrients (mg kg^{-1})				Cation Exchange Capacity ($cmolc\ kg^{-1}$)
			Sodium	Potassium	Calcium	Magnesium	
Planted	Biochar	4	0.336	0.493	**0.012**	**<0.001**	**0.022**
	Time	3	0.169	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Time *Biochar	12	0.274	0.569	**0.044**	0.088	**0.04**
	Residuals						
Unplanted	Biochar	2	0.678	**0.052**	0.874	0.139	0.622
	Time	3	0.216	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Time *Biochar	6	**0.011**	**0.006**	**0.035**	**0.03**	**0.028**
	Residuals						
Planted x Unplanted	Biochar	2	0.351	**0.011**	0.185	**<0.001**	0.123
	Time	3	0.056	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Plant	1	**0.047**	0.225	0.398	0.117	0.143
	Time *Biochar	6	0.121	**0.034**	0.302	0.218	0.262
	Time *Plant	3	0.536	0.537	0.878	0.59	0.811
	Plant *Biochar	2	0.495	0.268	0.069	**0.006**	**0.027**
	Time*Biochar*Plant	6	**0.023**	0.117	**0.005**	**0.001**	**0.003**
	Residuals						

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

Some available nutrients reflect the significant effect of biochar but inconsistent trends; Iron (planted: Time: $F_{3,60}$ = 80.39, *P* <0.001; unplanted: Time: $F_{3,36}$= 64.29, *P <0.001*); Cromium (planted: Time: $F_{3,60}$ = 291.13, *P* <0.001; unplanted: Time: $F_{3,36}$= 81.07, *P* <0.001); Manganese (planted: Time: $F_{3,60}$ = 19.19, *P* <0.001; unplanted: Time: $F_{3,36}$= 5.8, *P 0.028*);

Molybdenum (planted: Time: $F_{3,60}$ = 44.87, *P <0.001*; unplanted: Time: $F_{3,36}$= 58.81, *P <0.001*); Zinc (planted: Time: $F_{3,60}$ = 16.31, *P* <0.001; unplanted: Time: $F_{3,36}$= 6.68, *P* 0.018); Cobalt (planted: Time: $F_{3,60}$ = 45.94, *P* <0.001; unplanted: Time: $F_{3,36}$= 4.74, *P 0.028*); Nickel (planted: Time: $F_{3,60}$ = 3.92, *P 0.031*; unplanted: Time: $F_{3,36}$= 6.17, *P 0.027*) ; Arsenic (planted: Time: $F_{3,60}$ = 24.3, *P <0.001*; unplanted: Time: $F_{3,36}$= 11.3, *P <0.001*)

Table 6.5 p value of biochar amended soils for Available micronutrients in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Available micronutrients (µg kg^{-1})			
			Iron	**Copper**	**Chromium**	**Aluminium**
Planted	Biochar	4	0.281	0.781	0.075	**0.021**
	Time	3	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Time *Biochar	12	0.28	0.541	**0.046**	**0.027**
	Residuals	60				
Unplanted	Biochar	2	0.947	0.401	0.407	0.536
	Time	3	**<0.001**	0.288	**<0.001**	**<0.001**
	Time *Biochar	6	0.862	0.388	0.539	0.738
	Residuals	36				
Planted x Unplanted	Biochar	2	0.286	0.797	0.092	**0.019**
	Time	3	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Plant	1	0.908	0.095	0.275	0.958
	Time *Biochar	6	0.418	0.47	0.274	**0.035**
	Time *Plant	3	0.465	0.355	0.322	0.12
	Plant *Biochar	2	0.489	0.451	0.24	0.151
	Time*Biochar*Plant	6	0.336	0.352	0.341	0.267
	Residuals	72				

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

Table 6.6 p value of biochar amended soils for Available micronutrients in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Available micronutrients (µg kg^{-1})			
			Manganese	**Molybdenum**	**Zinc**	**Cobalt**
Planted	Biochar	4	0.638	0.469	0.693	0.384
	Time	3	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Time *Biochar	12	0.588	0.456	0.604	0.275
	Residuals	60				
Unplanted	Biochar	2	0.143	0.062	0.095	0.123
	Time	3	**0.028**	**<0.001**	**0.018**	**0.028**
	Time *Biochar	6	0.283	0.244	0.413	0.549
	Residuals	36				
Planted x Unplanted	Biochar	2	**0.049**	0.628	**0.055**	**0.048**
	Time	3	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	Plant	1	0.799	0.621	0.852	0.201
	Time *Biochar	6	0.094	0.777	0.147	0.367
	Time *Plant	3	0.82	0.813	0.967	0.564
	Plant *Biochar	2	0.712	0.087	0.545	0.201
	Time*Biochar*Plant	6	0.884	0.118	0.79	0.685
	Residuals	72				

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)
Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)
Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

These are the heavy metals which are significantly affected by time: Cadmium (planted: Time: $F_{3,60}$ = 53.4, *P <0.001*; unplanted: Time: $F_{3,36}$= 13.79, *P 0.001*) ; Cesium (planted: Time: $F_{3,60}$ = 73.82, *P <0.001*; unplanted: Time: $F_{3,36}$= 56.94, *P <0.001*) ; Barium (planted: Time: $F_{3,60}$ = 81.92, *P <0.001*; unplanted: Time: $F_{3,36}$= 43.87, *P <0.001*) ; Lead (planted: Time: $F_{3,60}$ = 62.5, *P <0.001*; unplanted: Time: $F_{3,36}$= 45.52, , *P <0.001*).

Table 6.7 p value of biochar amended soils for Available heavy metals in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Available heavy metals (µg kg^{-1})		
			Nickel	**Arsenic**	**Cadmium**
Planted	Biochar	4	0.693	0.331	0.311
	Time	3	**0.031**	**<0.001**	**<0.001**
	Time *Biochar	12	0.425	0.286	0.344
	Residuals	60			
Unplanted	Biochar	2	0.369	0.434	0.198
	Time	3	**0.027**	**<0.001**	**0.001**
	Time *Biochar	6	0.529	0.489	0.42
	Residuals	36			
Planted x Unplanted	Biochar	2	0.452	0.482	0.072
	Time	3	**0.008**	**<0.001**	**<0.001**
	Plant	1	0.804	0.914	0.561
	Time *Biochar	6	0.484	0.546	0.372
	Time *Plant	3	0.813	0.835	0.613
	Plant *Biochar	2	0.332	0.787	0.211
	Time*Biochar*Plant	6	0.374	0.422	0.286
	Residuals	72			

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

Some of the Available soil nutrients showed positive and significant response to biochar addition but the trend was inconsistent: Aluminium (planted: Time: $F_{3,60}$ = 304.81, *P <0.001*; unplanted: Time: $F_{3,36}$= 290.08, *P <0.001*);Magnesium (planted: Time: $F_{3,60}$ = 65.9, *P* <0.001; unplanted: Time: $F_{3,36}$= 56.54, *P <0.001*);

Table 6.8 p value of biochar amended soils for Available heavy metals in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Available heavy metals (µg kg^{-1})		
			Cesium	**Barium**	**Lead**
Planted	Biochar	4	0.135	0.179	0.539
	Time	3	**<0.001**	**<0.001**	**<0.001**
	Time *Biochar	12	0.159	0.145	0.505
	Residuals	60			
Unplanted	Biochar	2	0.158	0.086	0.255
	Time	3	**<0.001**	**<0.001**	**<0.001**
	Time *Biochar	6	0.455	0.106	0.332
	Residuals	36			
Planted x Unplanted	Biochar	2	0.036	0.074	0.482
	Time	3	**<0.001**	**<0.001**	**<0.001**
	Plant	1	0.491	0.71	0.793
	Time *Biochar	6	0.105	0.184	0.576
	Time *Plant	3	0.59	0.862	0.857
	Plant *Biochar	2	0.297	0.093	0.275
	Time*Biochar*Plant	6	0.311	**0.045**	0.224
	Residuals	72			

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

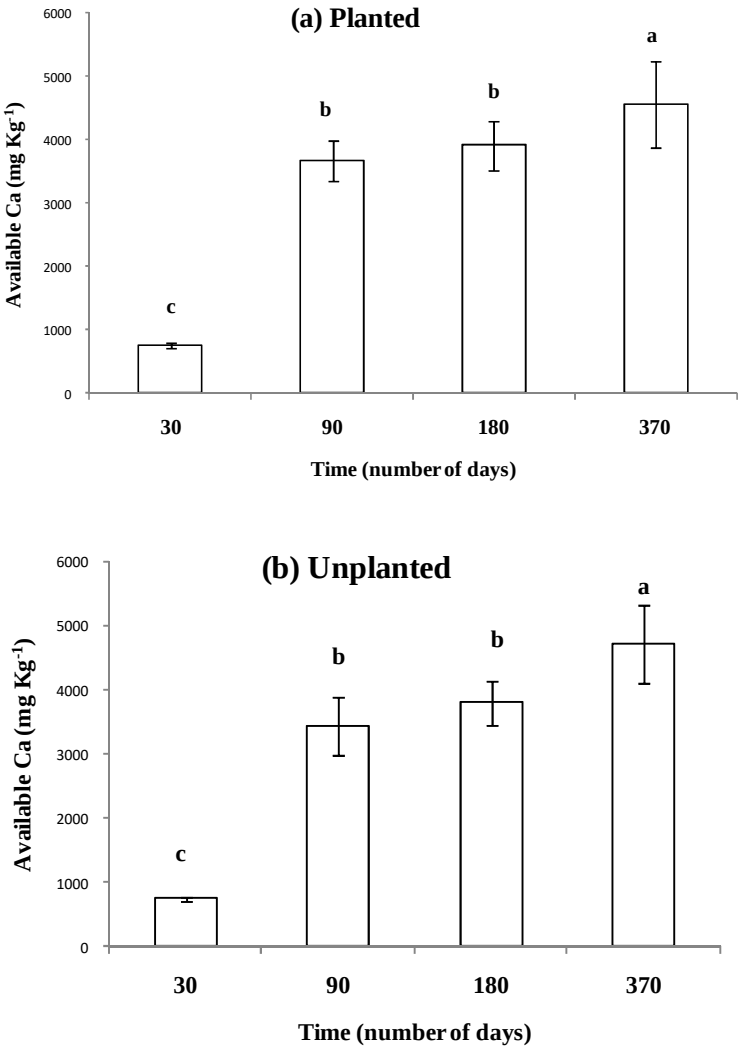


Figure 6.27 Effect of time on Available Ca (mg kg^{-1})
(a) with plants (Time: $F_{3,60}$ = 89.77, P <0.001) and (b) without plants (Time: $F_{3,36}$= 58.25, P <0.001) . Columns similarly superscripted and not significantly different.

Figure 6.27 represented the effect of time on Available Ca content over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted treatments (Figure 6.27: a) the maximum value was reported at the end of experiment (370 days) with the minimum observed at the start of experiment (30 days). In unplanted (Figure 6.27: b) the trend was same with planted treatment.

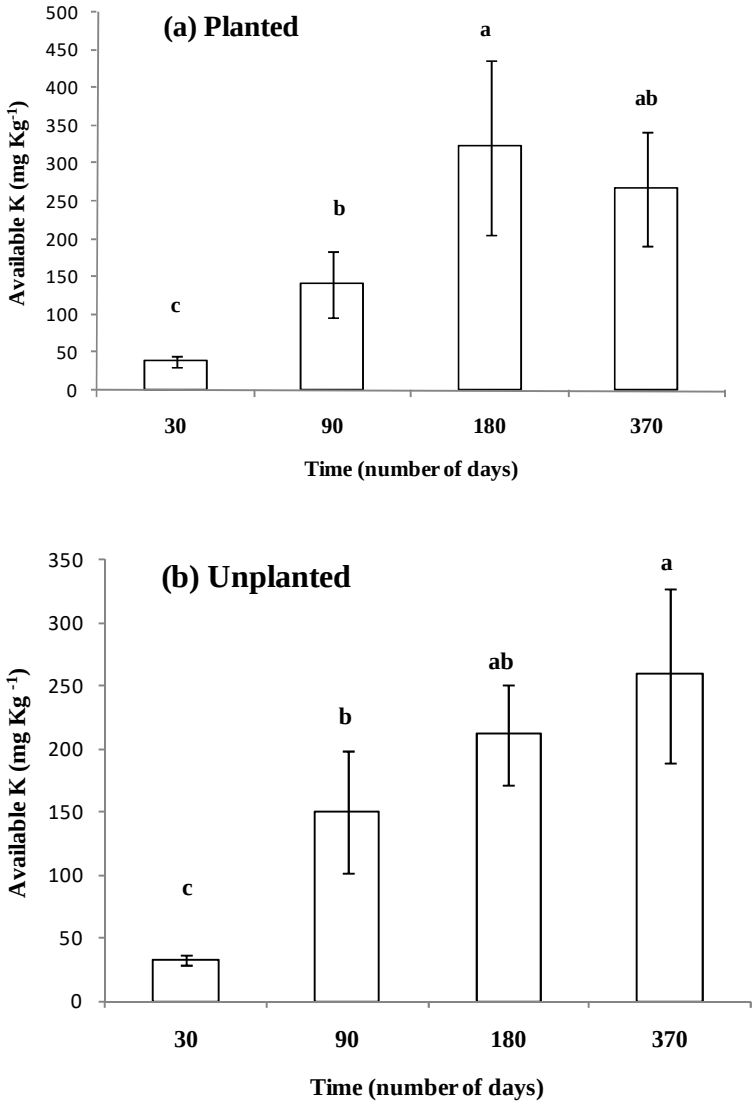


Figure 6.28 Effect of time on Available K (mg kg^{-1})
(a) with plants (Time: $F_{3,60}$ = 14.69, P <0.001) and (b) without plants (Time: $F_{3,36}$= 19.19, P <0.001) . Columns similarly superscripted and not significantly different.

Figure 6.28 represented the effect of time on available K content over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted treatments (Figure 6.28: a) the maximum value was reported at the end of experiment (370 days) with the minimum observed at the start of experiment (30 days). There was a gradual increase in available K with time. In unplanted (Figure 6.28: b) the trend was same as recorded in planted treatment.

Cation Exchange Capacity

The effect of time was proved significant in Cation Exchange Capacity (Planted: Time: $F_{3,60}$ = 2.24, P <0.04; Unplanted: Time: $F_{3,36}$= 57.98, P <0.001).

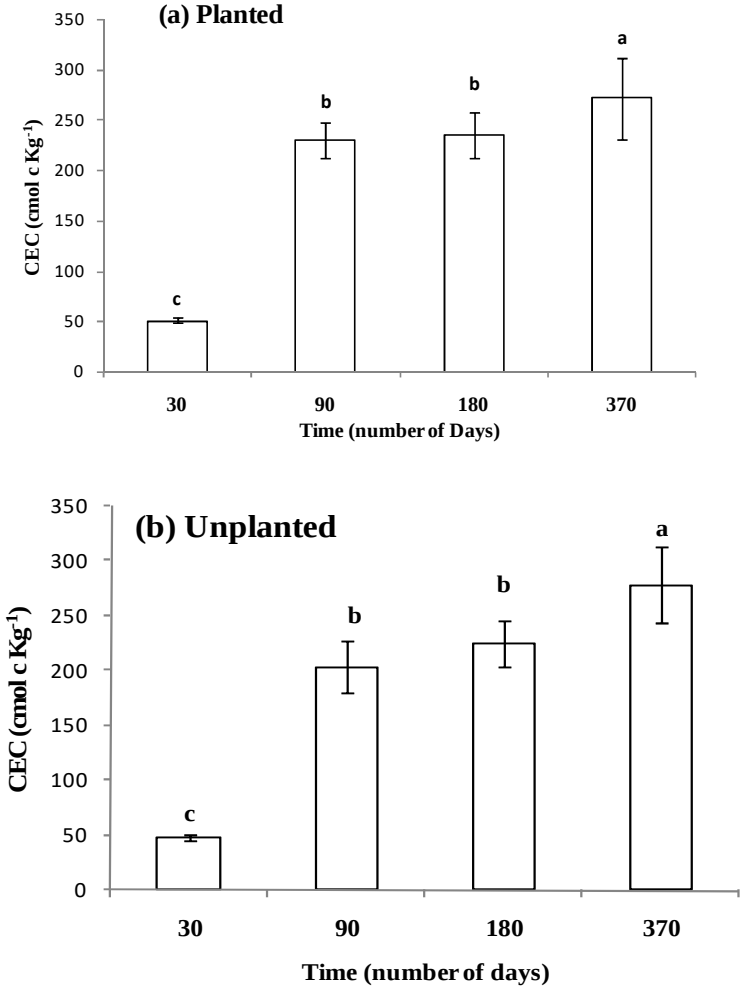


Figure 6.29 Effect of time on Cation Exchange Capacity (cmol c kg^{-1})
(a) with plants (Time: $F_{3,60}$ = 2.24, P <0.04) and (b) without plants (Time: $F_{3,36}$= 57.98, P <0.001) . Columns similarly superscripted and not significantly different.

Figure 6.29 represented the effect of time on CEC over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted treatments (Figure 6.29: a) the maximum value was reported at the end of experiment (370 days) with the minimum observed at the start of experiment (30 days). There was a gradual increase in CEC with time.

Effect of plant

Effect of plant was proved statistically significant for affecting availability of sodium (Plant: $F_{1, 20} = 4.48$, *P 0.047*)

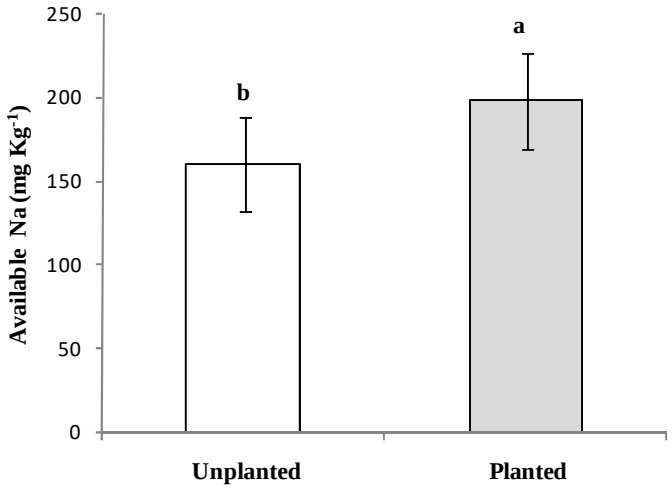


Figure 6.30 Effect of plant on soil Available Na (mg kg^{-1})
(Plant: $F_{1, 20} = 4.48$, *P* 0.047) . Columns similarly superscripted and not significantly different.

Presence of strawberry plant (Plant: $F_{1, 20} = 4.48$, *P* 0.047) was also proved significant for soil available sodium in all treatment with or without biochar application (Figure 6.30). The maximum activity was noted in planted than unplanted treatments.

Effect of Time x Biochar

Interaction of time x biochar was proved significant for affecting the availability of Potassium (Planted; Time x Biochar: $F_{12,60} = 0.83$, *P* 0.569) (Unplanted; Time x Biochar: $F_{6,36} = 5.64$, *P 0.006*) ; Calcium (Planted; Time x Biochar: $F_{12,60} = 2.2$, *P* 0.044) (Unplanted; Time x Biochar: $F_{6,36} = 2.87$, *P* 0.035) ; Aluminium (Planted; Time x Biochar: $F_{12,60} = 3.58$, *P* 0.004) (Unplanted; Time x Biochar: $F_{6,36} = 2.13$, *P* 0.145) ; Cation

Exchange Capacity (Planted; Time x Biochar: $F_{12,60}$ = 2.24, *P* 0.04) (Unplanted; Time x Biochar: $F_{6,36}$= 3.07, *P* 0.028) .

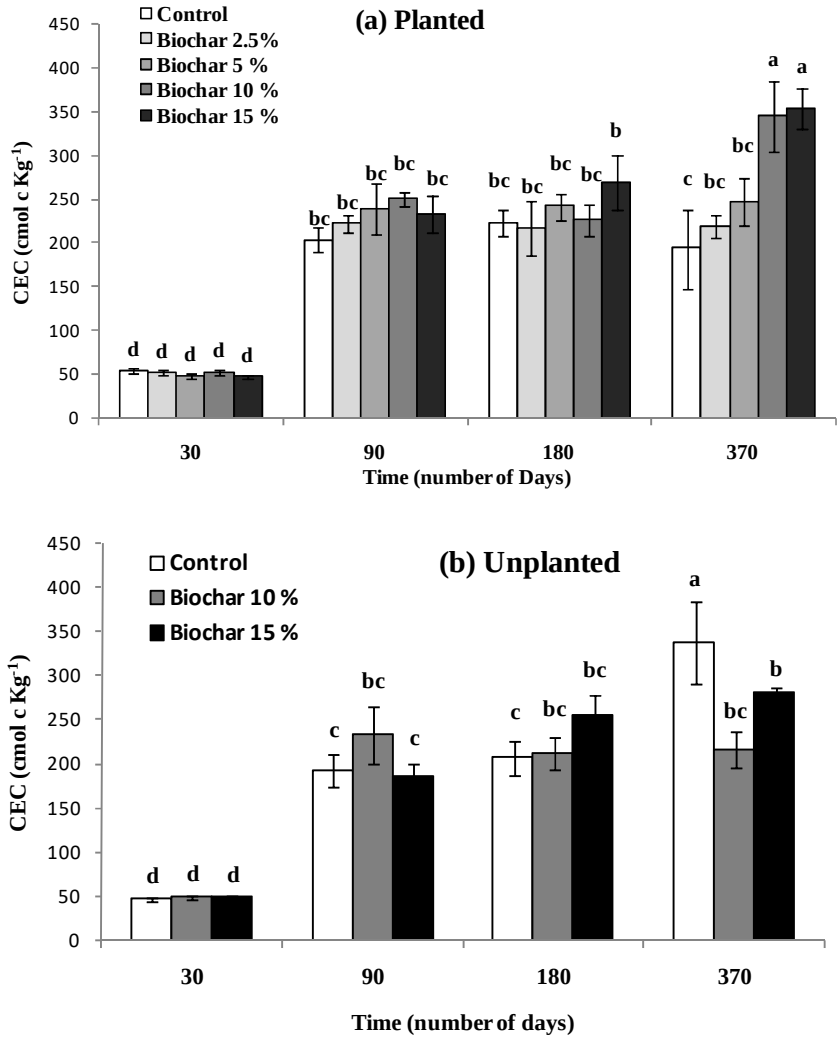


Figure 6.31 Effect of Time x Biochar on Cation Exchange Capacity (cmol c kg^{-1})
(a) with plants (Time x Biochar: $F_{12,60}$ = 2.24, *P* 0.04) and (b) without plants (Time x Biochar: $F_{6,36}$= 3.07, *P* 0.028) . Columns similarly superscripted and not significantly different.

Effect of Plant x Biochar

Interaction of Plant x Biochar was proved significant for Magnesium (Biochar x Plant: $F_{2, 20}$ = 6.75, *P 0.006*) and Cation Exchange Capacity (Biochar x Plant: $F_{2, 20}$ = 4.46, *P 0.025*)

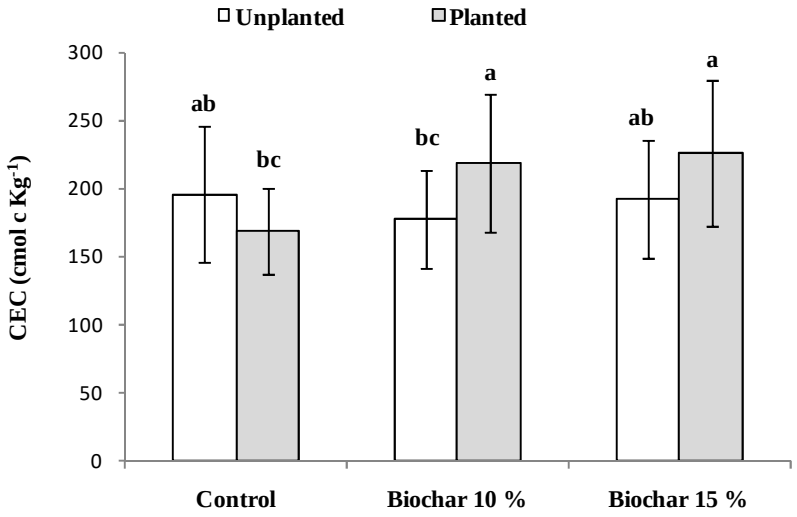


Figure 6.32 Effect of Biochar x Plant on Cation Exchange Capacity (cmol c kg^{-1})
(Biochar x Plant: $F_{2, 20} = 4.46$, P 0.025) . Columns similarly superscripted and not significantly different.

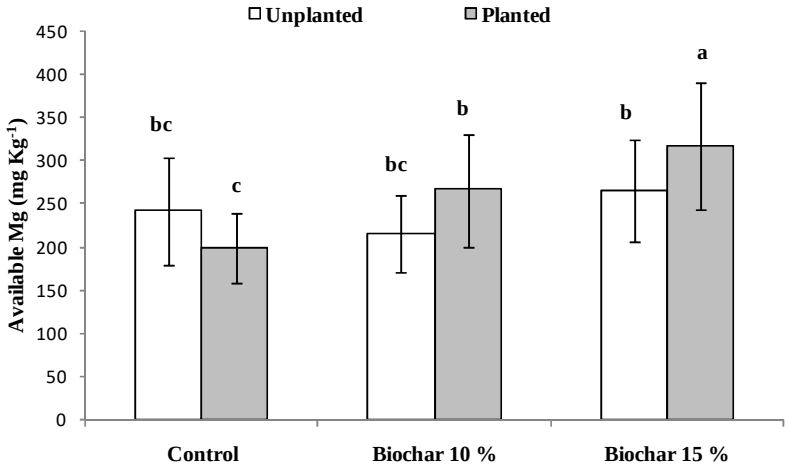


Figure 6.33 Effect of plant on soil Available Mg (mg kg^{-1})
(Biochar x Plant: $F_{2, 20} = 6.75$, P 0.006) . Columns similarly superscripted and not significantly different.

Effect of Time x Biochar x Plant

The interaction of Time x Biochar x Plant was statistically significant for affecting the availability of Sodium (Time x Biochar x Plant: $F_{6,72} = 3$, P *0.023*), Magnesium (Time

x Biochar x Plant: $F_{6,72} = 4.92$, $P = 0.001$) cation exchange capacity (Time x Biochar x Plant: $F_{6,72} = 4.53$, $P = 0.003$)

6.5.4 Soil Total Nutrients

Soil chemical properties were discussed and presented in following paragraphs:

Effect of Biochar

Effect of biochar was significantly proven to affect the availability of total nutrients in soil; Calcium (planted: Biochar: $F_{4,16} = 11.02$, $P < 0.001$; unplanted: Biochar: $F_{2,8} = 4.51$, P 0.049); Magnesium (planted: Biochar: $F_{4,16} = 3.83$, P 0.023; unplanted: Biochar: $F_{2,8} = 20.74$, $P < 0.001$); Sodium (planted: Biochar: $F_{4,16} = 4.68$, P 0.011; unplanted: Biochar: $F_{2,8} = 21.58$, $P < 0.001$); Aluminium (planted: Biochar: $F_{4,16} = 2.82$, P 0.06; unplanted: Biochar: $F_{2,8} = 6.61$, P 0.02); Cobalt (planted: Biochar: $F_{4,16} = 7.63$, P 0.001; unplanted: Biochar: $F_{2,8} = 36.86$, $P < 0.001$); Chromium (planted: Biochar: $F_{4,16} = 6.98$, P 0.002; unplanted: Biochar: $F_{2,8} = 22.35$, $P < 0.001$); Iron (planted: Biochar: $F_{4,16} = 3.8$, P 0.023; unplanted: Biochar: $F_{2,8} = 9.44$, P 0.008); Manganese (planted: Biochar: $F_{4,16} = 7.73$, $P < 0.001$; unplanted: Biochar: $F_{2,8} = 23.92$, $P < 0.001$); Zinc (planted: Biochar: $F_{4,16} = 14.12$, $P < 0.001$; unplanted: Biochar: $F_{2,8} = 39.73$, < 0.001); Arsenic (planted: Biochar: $F_{4,16} = 19.6$, $P < 0.001$; unplanted: Biochar: $F_{2,8} = 58.9$, $P < 0.001$); Barium (planted: Biochar: $F_{4,16} = 8.98$, $P < 0.001$; unplanted: Biochar: $F_{2,8} = 42.33$, $P < 0.001$); Cadmium (planted: Biochar: $F_{4,16} = 3.77$, P 0.024; unplanted: Biochar: $F_{2,8} = 14.91$, P 0.002); Cesium (planted: Biochar: $F_{4,16} = 3.97$, P 0.02; unplanted: Biochar: $F_{2,8} = 19.36$, $P < 0.001$); Nickel (planted: Biochar: $F_{4,16} = 4.47$, P 0.013; unplanted: Biochar: $F_{2,8} = 6.63$, P 0.02); Lead (planted: Biochar: $F_{4,16} = 21.06$, $P < 0.001$; unplanted: Biochar: $F_{2,8} = 59.32$, $P < 0.001$).

Table 6.9 p value of biochar amended soils for Total Macronutrients in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Total Macronutrients (mg kg^{-1})			
			Sodium	Potassium	Calcium	Magnesium
Planted	Biochar	4	**0.011**	0.958	**<.001**	**0.023**
	Time	3	**0.026**	**<.001**	**<.001**	0.415
	Time *Biochar	12	0.729	0.475	**0.007**	0.818
	Residuals					
Unplanted	Biochar	2	**<.001**	0.094	**0.049**	**<0.001**
	Time	3	0.376	**<.001**	0.147	0.88
	Time *Biochar	6	0.146	0.245	0.862	0.17
	Residuals					
Planted x Unplanted	Biochar	2	**<.001**	0.355	**<.001**	**<0.001**
	Time	3	0.071	**<.001**	**<.001**	0.83
	Plant	1	0.668	0.641	0.185	0.278
	Time *Biochar	6	0.192	0.151	0.061	0.092
	Time *Plant	3	0.961	0.885	0.164	0.761
	Plant *Biochar	2	0.574	0.774	**0.043***	0.387
	Time*Biochar*Plant	6	0.59	0.403	0.122	0.731
	Residuals					

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

Table 6.10 p value of biochar amended soils for Total Micronutrients in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Total Micronutrients (µg kg^{-1})			
			Manganese	**Molybdenum**	**Zinc**	**Cobalt**
Planted	Biochar	4	**<.001**	**<.001**	**<.001**	**0.001**
	Time	3	0.269	**0.052**	0.278	0.329
	Time *Biochar	12	0.272	0.527	0.058	0.113
	Residuals	60				
Unplanted	Biochar	2	**<.001**	0.1	**<.001**	**<.001**
	Time	3	0.801	0.226	0.562	0.545
	Time *Biochar	6	0.091	0.36	0.045	0.108
	Residuals	36				
Planted x Unplanted	Biochar	2	**<.001**	**<.001**	**<.001**	**<.001**
	Time	3	0.364	0.098	0.208	0.383
	Plant	1	0.536	0.533	0.296	0.23
	Time *Biochar	6	**0.046**	0.544	**0.003**	**0.031**
	Time *Plant	3	0.68	0.565	0.522	0.274
	Plant *Biochar	2	0.37	0.959	**0.029**	0.565
	Time*Biochar*Plant	6	0.258	0.185	0.349	0.506
	Residuals	72				

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

Table 6.11 p value of biochar amended soils for Total Micronutrients in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Total Micronutrients (µg kg^{-1})			
			Iron	Copper	Cromium	Aluminium
Planted	Biochar	4	**0.023**	0.273	**0.002**	**0.06**
	Time	3	**<.001**	0.356	0.793	0.261
	Time *Biochar	12	0.514	0.466	0.374	0.839
	Residuals	60				
Unplanted	Biochar	2	**0.008**	**<.001**	**<.001**	**0.02**
	Time	3	**<.001**	0.569	0.351	0.199
	Time *Biochar	6	0.217	0.67	**0.03**	0.428
	Residuals	36				
Planted x Unplanted	Biochar	2	**<.001**	0.186	**<.001**	**0.002**
	Time	3	**<.001**	0.348	0.538	0.271
	Plant	1	0.391	0.276	0.259	0.512
	Time *Biochar	6	0.133	0.417	**0.006**	0.218
	Time *Plant	3	0.869	0.33	0.686	0.657
	Plant *Biochar	2	0.98	0.363	0.56	0.639
	Time*Biochar*Plant	6	0.506	0.417	0.52	0.575
	Residuals	72				

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

Table 6.12 p value of biochar amended soils for Total heavy metals in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Total heavy metals (µg kg^{-1})		
			Cesium	Barium	Lead
Planted	Biochar	4	**0.02**	**<.001**	**<.001**
	Time	3	0.411	0.635	0.507
	Time *Biochar	12	0.707	0.858	**0.01**
	Residuals	60			
Unplanted	Biochar	2	**<.001**	**<.001**	**<.001**
	Time	3	0.487	0.527	0.471
	Time *Biochar	6	**0.013**	0.119	**0.023**
	Residuals	36			
Planted x Unplanted	Biochar	2	**<.001**	**<.001**	**<.001**
	Time	3	0.498	0.19	0.446
	Plant	1	0.402	0.477	0.185
	Time *Biochar	6	**0.025**	0.19	**<.001**
	Time *Plant	3	0.711	0.657	0.377
	Plant *Biochar	2	0.555	**0.014**	**0.003**
	Time*Biochar*Plant	6	0.27	0.863	0.192
	Residuals	72			

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry Plants)

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)

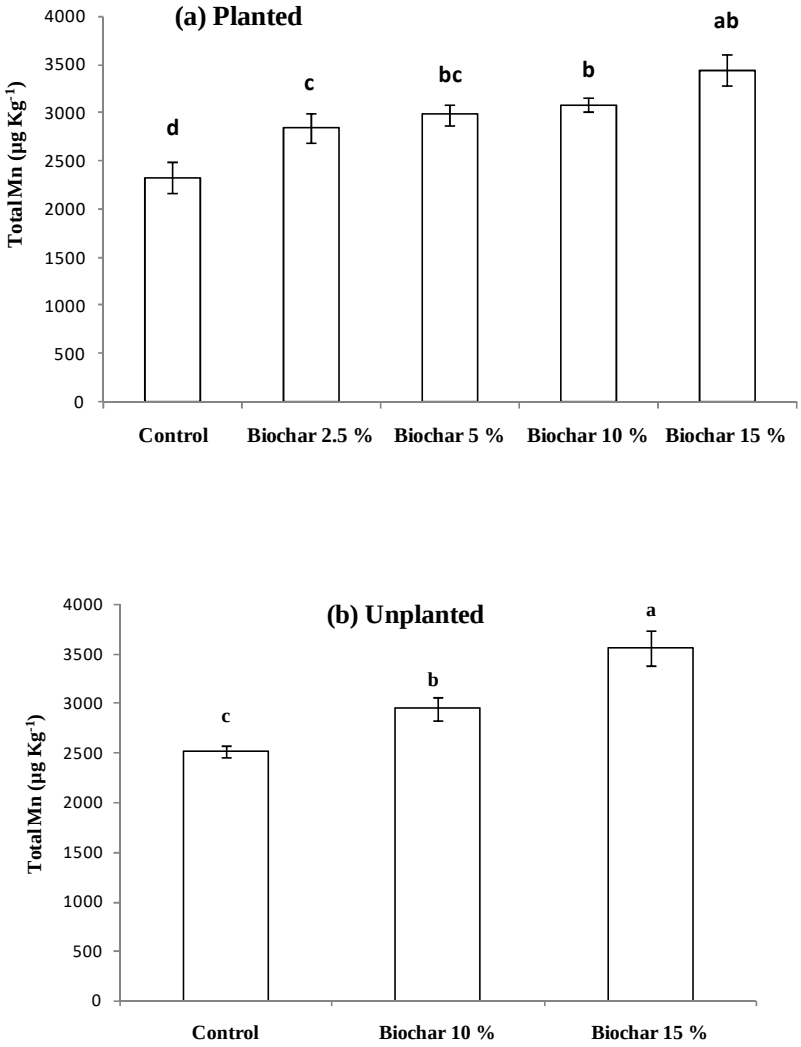


Figure 6.34 Effect of Biochar on Total Mn (µg kg^{-1})
(a) with plants (Biochar: $F_{4,16} = 7.73$, $P < 0.001$) and (b) without plants (Biochar: $F_{2,8} = 23.92$, $P < 0.001$) . Columns similarly superscripted and not significantly different.

In planted (Figure 6.34: a) the lowest Total Mn was recorded in control treatment with no biochar, with the increasing values reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The highest amount was noted in the treatment where the highest rate of biochar 15% was applied. In unplanted (Figure 6.34: b) the same increasing trend was noted like the planted treatments.

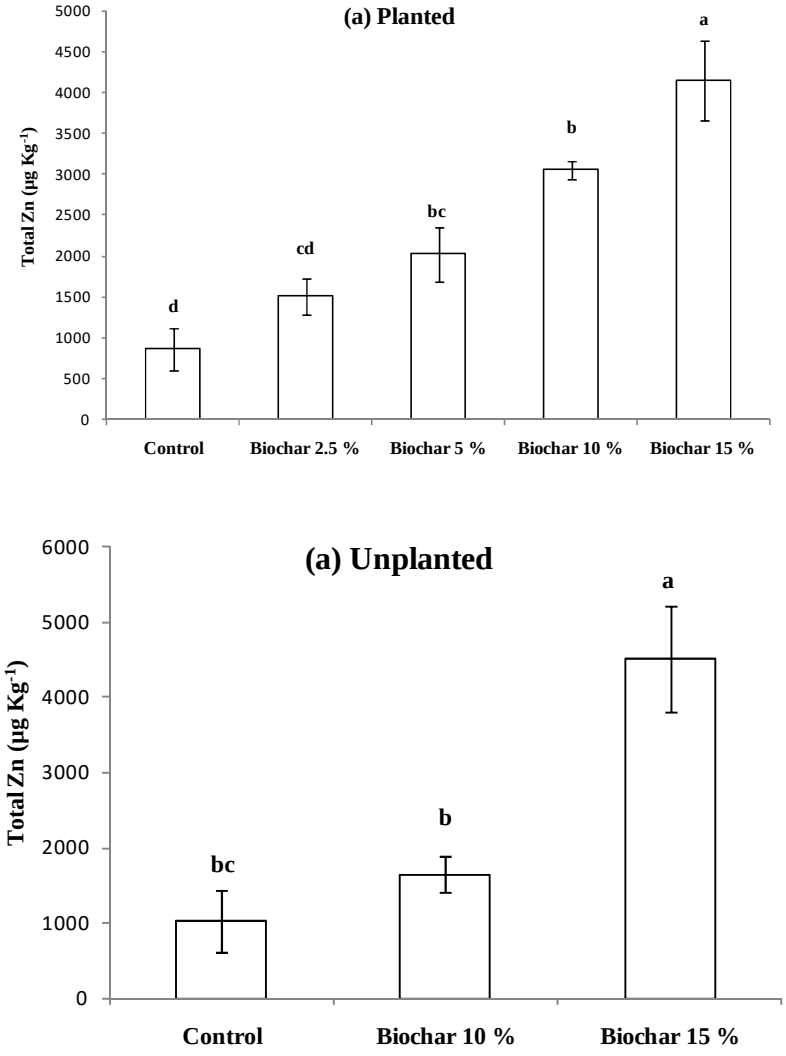


Figure 6.35 Effect of Biochar on Total Zn (µg kg^{-1})
(a) with plants (Biochar: $F_{4,16}$ = 14.12, *P* <0.001) and (b) without plants (Biochar: $F_{2,8}$= 39.73, *P* <0.001) . Columns similarly superscripted and not significantly different.

In planted (Figure 6.35: a) the lowest Total Zn was recorded in control treatment with no biochar, with the increasing values reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The highest amount was noted in the treatment where the highest rate of biochar 15% was applied. In unplanted (Figure 6.35: b) the same increasing trend was noted like the planted treatments.

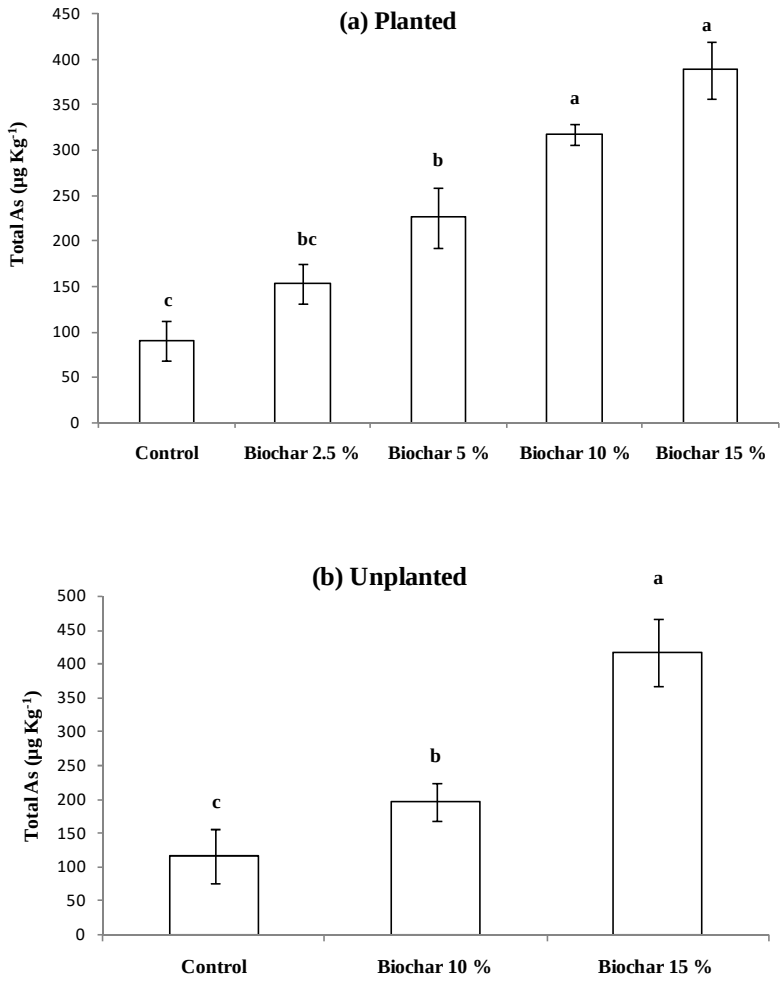


Figure 6.36 Effect of Biochar on Total As (µg kg^{-1})
(a) with plants (Biochar: $F_{4,16}$ = 19.6, P <0.001) and (b) without plants (Biochar: $F_{2,8}$= 58.9, P <0.001) . Columns similarly superscripted and not significantly different.

In planted (Figure 6.36: a) the lowest Total As was recorded in control treatment with no biochar, with the increasing values reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The highest amount was noted in the treatment where the highest rate of biochar 15% was applied. In unplanted (Figure 6.36: b) the same increasing trend was noted like the planted treatments.

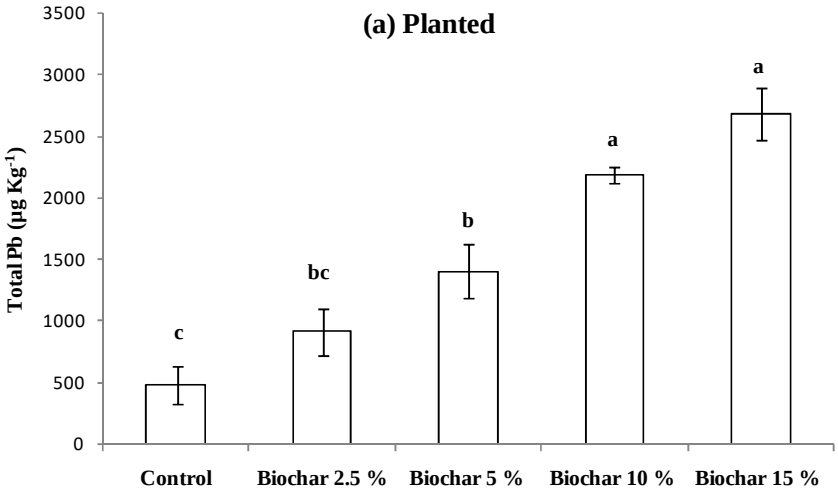
Table 6.13 p value of biochar amended soils for Total heavy metals in soil alone or biochar amended soil in presence or absence of Strawberry plants

Group	Experimental Factors	d.f	Total heavy metals (µg kg^{-1})			
			Nickel	Arsenic	Strontium	Cadmium
Planted	Biochar	4	**0.013**	**<.001**	**<.001**	**0.024**
	Time	3	0.286	0.442	0.462	0.173
	Time *Biochar	12	0.823	**0.022**	0.524	0.358
	Residuals	60				
Unplanted	Biochar	2	**0.02**	**<.001**	**<.001**	**0.002**
	Time	3	0.678	0.491	0.913	0.283
	Time *Biochar	6	0.271	**0.025**	0.543	0.257
	Residuals	36				
Planted x Unplanted	Biochar	2	**<.001**	**<.001**	**<.001**	**<.001**
	Time	3	0.708	0.356	0.65	**0.06**
	Plant	1	0.431	0.201	0.407	0.292
	Time *Biochar	6	0.359	**<.001**	0.499	0.135
	Time *Plant	3	0.571	0.615	0.645	0.413
	Plant *Biochar	2	0.299	**0.003**	0.198	0.441
	Time*Biochar*Plant	6	0.338	0.24	0.322	0.651
	Residuals	72				

Planted :(Treatments with 0, 2.5, 5, 10 and 15 % Biochar with Strawberry

Unplanted :(Treatments with 0, 10 and 15 % Biochar without Strawberry Plants)

Planted x Unplanted :(Treatments with 0, 10 and 15 % Biochar were compared for planted and unplanted)



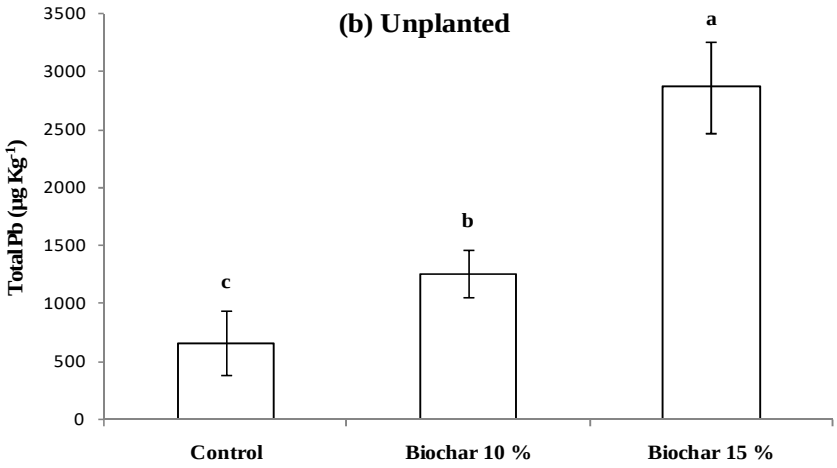


Figure 6.37 Effect of Biochar on Total Pb (µg kg^{-1})

(a) with plants (Biochar: $F_{4,16}$ = 21.06, *P* <0.001) and (b) without plants (Biochar: $F_{2,8}$= 59.32, *P* <0.001) . Columns similarly superscripted and not significantly different.

In planted (Figure 6.37: a) the lowest Total Pb was recorded in control treatment with no biochar, with the increasing values reported in all the other treatments where biochar was applied @ 2.5, 5, 10 and 15%. The highest amount was noted in the treatment where the highest rate of biochar 15% was applied. In unplanted (Figure 6.37: b) the same increasing trend was noted like the planted treatments.

Effect of Time

Effect of time was proved significant for the following available nutrients. Soil available nutrients contents increase in soil with time: Potassium (planted: Time: $F_{3,60}$ = 14.69, *P* <0.001; unplanted: Time: $F_{3,36}$= 19.19, *P* <0.001) ; Iron (planted: Time: $F_{3,60}$ = 15.15, *P* <0.001; unplanted: Time: $F_{3,36}$= 12.18, *P* <0.001).

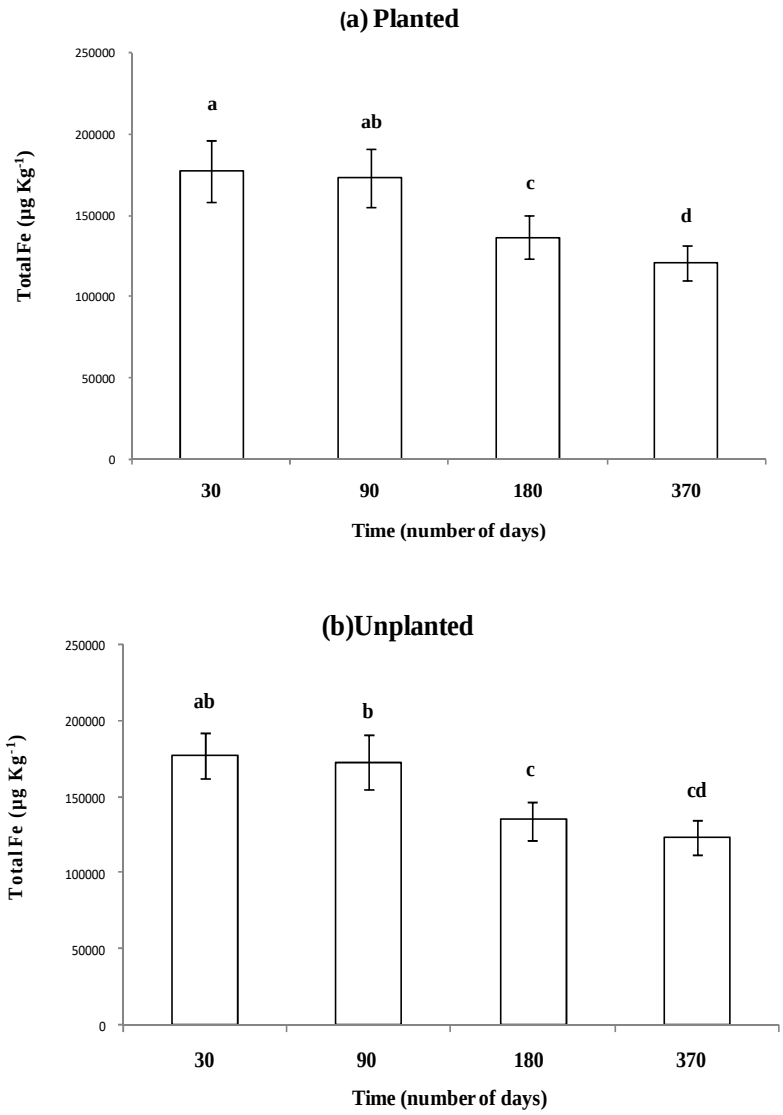


Figure 6.38 Effect of time on Total Fe (µg kg^{-1})
(a) with plants (Time: $F_{3,60}$ = 15.15, P <0.001) and (b) without plants (Time: $F_{3,36}$= 12.18, P <0.001) . Columns similarly superscripted and not significantly different.

Figure 6.38 represented the effect of time on Total Fe content over a period of one year. Time was statistically proved significant in both planted and unplanted treatments. In planted treatments (Figure 6.38: a) the minimum value was reported at the end of experiment (370 days) with the maximum observed at the start of experiment (30 days). There was a gradual decrease with time. In unplanted (Figure 6.38: b) the trend was same like planted treatment.

Effect of Time x Biochar

Interaction of time x biochar was proved significant for affecting the availability of Lead (Planted; Time x Biochar: $F_{12,60}$ = 2.77, *P* 0.01) (Unplanted; Time x Biochar: $F_{6,36}$= 3.69, *P 0.023*)

Effect of Plant x Biochar

Interaction of Plant x Biochar was proved significant for Calcium (Biochar x Plant: $F_{2, 20}$ = 3.69, *P* 0.043); Arsenic (Biochar x Plant: $F_{2, 20}$ = 7.78, *P* 0.003); Lead (Biochar x Plant: $F_{2, 20}$ = 7.64, *P* 0.003)

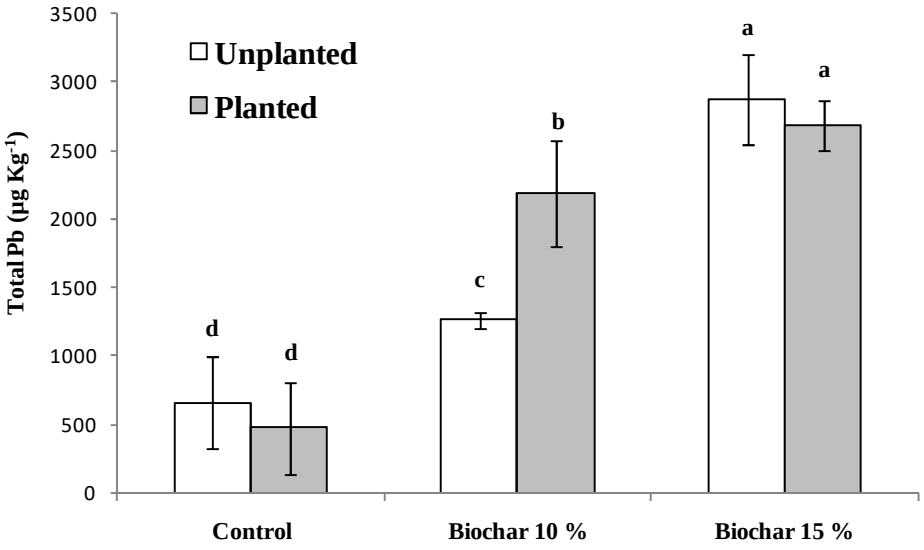


Figure 6.39 Effect of plant x biochar on Total Pb (µg kg^{-1})
(Biochar x Plant: $F_{2, 20}$ = 7.64, *P* 0.003) . Columns similarly superscripted and not significantly different.

Figure 6.39 reflected the effect of plant x biochar interaction on Total Pb content in soil. The highest values are recorded for the treatment with 15% biochar with the lowest recorded in control treatment with no biochar.

6.6 Discussions

Biochar has been used as a potential soil amendment because of its well known role in improving plant productivity and soil fertility (Lehmann and Joseph, 2009; Sohi *et al*., 2010; Li *et al*., 2020 Seki *et al*, 2021; Liao *et al*., 2022), adding essential nutrients and transforming heavy metals and contaminants in agricultural soils (Zhu *et al*., 2017; Singh *et al*., 2022). The effects of biochar application on soil properties are the function of various factors such as pyrolysis temperature, feedstock source, soil condition, and biochar application rates (Mukherjee and Zimmerman, 2013).

In the present study, obtained results reflected that biochar has positive effect on soil gravimetric water content. It was well documented in literature that biochar application could improve water holding capacities of soil (Razzaghi *et al*., 2020; Xia *et al*., 2022). That results in an improved environment for microbial community in soil with enough aeration, water and nutrients (Ameloot *et al*. 2013; McCormack *et al*., 2013; Haider *et al*., 2022, Xia *et al*., 2022). This change in soil water content largely determines the nature and type of composition, abundance and activities of microorganisms (Chan *et al*., 2008; Lehmann *et al*., 2011). As a result of such changes, the major soil processes driven by microbes such as the nutrient transformations and formation and mineralization of carbon could be altered by the addition of biochar to soil (Ullah *et al*., 2020)

Biochar application significantly reduced bulk density and increased water filled pore space in the present study .Porosity and bulk density are inversely related so when we add porous material like biochar, it reduces bulk density of soil due to the increase in pore spaces for water and air. However, the extent of effect was determined by the application rate and physical properties of biochar (Githinji, 2014; Kakaire *et al*., 2015; Blanco-Canqui, 2017).

Another important factor that causes the major decrease in soil bulk density is soil texture and the extent is high in coarse textured as compared to fine textured soils. One possible reason could be the difference in the size and density of biochar, sand and clay particles (Lu and Zong , 2018; Blanco-Canqui, 2017). It was reported in literature that biochar contains different sizes (micr, meso and macro) of longitudinal pores, which varies with feedstock, pyrolysis temperature and duration of combustion cycle. Wood derived high temperature biochars have large and stable pores due to high lignin content as compared to biochar derived from manures and produced at low pyrolysis temperature (Leng *et al*., 2020; Tomczyk *et al*., 2020).

Biochar has significant effect on soil pH, organic matter and cation exchange capacity (Figure 6.9). Considerate increase was reported in soil organic matter and pH of soil with increasing rates of biochar in present study. Biochar was reported to increase soil pH in various studies (Syuhada *et al*., 2016; Atkinson, 2018). The possible reasons for this increase in soil pH could be reduction in exchangeable cations on negatively charged biochar surfaces either by their adsorption or chelation by soluble organic compounds released by biochar in soil solution (Butnan *et al*., 2015; Syuhada *et al*., 2016). Increase in pH could be due to the presence of basic cations in biochar and their conversion to alkaline substances such as oxides, carbonates and hydroxides during pyrolysis. Biochar acts like a liming substance because of the solubilisation and dissolution of these compounds when applied to the soil. High pyrolysis biochar are alkaline in nature compared to biochar produced at low temperature (Cantrell *et al*., 2012; Yuan *et al*., 2011; Wang *et al*., 2013).

When biochar added to the soil, it increases CEC due to the presence of oxygen containing functional groups like carboxyl, carbonyl, and hydroxyl (Uchimiya *et al*., 2010). In the

present study, high values of CEC were reported in soils with high biochar rates. Herbaceous materials have high CEC than biochars derived from wood or other wastes (Kloss *et al*., 2012; Wang *et al*., 2013)

Biochar has a strong role in soil nutrient release and availability when added to soil. Biochar has great affinity to adsorb nutrients to its surface and trap in its pores with organic complexes formed with clays and minerals (Hagemann *et al*., 2017; Joseph *et al*., 2018; Li *et al*., 2020). This mechanism will prevent nutrients from leaching, retain, facilitate and make them available for plant uptake. The adsorbed nutrients retain in biochar for long period of times making biochar a successful single nutrient management technique (Mia *et al*., 2017a). In the present study biochar has significant effect on soil total nutrients only and time has significant effect on available soil nutrients. On aging biochar releases the adsorbed and trapped nutrients which make them available for easy uptake by plants (Karer *et al*., 2013; Alburquerque *et al*., 2013; Akhtar *et al*., 2015).

6.7 Conclusions

In order to acquire the status of soil amendment biochar need to qualify different criteria. The positive or negative effects of biochar on soil properties and nutrient availability determines its usage and application on larger scale. The high temperature biochar full of contaminants is not recommended for agricultural use. Further studies need to identify the best and safe use of biochar for soil improvement and carbon sequestration purposes.

Chapter 7 Plant mediated changes in surface chemistry of biochar

7.1 Overview

In this Chapter, Fourier-transform infrared spectroscopy (FTIR) spectra and functional group chemistry of topsoil, biochar, biochar fractions and biochar-amended soils derived from the main strawberry experiment (see Chapter 2 for set-up) are presented and discussed. The spectra obtained from FTIR were primarily classed as aliphatics, aromatics and carbohydrates and changes in these groups were followed for a year in order to quantify the changes in surface chemistry of soil, biochar and biochar-amended soil. The effects of planting, biochar addition to the soil, time and their interactions on nature and type of functional groups in FTIR spectra are reported.

7.2 Introduction

Biochar, a carbon rich solid product of pyrolysis (Wang *et al*., 2020), can persist in the environment for centuries due to its aromatic and inert nature (Wan *et al*., 2020), however the ^{14}C ages of biochar were found about 1160 and 5040 years in literature (Schmidt *et al*., 2002; Ahmad *et al*., 2014; Palansooriya *et al*., 2019). Despite its recalcitrant nature, it undergoes mineralization by number of oxidation and reduction processes by active biotic and abiotic factors of environment (Guggenberger *et al*., 2008; Qiu *et al*., 2019).

These processes have a direct, prominent role in short-term and long-term biochar degradation and are responsible for variations in biochar surface properties (Wu *et al*., 2020).

During pyrolysis, biomass undergoes a variety of physical, chemical and molecular changes, which principally cause major volume and mass losses, shrinking without any notable change in the original structure of the feedstock. In addition, pyrolysis involves alterations in ratios of C/N, O/C, and H/C, pore size, surface area, cation exchange capacity, crystalline structure and functional groups such as a rise in stable aromatic carbon–carbon double bonds (C=C) and a decrease in unstable O-H and CH_3 bonds (Kloss *et al*., 2012;Wu *et al*., 2020).

Pyrolysis combustion results in degradation, conversion and formation of the organic and inorganic components of biomass, which directly affect functional group chemistry of biochar (Tan *et al*., 2017; Shan *et al*., 2020; Adesemuyi *et al*., 2020). According to Hanudin (2004), organic material contains many compounds composed of organic and inorganic functional groups and the pyrolysis process increases occurrences of inorganic (resistant) surface or groups on biochar (Elkhalifa *et al*., 2019).

The chemical composition of biochar can be investigated using spectroscopic techniques like Fourier Transform Infrared Spectroscopy (Chen *et al*., 1998). FTIR analysis can identify the functional groups by producing an infrared absorption spectrum by collecting the sample signals, from the infrared frequencies. The emergence of new functional groups would lead to either an increase in the higher degree of aromaticity, which would also contribute to the stability of the organic C, more than before it was processed to become biochar.

The FTIR analysis has very complex procedures (preparation of sample, standardization against ambient air CO_2 and moisture, background subtraction, normalization and band assignments) than other IR techniques, which means that the data is only semi-quantitative. FTIR is a label-free non-destructive technique, which is critical to provide insight of denaturation, aggregation and quantity level of different chemical metabolites (Tiernan *et al*.,

2020). However, the FTIR data still provide clear indications of which functional groups are present or absent.

7.3 Aims and Objectives/Hypothesis

The main questions addressed in this Chapter are;

(i) What are the dominant functional groups present in the biochar, topsoil and biochar-amended soils?

(ii) How does the presence of plants (in this case strawberry) affect the nature and type of surface chemistry of biochar amended-soil?

We hypothesized that the change in surface chemistry of soil amended with varying amount of biochar, influences plant growth and soil properties.

7.4 Results

FTIR spectra for biochar amended soils with or without strawberry plants were obtained twice to identify the changes in surface chemistry and functional groups of biochar amended soils over time. The two timings were:

- Near the start of experiment i.e., initial (30 days from set-up)
- At the end of experiment i.e., final (after one year)

Organic based carbonaceous material like biochar normally contains many compounds composed of aromatic, aliphatic or hydrocarbon origin functional groups (Hanudin, 2004; Adesemuyi *et al.*, 2020). In order to indentify the chemistry behind FTIR spectra changes over time, functional groups were grouped into three categories; aromatic; aliphatic and carbohydrates.

7.4.1 Aromatics

A stable and resistant compound that has a ring structure with a double bond was defined as an aromatic. Aromatics could be non-polar aromatics, mono-polar aromatics or bipolar aromatics (phenols) (Stephen, 2004). In the present study, aromatic functional groups found in the biochar spectra, top soil and biochar-amended soils were: Carboxylate (O-H); carboxyl (C-O); carbonyl (C=O); acetyl ester group; aromatic ring (C-C) and phenols.

Table 7.1 Assignment of aromatic bands studied in soil and biochar-amended soil (4000-400 cm^{-1} spectral range)

Wave number (cm^{-1})	Functional Group	Assignment	Reference
782	CH	Aromatic.	Masto *et al*., (2013), Harris *et al*.,(2013)
815	C-H	Lignin	Uchimiya *et al*., (2011)
877	C-C	Aromatic stretching	Melo *et al*., (2013)
1260	CO-,-OH, RCO-O	Phenol group	Wang *et al*., (2009)
1321	CH2	Deformation	Melo *et al*., (2013)
1420	C=C	stretching of aromatic rings	Qayuum *et al*.2012
1427	C-O	Aromatic ring stretching	Masto *et al*., (2013)
1430	C-H	lignin	Chen *et al*.,(2010)
1513	C=C	C=C stretching of aromatic rings	Qayuum *et al*.,(2012)
1600	C=C	Aromatic vibration for lignin formation	Chun *et al*., (2004)
1604	C=C, C=O	lignin	Uchimiya *et al*.,(2011)
1616	C=O	Stretching vibrations of ketones, aldehydes and carbonyl group in aromatics	Chun *et al*., (2004)
1700	C=O	aromatic carbonyl stretching	Chun et al., (2004)

7.4.2 Aliphatics

Aliphatics aremajor structural group of organic molecules in which atoms arenot joined to form a ring. The most representative aliphatic bands (Table 7.2) in the samples studied were:Polar aliphatics (alkanes);mono-polar aliphatics (ethers and alkenes);llkynes and bipolar aliphatics (alcohols).

Table 7.2 Assignment of aliphatic bands studied in soil and biochar amended soil (4000-400 cm^{-1} spectral range)

Wave number (cm^{-1})	Functional Group	Assignment	Reference
3000.131	C=O	stretching	Ozeimen and Menoboyu,(2010)
2960.592	C-H	stretching	Ozeimen and Menoboyu,(2010)
2925.875	C-H	OH vibrations	Ozeimen and Menoboyu,(2010)
2919.125	CH3	Methyl and Methylene Aliphatic bonds	Ozeimen and Menoboyu,(2010)
2859.334	CH	Aliphatics	Wu *et al*., (2012)
2850.655	C-H	stretching	Ozeimen and Menoboyu,(2010)
1165	C-O-C	Cellulose and Hemicellulose	Sun and Hughes, (1998)

Table 7.3 Assignment of carbohydrates bands studied in soil and biochar amended soil (4000-400 cm^{-1} spectral range)

Wave number (cm^{-1})	Functional Group	Assignment	Reference
3424	O-H	Cellulose	Uchimiya *et al*., (2011)
3400	O-H,N-H	Stretching vibrations of amino and hydroxyl groups	Sun and Hughes, (1998)
2920	C-H	Methyl and Methylene	Qayuum *et al*., (2012)
1732	C=O	Hemicellulose	Masto *et al*., (2013)
1514	C=C	Lignin	Bilba and Ouensanga, (1996)
1530	C=N,C=C	chain elongation	Yang *et al*., (2007)
1460	C-H	Lignin	Movasaghi *et al*. (2008).
1375	C-H	Cellulose and Hemicellulose	Pandey and Pitman, (2004)
1330	C-N	Polysaccharides	Chen *et al*., (2010)
1108	C-OH	ketone or ester bonding	Pandey and Pitman, (2004);Domingues *et al*., (2014)
1060	C-O	Cellulose and Hemicellulose	Movasaghi *et al.* (2008).
1058	C-C	Polysaccharides	Harris *et al*.,(2013)
1032	OH	Cellulose, Hemicellulose and Lignin	Mothe and de Miranda,(2009)
913	Al-OH-Al	hydroxyl group	Chen *et al*., (2008a)
899	ß-bonds	Cellulose	Hao *et al*.,(2013)
815	C-H	Lignin	Movasaghi *et al*., (2008),Chen *et al*.,(2008a)

7.4.3 Carbohydrates

Carbohydrates are the most abundant, widespread organic hydrocarbons. They could be alkenes (C−H), alkynes (C=C) and amines (C−N). The bands observed in biochar, soil and biochar-amended soils were presented in Table 7.3.

7.5 FTIR spectra

The spectra obtained for biochar, biochar fractions and biochar amended soils are presented and discussed in the following paragraphs:

7.5.1 FTIR spectra of biochar

The Fourier Transform Infrared (FT-IR) spectra between 500 cm^{-1} and 4000 cm^{-1} in the absorption mode of the whole biochar sample is shown in Figure 7.1. There were several functional groups shown within the wavelengths investigated in this study.

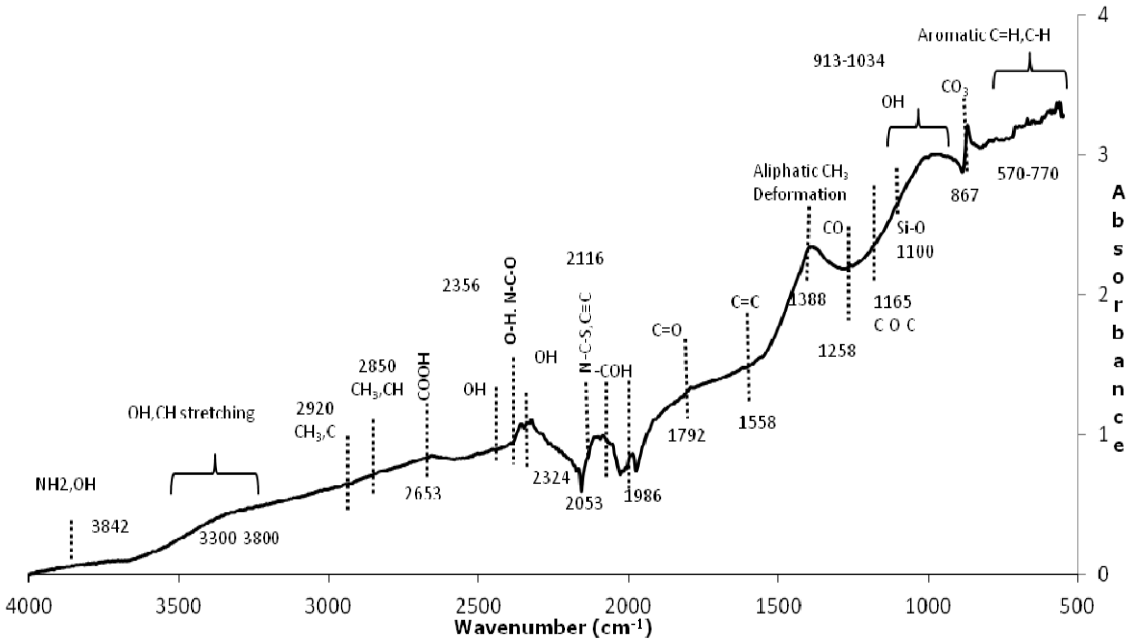


Figure 7.1 FTIR spectra of biochar recorded between 500 cm^{-1} and 4000 cm^{-1} wave numbers

The absorption band at 3842cm^{-1} of the biochar spectra was ascribed to the mixed stretching vibration of amino (NH_2) and hydroxyl (OH) groups (Melo *et al*., 2013; Peng *et al*., 2013). Stretching vibration of hydroxyl functional groups produced a broad band at 3300-3500 cm^{-1}. Cellulose is a principal polymer of wood biochar (Calderón *et al*., 2011; Chia *et al*., 2012;Qayuum *et al*., 2012; Hao *et al*., 2013) and contains hydroxyl groups, which are

recalcitrant and locked in biochar even after pyrolysis. A few weak bands were observed in the strong aliphatic zone i.e., 2920 cm^{-1} and 2850 cm^{-1} in the biochar spectra, which shows biochar aromatization. This was further supported by the presence of distinct aliphatic methyl band (CH_3) at 1388 cm^{-1} that reflects the incomplete carbonization of biochar (Ghani *et al*., 2013).

Table 7.4 Band assignment for FTIR spectra of whole biochar

Wavenumber (cm^{-1})	Functional group	Assignment	References
3842	NH_2, OH	Amino and hydroxyl stretching	Melo *et al*., (2013)
3300-3500	O-H, C-H	Stretching vibrations of hexagonal group	Qayuum *et al*.(2012);Chia *et al*., (2012).
2920	CH_3-C	Aliphatic CH stretching	Ghani *et al*., (2013)
2850	CH_3-CH	Aliphatic CH group	Ghani *et al*., (2013)
2653	COOH	Alcholic group	Harris *et al*.,(2013)
2356	OH,N-C-O	O-H stretching vibration, N-C-O stretching	Saleh *et al*., (2013);Hao *et al*., (2013)
2324	O-H	OH strtech in carboxylic acid	Hao *et al*.,(2013)
2116	N-C-S,C≡C	Stretching	Garside and Wyeth, (2003)
2053	COH	COH in aliphatics	Janik *et al*. (2007)
1986	COH	COH in aliphatics	Nguyen *et al*. (1991)
1792	C=O	Stretching vibrations of ketones, aldehydes and carbonyl group in aromatics	Chun *et al*., (2004); Chia *et al*., (2012)
1558	C=C	Aromatic vibration for lignin formation	Saleh *et al*., (2013)
1388	CH3	Aliphatic CH3 deformation	Saleh *et al*., (2013)
1258	C-O	Stretching of aromatic carbon	Ozeimen and Menoboyu,(2010)
1165	C-O-C	Cellulose and Hemicellulose	Sun and Hughes, (1998)
1100	Si-O_2	Stretching	Makul and Agrawal, (2010)
1034-913	Si-O,=OH	Stretching	Qayuum *et al*. (2012)
867	CO3	Carbontes	Spokas *et al*., (2011)
770-570	C-H,C=H	Aromatic and alkyl bonds or silicon or metal group	Harris *et al*.,(2013);Masto *et al*., (2013)

The prominent peaks at 2653 cm^{-1},2053 cm^{-1} and 1986 cm^{-1} were assigned to either the acetyl and ester groups of the hemicelluloses or the ester linkage of acid carboxylic group of lignin (Sain and Panthapulakkal, 2006; Sun *et al*., 2005). These peaks were less intense in

high temperature biochars because of the removal of most hemicelluloses and cellulose from the feedstock (Harris *et al*., 2013; Janik *et al*., 2007; Nguyen *et al*., 1991).

The O–H stretching mode of hexagonal groups and adsorbed water can be assigned to the bands observed at 2356 cm^{-1} and 2324 cm^{-1}. The position and asymmetry of the bands at these particular wave numbers indicate the presence of strong hydrogen bonds (Saleh *et al*., 2013; Hao *et al*., 2013). An additional unsymmetrical peak at 1792 cm^{-1} was noted which is comprised of a variety of C=O containing functional groups including ketones, carboxylic acids esters, and anhydrides (Qiu *et al*., 1996; Chun *et al*., 2004; Calderón *et al*., 2011; Chia *et al*., 2012).

The aromatic C-H bond and alkyl C=C bond stretching found at 1558 cm^{-1} and 770 cm^{-1}, were derived from original aromatic rings in the lignin of biochar samples, as well as newly aromatized and carbonized materials formed during pyrolysis (Gomez-Serrano *et al*., 1996; Saleh *et al*., 2013; Masto *et al*., 2013). The band reflecting SiO_2 stretching was observed at 1100 cm^{-1} (Makul and Agrawal, 2010).

The peak at 1258 cm^{-1} was due to the aromatic C-O structures and phenolic OH stretching (Özçimen & Meriçboyu, 2010). The peaks between 867 and 880cm^{-1} correspond to carbonates which can be clearly observed in the biochar spectra (Spokas *et al*., 2011). The broad peak between 1034 and 913 cm^{-1} represented the OH stretching vibration (Qayuum *et al*., 2012; Sheng *et al*., 2016).

The small band obtained at 2116 cm^{-1} was assigned to the carboxyl group stretching vibration or to C=C in plane aromatic vibrations from lignin formations (Garside and Wyeth, 2003).The biochar spectrum is dominated by aromatic organic and inorganic functional groups, which in turn determine the chemical properties of biochar. The presence of oxygen-

containing functional groups at lower wave numbers indicated the strong hydrogen bonding and great affinity of biochar for cations.

7.5.2 Biochar fractions

The high pyrolysis wood biochar was sieved into different size fractions and all of them were run through FTIR to obtain their individual spectra.

Table 7.5 Intensities observed in FTIR spectra for different size fractions of biochar

Wavenumber (cm^{-1})	Biochar (whole)	BC 1 mm	BC 2 mm	BC 53 µm	BC 106 µm	BC 212 µm	BC 300 µm	BC 425 µm	BC 500 µm	BC 710 µm
3842	0.0666	0.0442	0.0363	0.0460	0.0392	0.0399	0.0306	0.0372	0.0338	0.0467
3300-3500	0.2265	0.1306	0.1161	0.1633	0.1256	0.1356	0.1229	0.1246	0.1104	0.1189
2920	0.6530	0.3600	0.3447	0.4969	0.3701	0.4110	0.3526	0.3723	0.3273	0.3379
2850	0.7122	0.3964	0.3859	0.5132	0.4138	0.4543	0.3951	0.4257	0.3709	0.3716
2653	0.8489	0.4950	0.4879	0.6137	0.5141	0.5641	0.4920	0.5143	0.4600	0.4620
2356	1.0726	1.1974	0.7532	0.6039	0.7903	0.6310	0.6035	0.6369	0.4282	0.3995
2324	1.1066	0.9876	0.7262	0.7336	0.7627	0.6960	0.6371	0.6647	0.5234	0.5148
2116	0.9773	0.7194	0.7194	0.8265	0.7225	0.7947	0.7054	0.7163	0.6628	0.6642
2053	0.9308	0.7646	0.7437	0.8553	0.7398	0.8081	0.7076	0.7315	0.6878	0.6824
1986	0.8614	0.7321	0.7581	0.8099	0.7355	0.8114	0.7092	0.7218	0.6768	0.6754
1792	1.3373	1.0323	1.0307	1.1231	1.0166	1.0927	0.9884	1.0088	0.9488	0.9444
1558	1.5493	1.3791	1.3569	1.4402	1.3276	1.3913	1.3132	1.3211	1.2619	1.2813
1388	2.3391	1.6803	1.6888	1.9550	1.8127	1.8734	1.7712	1.7690	1.6456	1.6213
1258	2.2084	1.8454	1.8544	1.9738	1.8799	1.9468	1.8555	1.8690	1.7756	1.7676
1165	2.4010	2.0907	2.0820	2.2230	2.1060	2.1703	2.0727	2.0731	1.9937	1.9892
1100	2.6599	2.2899	2.2492	2.4611	2.3198	2.3798	2.2799	2.3541	2.1680	2.1747
1034-913	2.9794	2.5234	2.5168	2.6849	2.5602	2.6236	2.5250	2.5474	2.4371	2.4431
867	3.2003	2.8427	2.7878	3.1146	2.8741	2.9338	2.8265	2.8095	2.7080	2.7305
770-570	3.2070	3.3633	3.3666	3.2348	3.3411	3.3125	3.3588	3.3580	3.4178	3.3993

These biochar fractions were: 1mm; 2 mm; 53 µm; 106 µm; 212 µm; 300 µm; 425 µm; 500 µm; 710 µm. Moreover, almost similar intensities were recorded for all biochar size fractions for identified wave numbers (Table 7.5).

All the biochar fractions exhibit similar spectras the whole biochar. The major functional groups identified were presented in Table 7.5. This similarity was found to be consistent for all the samples investigated.

7.5.3 Topsoil

The FTIR spectra of topsoil (Figure. 7.2) showed that the tiny and sharp peaks in the region between 3657-3931 cm^{-1} are due to stretching vibrations of NH_2 and hydroxyl groups (Melo *et al*., 2013).

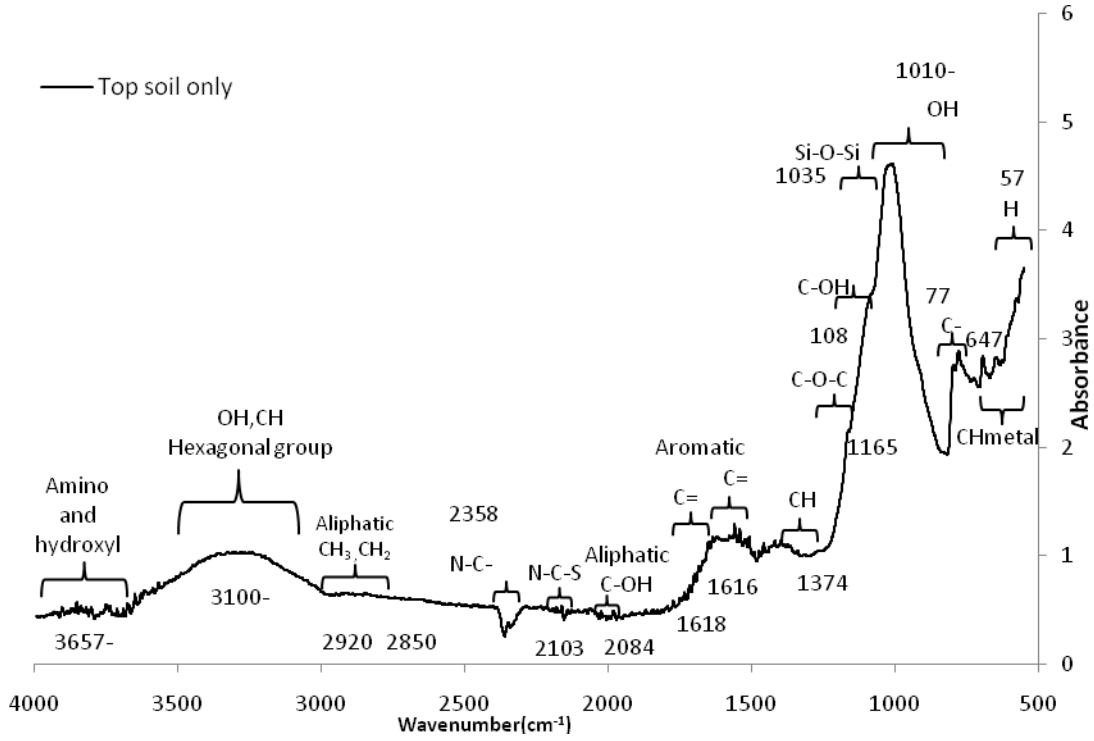


Figure 7.2 FTIR spectra of topsoil (500-4000 cm^{-1} wave numbers)

The broad band in the topsoil spectra at 3400-3100 $cm^{-1}$was produced by O-H, C-H stretching vibrations (Qayuum *et al*., 2012; Hao *et al*., 2013). Ozcimen and Mericboyu (2010) and Ghani *et al*., (2013) reported aliphatic CH_3 and CH_2 distinct peaks at 2920 cm^{-1} and 2850 cm^{-1}. A small peak was observed at 2358 cm^{-1} which is the result of N-C-O presence (Hao *et*

al., 2013).At 2103 cm^{-1} N-C-S and OH group stretch reflected the structural change in carboxylic acid (Nguyen *et al*., 1991). Janik *et al.,* (2007) reported the presence of COH group of aliphatics at 2084 cm^{-1}.

Table 7.6 Band assignment for most representative bands observed in topsoil FTIR spectra

Wavenumber (cm^{-1})	Functional group	Assignment	References
3931-3657	NH_2, OH	Amino and Hydroxyl stretching	Melo *et al*., 2013
3400-3100	O-H, C-H	Stretching vibrations of hexagonal group	Qayuum *et al*.(2012);Hao *et al*.,(2013)
2920	CH3	Methyl and Methylene Aliphatic bonds	Ozeimen and Menoboyu,(2010)
2358	N-C-O	N-C-O stretching	Hao *et al*., (2013)
2103	N-C-S	OH strtech in carboxylic acid	Nguyen *et al*. (1991)
2084	COH	COH in aliphatics	Janik *et al.* (2007)
1618	C=C	Aromatic vibration for lignin formation	Chun *et al*., (2004)
1616	C=O	Stretching vibrations of ketones, aldehydes and carbonyl group in aromatics	Chun *et al*., (2004)
1374	CH3	Aliphatic CH3 deformation	Saleh *et al*., ()
1558	C=C	Aromatic vibration for lignin formation	Saleh *et al*., ()
1374	CH3	Aliphatic CH3 deformation	Saleh *et al*., ()
1165	C-O-C	Cellulose and Hemicellulose	Sun and Hughes, (1998)
1085	C-OH	COH in Aliphatics	Janik *et al*. (2007)
1035	Si-O-Si	Si-O-Si stretching	Qayuum *et al*.2012
1010-1031	0H	Aliphatic Ethers C-O and alcohol C-O stretching	Domingues *et al*.2014
777	CH	CH aromatic and alkyl bonds or silicon group	Saleh *et al*., ()
740	CH	Aromatic C-H bond	masto *et al*., 2013; Harris *et al*., 2013
647	CH-Metal	C-H bond with metal	Uras *et al*.2012
575	H	aromatic structural changes such as H2	Uras *et al*.2012

Several bands were associated with aromatic vibrations such as: 1618 cm^{-1} (C=C aromatic vibration for lignin formation) Chun *et al*., (2004); 1558 cm^{-1} (C=C); 777 cm^{-1} (CH aromatic and alkyl bonds or silicon group) Saleh *et al*., (2013); 740 (aromatic C-H bond) Masto *et al*. (2013);575 cm^{-1} (H aromatic structural changes) Uras *et al*.(2012).The oxygen-containing

functional groups like ketones, aldehydes and carbonyl groups were identified at 1616 cm^{-1} (Chun *et al*., 2004).

A small peak was observed at 647 cm^{-1}, which is characteristic of a C-H bond with a metal (Uras *et al*., 2012).The FTIR spectra of topsoil were characterized by principal aliphatic bands at the following wavelengths: 1374 cm^{-1} (CH_3 aliphatic deformation) Saleh *et al*., (2013); 1085 cm^{-1} (C-OH in aliphatics) Janik *et al*., (2007); 1010-1031 cm^{-1} (aliphatic Ethers C-O and alcohol C-O stretching) Domingues *et al*.(2014).

7.6 Effect of biochar on FTIR spectra of biochar amended soil

The FTIR spectrum of soil amended with biochar at different rates exhibited glimpses from whole biochar and topsoil spectra (Figure 7.3 a).

The typical bands were observed in which the biochars showed very broad continuum absorption with few prominent peaks in the range of 500–4000 cm^{-1} (Table 7.7). All four of the biochar treatments (BC 2.5 %, 5 %, 10% and 15 %) in soil behaved similarly and produced the same pattern of bands/peaks at assigned wave numbers but with different intensities (Figure 7.5 b). The maxima of all the functional groups (aliphatic, aromatic and carbohydrates) were recorded in the control soil (0 % biochar) with or without plants.

Aromatics (C-C and CH) were recorded at higher intensity than oxygen-containing compounds (carboxyl and carbonyl), followed by aliphatics and a few hydrocarbons. On comparison of control (BC0 %) with BC 10 % and 15 %, the greatest change was observed in the zones of 1400-1600 cm^{-1} and 3752 cm^{-1} which are characteristics of aromatic C-C, amino and hydroxyl stretching.

Table 7.7 Band assignment for most representative bands observed in biochar amended soil FTIR spectra

Wavenumber (cm^{-1})	Functional group	Assignment	References
649	CH-metal	C-H bond with metal	Uras *et al*.,(2012)
693	S-O	Stretching	26.Uras *et al*.,(2012)
777	C-H	CH aromatic and alkyl bonds or silicon group	Saleh *et al*., (2013)
875	C-C	Aromatic stretching	Melo *et al*., (2013)
1008	C-O-C	Cellulosic ethers dehydration	Melo *et al*., (2013)
1085	C-OH	COH in Aliphatics	Janik *et al*. (2007)
1165	C-O-C	Cellulose and Hemicellulose	Sun and Hughes, (1998)
1421	CH2	Deformation	Melo *et al*., (2013)
1400-1600	C-C	Aromatic stretching	Uras *et al*., 2012
2086	C-OH	COH in aliphatics	Janik *et al*., (2007)
2287	N-C-S	OH strtech in carboxylic acid	Nguyen *et al*., (1991)
2350	N-C-O	N-C-O stretching	Hao *et al*., (2013)
2655	CH3	Methyl and Methylene Aliphatic bonds	Ozeimen and Menoboyu,(2010)
2800-2900	CH	Aliphatics	Wu *et al*., (2012)
3444	OH, H-H	Stretching	Chen *et al*., (2011)
3752	OH.NH2	Amino and hydroxyl stretching	Melo *et al*., (2013)
3876	NH2, OH	Amino and hydroxyl stretching	Melo *et al*., (2013)

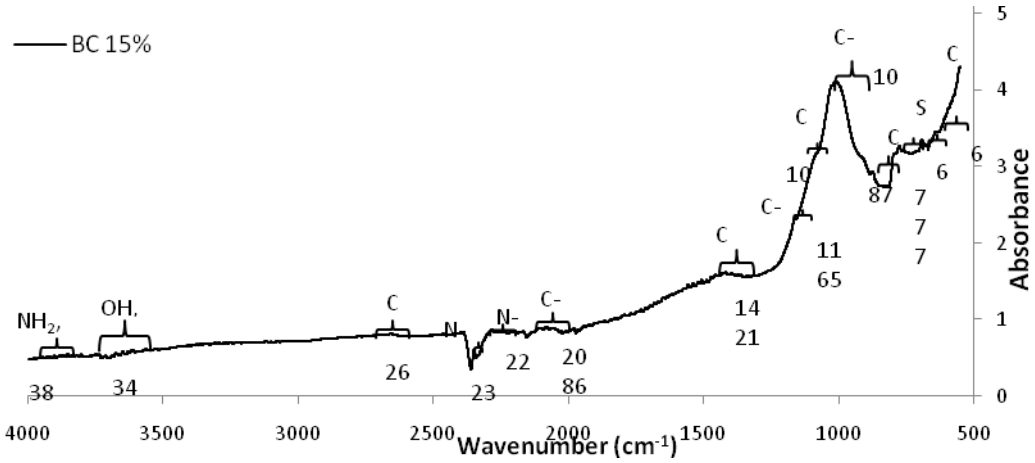


Figure 7.3 (a) FTIR spectra of topsoil amended with biochar at different application rates

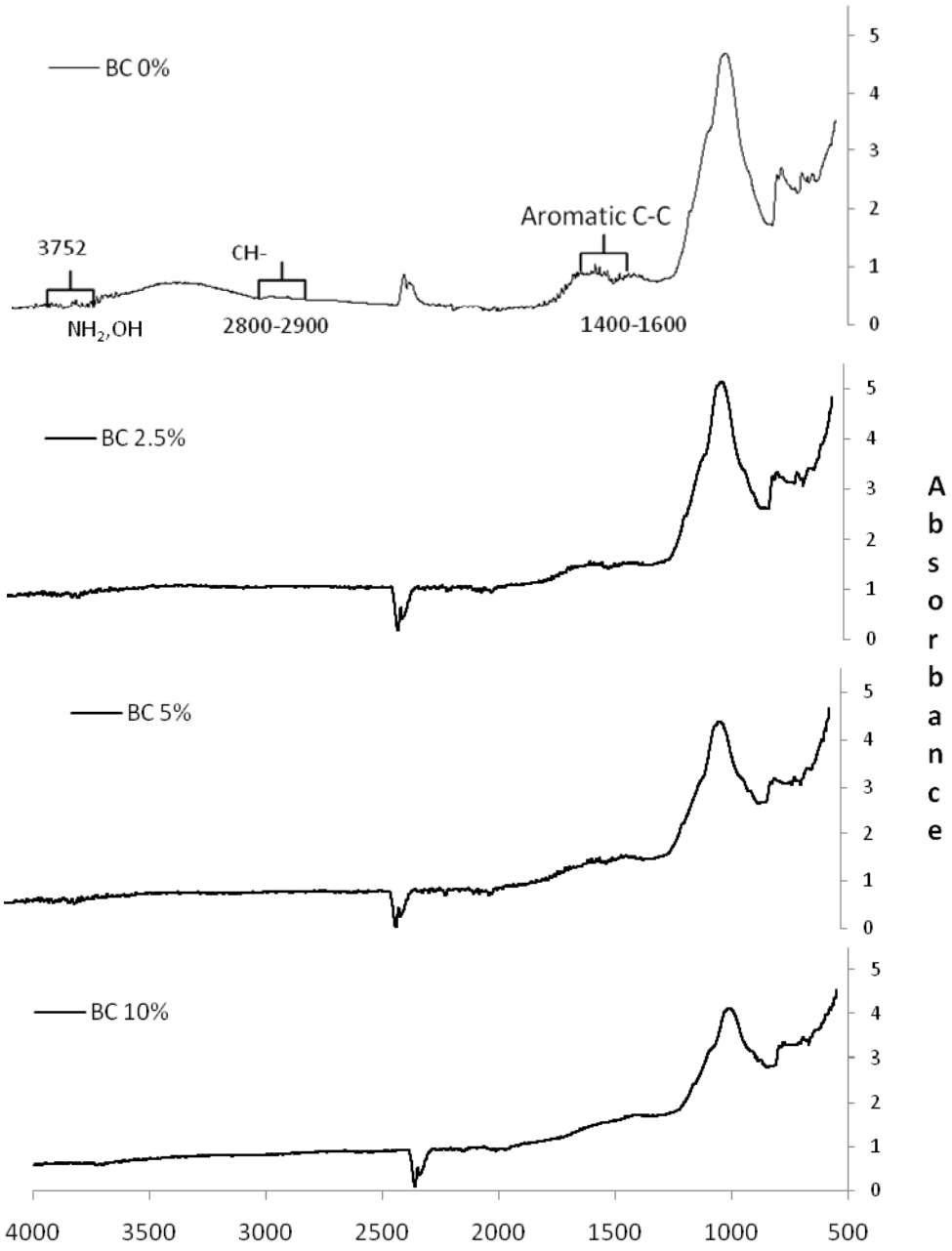


Figure 7.3 (b) FTIR spectra of Top soil amended with biochar at different application rates

The prominent peaks observed in the BC 15 % spectra were: 693 cm^{-1} (S-O) Uras *et al*., (2012);777 cm^{-1} (C-H) Saleh *et al*., (2013);1008 cm^{-1} (C-O-C) Melo *et al*., (2013);1085 cm^{-1} (C-OH) Janik *et al*., (2007); 1165 cm^{-1} (C-O-C) Sun and Hughes (1998); 1421 cm^{-1} (CH_2) Melo *et al*., (2013); 1400-1600 cm^{-1}(C-C) Uras *et al*., (2012); 2655 cm^{-1} (CH_3) Ozcimen and

Mericboyu (2010);2800-2900 cm^{-1} (CH) Wu *et al*., (2012);3444 cm^{-1} (OH, H-H) Chen *et al*., (2011); 3876 cm^{-1} (NH_2, OH) Melo *et al*., (2013).

7.7 Effect of the plants on FTIR spectra of biochar amended soil

The FTIR spectra for biochar-amended soils with or without plants are shown in Figure 7.4 with band assignments in the near IR region wave number 4000–500 cm^{-1} indicated in Table 7.8.

Table 7.8 Band assignment for most representative bands observed in biochar amended soils in absence or presence of strawberry plants

Wavenumber (cm^{-1})	Functional group	Assignment	References
549	Si-O-Si	Si-oxygen network	Saleh *et al*., (2013)
575	C-Cl	alkyl halides stretching	Ozeimen and Menoboyu,(2010)
674	C-H	aromatic and alkayl bend	Ozer *et al*., 2007
695	S-O	Stretching	26.Uras *et al*.,(2012)
777	C-H	CH aromatic and alkyl bonds or silicon group	Saleh *et al*., (2013)
875	C-C	Aromatic stretching	Melo *et al*., (2013)
1003	C-O-C	Cellulosic ethers dehydration	Melo *et al*., (2013)
1087	C-OH	COH in Aliphatics	Janik *et al*. (2007)
1091	C-O	aliphatic ether C O or alcohol C O stretching	24.Uras *et al*.,(2012)
1165	C-O-C	Cellulose and Hemicellulose	Sun and Hughes, (1998)
1418	OH	phenolic O–H bending	Saleh *et al*., (2013)
1558	C=C	Aromatic vibration for lignin formation	Saleh *et al*., (2013)
1634	C=O	Acetyl and ester groups of hemicelluloses and cellulose	Sain and Panthapulakkal, (2006); Sun *et al*.,(2005)
1771	C-O	carbonyl and carboxyl stretching vibrations of aromatics	Ascough *et al*., (2011)
2121	C≡C	Alkynes stretch	Saleh *et al*., (2013)
2175	CH,OH	stretching vibrations of CH and OH	Uras *et al*.,(2012)
2667	CH3	Methyl and Methylene Aliphatic bonds	Ozeimen and Menoboyu,(2010)
2933	CH	Aliphatic CH stretching	Ozeimen and Menoboyu,(2010)
3141	O-H, C-H	Stretching vibrations of hexagonal group	Qayuum *et al*.(2012);Hao *et al*.,(2013
3420	OH, H-H	Stretching	Chen *et al*., (2011)
3946	NH_2, OH	Amino and Hydroxyl stretching	Melo *et al*., 2013

The presented spectra indicated an overall shift in intensity for all the functional groups. This shift is negative in the case of the control (0% biochar) with plants, whereas a decrease was noted in intensities of all wave numbers except some aromatics. The peaks recorded at 1091 cm^{-1}(aliphatic ether CO or alcoholic CO), 1165 cm^{-1} and 1003 cm^{-1} (cellulosic C-O-C) were the same in both spectra and planting had no impact. The broad peak at 3141 cm^{-1} (O-H, C-H stretching) observed in the no-plant spectra was not visible in plus-plant spectra.

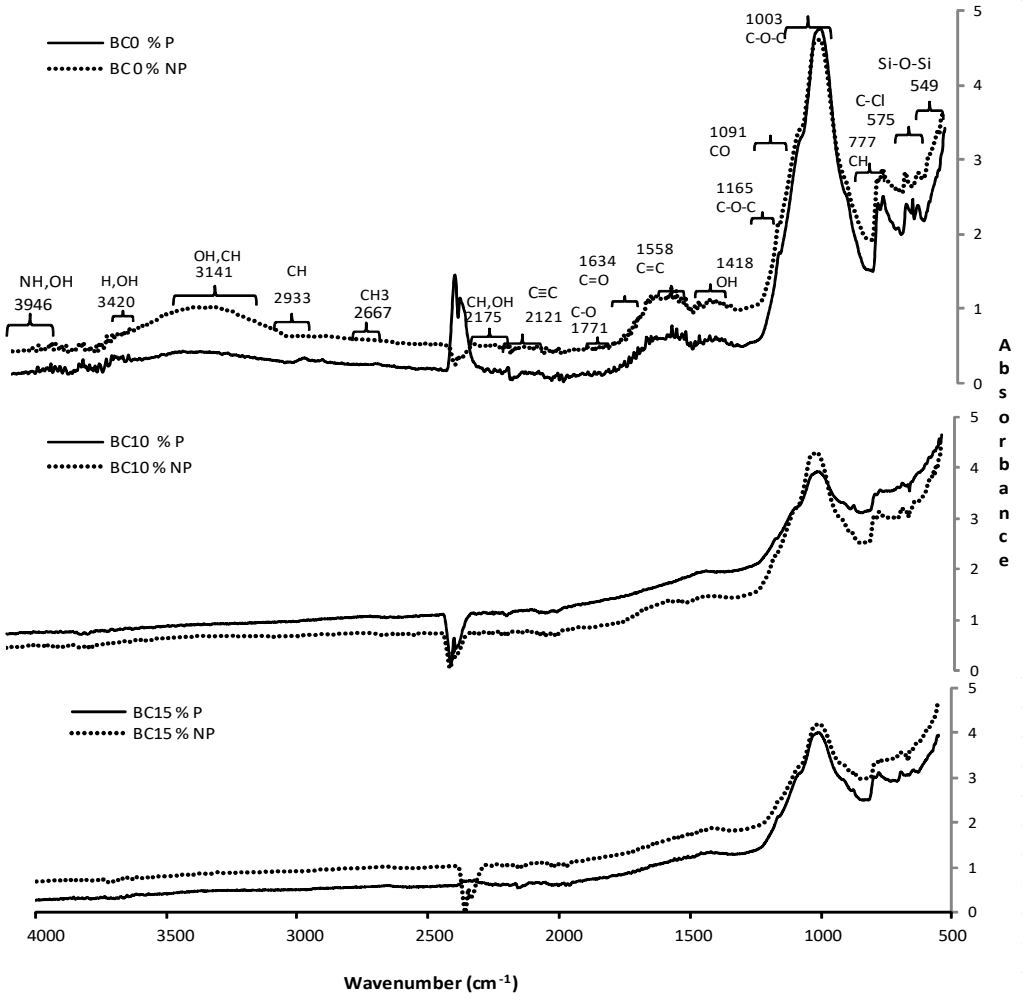


Figure 7.4 FTIR spectra of biochar amended topsoil in the absence or presence of strawberry plant

All the other prominent bands in both the spectra with their assignments and references are presented in Table 7.8.The similar pattern of decrease due to plants was found in soils where

biochar @15% had been applied, however, the percent decrease in intensity was less than in control soils. The spectra obtained from the soils with a plant and 15 % biochar is in contrast with the other two soils with 0 and 10 % biochar. An overall lift in spectra was observed with no change or shift in wave numbers of functional groups.

7.8 Effect of time on FTIR spectra of soil plus biochar treatments

No major change was observed for FTIR spectra of control soil (0 % biochar) for the initial versus the final samples taken for both planted and unplanted treatments (Figure 7.5), except in two regions. A clear spread in intensity was observed at 3400-3100 cm^{-1} after 12 months, which was more obvious in unplanted treatments, where the bands were associated with vibrations of aromatic structures such as CH. This increase was accompanied with the more specific change in H bonded O-H detected at 3407 cm^{-1} indicating the polymeric nature of fresh biochar. This could be due to the orderly arrangement of the crystalline phase in the biochar and soil complex where all the sites were exposed to microbes (Cheah *et al*., 2013).

A small decrease was observed in aromatic C=C at 1024 cm^{-1} after 12 months in FTIR spectra for the control soil with 0 % biochar and no plant. At the same time, a broad increase in OH stretching region at 3400-3100 cm^{-1} was observed. This increase was supported by the findings of Cheng *et al*., (2008) who found that biochar after exposure to soil and microbial degradation may experience increases in oxygen containing functional groups like OH which might come from degradation of aromatic structures. In the biochar 10 % amended soil without a plant, there was an overall decrease in spectra at the final analysis, except for some oxygen containing groups like 1024 cm^{-1} (OH) and 1060 cm^{-1} (CO) (Figure 7.6). However, in the case of the 15 % biochar amendment, a contrasting trend was observed at the end of experiment with an increase in all functional groups (Figure 7.6).

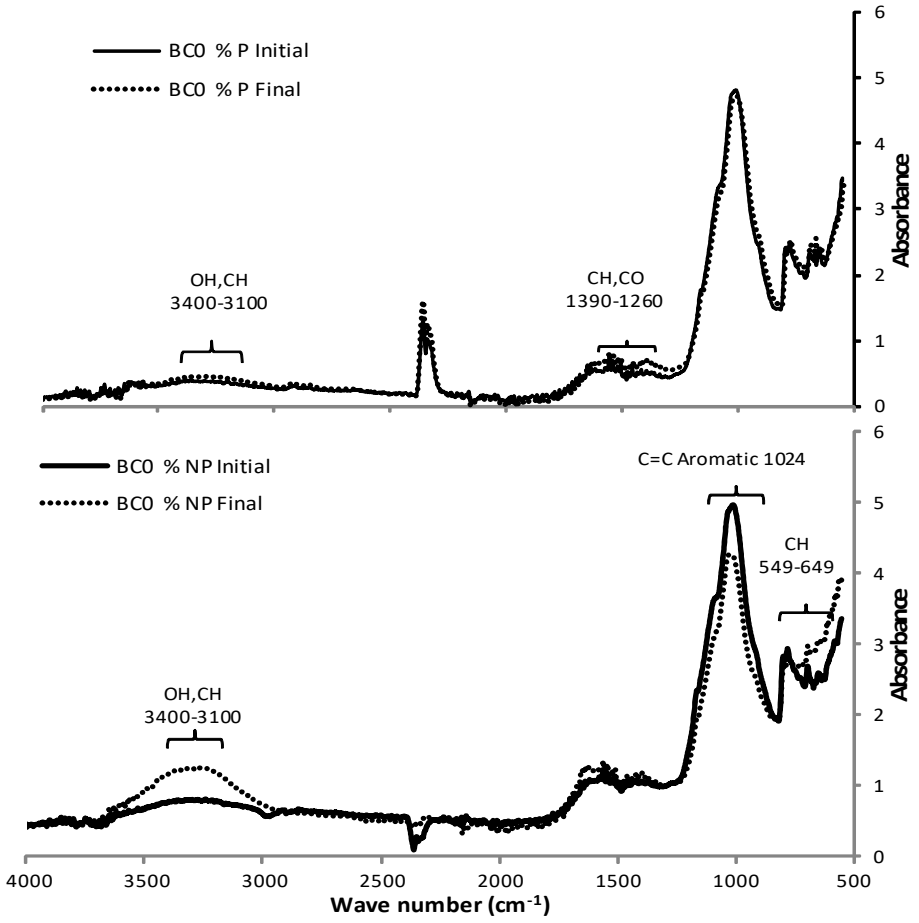


Figure 7.5 FTIR spectra of control soil (0 % biochar) for initial and final sampling times in the presence and absence of a strawberry plant

There was an obvious increase in oxygen containing groups mainly because of rearrangement of molecules in aliphatics and carbohydrates. The end result was an increase in aromaticity. Major shifts in the spectra for all wave numbers were observed in soils, where they were amended with biochar @ 2.5, 5, 10 and 15 % as compared to the control (0 % biochar) in the presence of the strawberry plant (Figure 7.7). After 12 months, a marked decrease in 2.5 %, 10 % and 15 % in the spectral bands between 500 and 4000 cm^{-1} was noted, However in 5 % biochar a contrasting increase was observed.

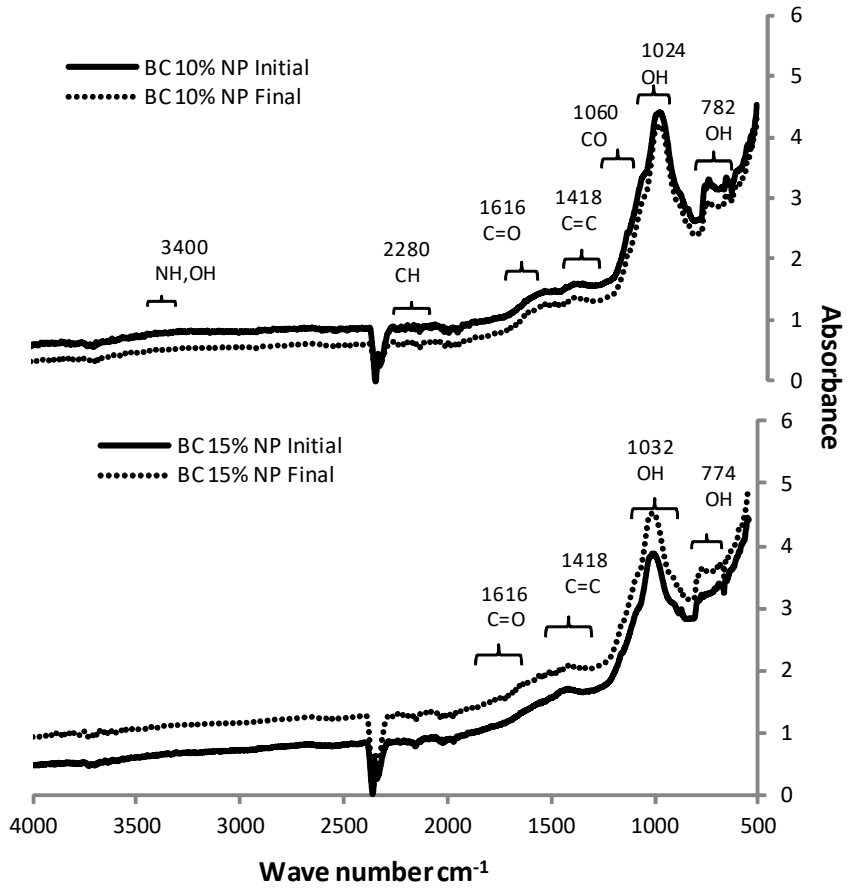


Figure 7.6 FTIR spectra for 10 and 15 % biochar amended soils without strawberry plants

The H bonded O-H detected at 3407 cm^{-1} indicated the polymeric nature of biochar. The decrease in intensity in BC 2.5, 10 and 15 % treatments might be due to the orderly rearrangement of the crystalline phase right after biochar exposure to microbial and plant mediated rhizosphere transformations.

The increase in intensity was observed in BC 5 % (Figure 7.7) at 3400-3200 cm^{-1} due to the existence of surface hydroxyl group and chemisorbed water. The asymmetrical appearance of this band at lower wave numbers indicates the presence of strong hydrogen bonds.

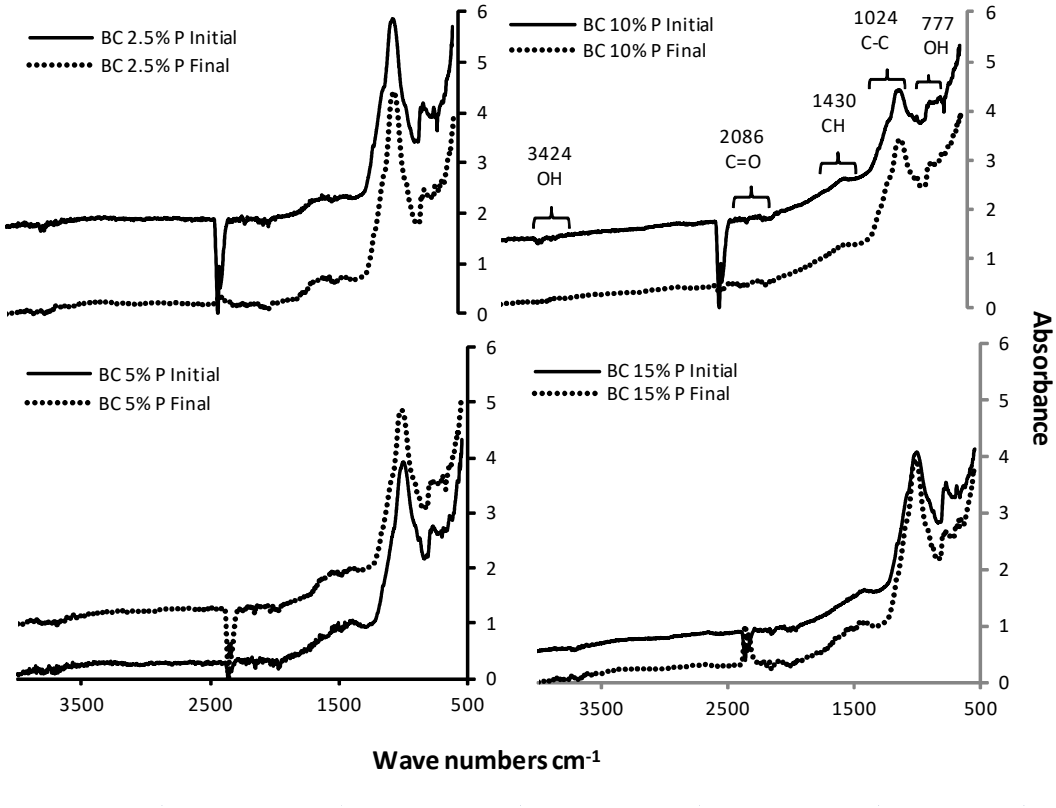


Figure 7.7 FTIR spectra for unplanted biochar amended soils (2.5, 5, 10 and 15 % biochar)

The presence of absorption bands characteristic of CH_3 or CH_2 structures (2960, 2925, 1460, 1430, 1321 and 815 cm^{-1}) in all the spectra suggests the existence of some aliphatic species. The decrease in the band intensity in biochar amended soils over time has also been considered as an indicator for degradation (Schmidt*et al*., 2002) and a similar trend was observed in the spectral bands at 2850 cm^{-1}.The changes indicate the loss of more labile aliphatic and polysaccharide components of the biochar, and resulting in the retention of more stable aromatic structure (Rutherford *et al*., 2012). Below 2000 cm^{-1} the FTIR spectrum for all the biochar amended soils exhibits tiny absorption peaks typical of oxygen or nitrogen species.

The presence of bands at 1260 cm^{-1}, 1427 cm^{-1}, 1060 cm^{-1} can be attributed to the stretching vibrations of C-O in most probably carboxylic ester or ketene ester structures. Remarkable

decreases in intensity at most of these wave numbers in spectra for biochar-amended soils indicate the removal of acetyl ester groups (Schwanninger *et al*., 2004; Stefke *et al*., 2008).The increase of aromatic functional groups (C=C) as shown by the results of the analysis of FTIR absorption for 5 % biochar treatment at 1514.12 cm^{-1} and 1530.70 cm^{-1}, is in line with the results of Cheng *et al*., (2008). These authors reported that after 12 months of soil application, biochar acquired oxygen functionalities like hydroxyl, carboxylic and phenolic groups.

The FTIR absorption peaks at 1600 cm^{-1}, 1604 cm^{-1} and 1420 cm^{-1} appear with higher intensity in BC 5 % and at lower intensity in 2.5, 10 and 15 % BC treatments. These absorbance differences showed the change in biochar chemical composition, mainly due to decreases in acidic groups (Chun *et al*., 2004). The complicated nature of the absorption bands at 1732 cm^{-1}, 1616 cm^{-1} suggest that these bands were due to aromatic rings and double bond variations which overlapped other less intense bands such as nitrogen-containing groups.

The band at 1700 cm^{-1} indicates a reduction of carbonyl and carboxyl functional groups. An alkene C=C stretch group was detected at 1530 cm^{-1}.The FTIR spectra for all functional groups clearly indicated various some small shifts in wave numbers but more obvious are the changes in the relative absorbance.

The most noticeable was the decrease of the absorbance at 1032 cm^{-1} for sucrose. The C-O stretch (1060 cm^{-1}) combined with H bonded O-H suggested the presence of an alcohol group in all biochar spectra.

7.9 Effect of biochar, plant, time and their interactions on functional group chemistry

In samples taken from the experimental pots, the effect of biochar application as a single factor on most carbohydrate, aromatic and aliphatic functional groups was significant (P <0.05) (Table 7.9). In contrast, planting as a single factor did not have a significant effect on these functional groups.

Table 7.9 Effect of biochar as a single factor on presence of prominent functional groups

Aliphatic		Aromatic		Carbohydrates	
Wave number (cm^{-1})	F value	Wave number (cm^{-1})	F value	Wave number (cm^{-1})	F value
3000.131	0.022 *	1616	0.002*	3424	0.286 NS
2960.592	0.013 *	1604	<0.001**	3400	0.330 NS
2925.875	0.026*	1600	<0.001**	2920	0.027 *
2919.125	0.027*	1513	<0.001**	1732	<0.001**
2859.334	0.012*	1430	<0.001**	1700	<0.001**
2850.655	0.012*	1427	<0.001**	1530	<0.001**
1165	0.006*	1420	<0.001**	1514	<0.001**
		1321	<0.001**	1460	<0.001**
		1260	<0.001**	1375	<0.001**
		877	<0.001**	1330	<0.001**
		815	<0.001**	1108	0.234 NS
		782	<0.001**	1060	0.023*
				1058	0.016*
				1032	<0.001**
				913	<0.001**
				899	<0.001**
				815	<0.001**

The differences among aliphatics, aromatics and hydrocarbons observed at the start and at the end of the experiment were significant for most of the functional groups at selected wave numbers (cm^{-1}) (Table 7.10).

Although plants as a single factor did not affect functional group chemistry, there were significant biochar × plant interactions (Table 7.11).

Table 7.10 Effect of time as a single factor on presence of prominent functional groups

Aliphatic		Aromatic		Carbohydrates	
Wave number (cm^{-1})	F value	Wave number (cm^{-1})	F value	Wave number (cm^{-1})	F value
3000.131	0.007	1616	0.017	3424	0.028
2960.592	0.005	1604	0.015	3400	0.034
2925.875	0.005	1600	0.015	2920	0.005
2919.125	0.005	1513	0.010	1732	0.002
2859.334	0.004	1430	0.006	1700	0.003
2850.655	0.004	1427	0.006	1530	0.013
1165	<0.001	1420	0.008	1514	0.010
		1321	0.004	1460	0.006
		1260	0.004	1375	0.008
		877	0.001	1330	0.0084
		815	0.001	1108	0.002
		782	<0.001	1060	0.008
				1058	0.010
				1032	0.049
				913	0.003
				899	0.002
				815	0.001

NS Non-Significant, * Significant (<0.05), ** Highly Significant (<0.001)

Table 7.11 Effect of biochar × plant interaction on presence of prominent functional groups

Aliphatic		Aromatic		Carbohydrates	
Wave number (cm^{-1})	F value	Wave number (cm^{-1})	F value	Wave number (cm^{-1})	F value
3000.131	0.023	1616	0.002	3424	0.026
2960.592	0.023	1604	0.002	3400	0.028
2925.875	0.026	1600	0.002	2920	0.028
2919.125	0.027	1513	<0.001	1732	0.002
2859.334	0.021	1430	<0.001	1700	0.002
2850.655	0.024	1427	<0.001	1530	0.001
1165	0.017	1420	<0.001	1514	<0.001
		1321	<0.001	1460	<0.001
		1260	<0.001	1375	<0.001
		877	0.001	1330	<0.001
		815	<0.001	1108	0.420
		782	0.007	1060	0.912
				1058	0.908
				1032	0.390
				913	0.054
				899	0.018
				815	<0.001

The results showed that a biochar × time interaction was significant for aliphatic and carbohydrates and specific aromatic functional groups (Table 7.12).

Table 7.12 Effect of biochar ×time interactions on presence of prominent functional groups

Aliphatic		Aromatic		Carbohydrates	
Wave number (cm^{-1})	**F value**	**Wave number (cm^{-1})**	**F value**	**Wave number (cm^{-1})**	**F value**
3000.131	**<0.001**	**1616**	**0.003**	**3424**	<0.001
2960.592	**<0.001**	**1604**	**0.004**	**3400**	<0.001
2925.875	**<0.001**	**1600**	**0.003**	**2920**	<0.001
2919.125	**<0.001**	**1513**	**0.005**	**1732**	<0.001
2859.334	**<0.001**	**1430**	**0.007**	**1700**	<0.001
2850.655	**<0.001**	**1427**	**0.007**	**1530**	<0.001
1165	**<0.001**	**1420**	**0.006**	**1514**	<0.001
		1321	**0.006**	**1460**	<0.001
		1260	**0.006**	**1375**	<0.001
		877	**0.021**	**1330**	<0.001
		815	**0.004**	**1108**	<0.001
		782	**0.006**	**1060**	<0.001
				1058	<0.001
				1032	<0.001
				913	<0.001
				899	<0.001
				815	<0.001

Upon statistical analysis, plant and time interaction was proved significant for some of the aliphatics, aromatic and carbohydrates in strawberry plants grown in biochar amended soils (Table 7.13).

The only plant× time ×biochar interactions on surface chemistry of functional groups were for wave numbers 1165 cm^{-1} (aliphatics), 877 cm^{-1} (aromatics), 913 and 899 cm^{-1} (carbohydrates).

Table 7.13 Effect of plant × time interaction on presence of prominent important functional groups

Aliphatic		Aromatic		Carbohydrates	
Wave number (cm^{-1})	F value	Wave number (cm^{-1})	F value	Wave number (cm^{-1})	F value
3000.131	**0.004**	**1616**	**0.003**	**3424**	**0.004**
2960.592	**0.004**	**1604**	**0.004**	**3400**	**0.005**
2925.875	**0.007**	**1600**	**0.003**	**2920**	**0.007**
2919.125	**0.007**	**1513**	**0.005**	**1732**	**0.004**
2859.334	**0.006**	**1430**	**0.007**	**1700**	**0.003**
2850.655	**0.008**	**1427**	**0.007**	**1530**	**0.004**
1165	**0.023**	**1420**	**0.006**	**1514**	**0.005**
		1321	**0.006**	**1460**	**0.006**
		1260	**0.006**	**1375**	**0.006**
		877	**0.021**	**1330**	**0.006**
		815	**0.004**	**1108**	0.056
		782	**0.006**	**1060**	0.073
				1058	0.077
				1032	0.181
				913	**0.048**
				899	**0.033**
				815	**0.004**

7.10 Discussion

The FTIR spectrum for the wood biochar showed that it contained functional groups including ketones, carboxylic acids esters, anhydrides, aliphatic, CH_3 , CH_2 , C=C, aromatic and carbonyls. These functional groups are characteristic of cellulose and lignin which are principal polymers of wood biochar (Chia *et al*., 2012; Qayuum *et al*., 2012; Hao *et al*., 2013) and are recalcitrant and locked into biochar even after pyrolysis. During pyrolysis at high temperature (1100°C), cellulose and lignin aliphatic compounds are predominantly converted into aromatic carbon compounds. When this high temperature wood biochar was applied to soil, due to microbial and plant mediated transformation, it becomes more aromatic due to the loss of aliphatic and labile compounds and broadening of aromatic bands as observed in the present study.

The maxima of all the functional groups (aliphatic, aromatic and carbohydrates) were recorded in the control soil (0 % biochar) with or without plants. Major shifts in the spectra for all wave numbers were observed in soils amended with biochar @ 2.5, 5, 10 and 15 % w/w as compared to the control (0 % biochar) in the presence of strawberry plant.

After 12 months, a marked decrease in spectral bands between 500 and 4000 cm^{-1} was noted in treatments with 2.5 %, 10 % and 15 % biochar. The FTIR spectrum exhibited the same pattern for all treatments where biochar was applied at different rates with slight variation at some wave numbers. All spectra produced peaks at similar wave numbers but some had notable changes in intensity when the initial and final spectra were compared. This suggests that there was a shift in the amount of the functional groups due to inter-conversions of aliphatic, aromatics and hydrocarbons, but no new functional groups were reported over time.

The possible reasons for increased intensity of the particular functional groups could be:

- For oxygen-containing groups, there could have been a rearrangement of molecules in the aliphatics and carbohydrates.
- For aromatic functional groups, some of the biochar-associated aliphatic carbon (largely from cellulose) was vulnerable and lost rapidly.
- Carbohydrate decomposition could have occurred, and utilization by plants and microbes could result in an increase in some carboxylic and carbonyl groups being formed over time.
- Another reason for increased intensity over time could be the orderly rearrangement of crystalline biochar structure after microbial exposure and consequent decomposition.
- The increase in intensity could be related to the existence of surface hydroxyl groups and chemisorbed water.

- An increase in the intensity of the bands containing pyridine, pyridine-N oxide or pyridine structures, was due to the incorporation of nitrogen into the carbon aromatic lattice.
- Biochar after exposure to soil and microbial degradation may experience an increase in oxygen-containing functional groups, which might come from degradation of aromatic structures (Cheng *et al.,* 2008).

The shift in functional groups intensity could be accompanied by the more specific changes in H bonded O-H polymers in the biochar-soil complex where all the sites were exposed to microbes (Cheah *et al*., 2013). The increase of the aromatic functional group (C=C) is in line with the results of Cheng *et al*., (2008). These authors reported that after 12 months of soil application, biochar acquired oxygen functionalities like hydroxyl, carboxylic and phenolic groups, which could improve its character as a soil amendment, have a positive effect on soil structural properties, lead to soil aggregation, and increasing the total C content (either organic or inorganic) and form mineral-organic complexes.

The possible reasons for the decreased intensity observed in treatments amended with the 5 % biochar could be:

- Decreased band intensity over time might be an indicator of degradation (Schmidt *et al*., 2002). The changes indicate the loss of more labile aliphatic and polysaccharide components of the biochar, and result in the retention of more stable aromatic structure (Rutherford *et al*., 2012).
- Decreases intensity of spectra indicates the removal of acetyl ester groups (Schwanninger *et al*., 2004; Stefke *et al*., 2008) at some specific wave numbers.

- The absorbance differences in initial and final data over time showed the change in biochar chemical composition, mainly due to decreases in acidic groups (Chun *et al*., 2004).
- A marked decrease in intensity was observed for some bands which mark the loss of polysaccharides resulting in abrupt spectral changes with replacement of the sharp, primarily carbohydrate bands to aromatics.
- The bands assigned to the O-H stretching vibration and the aliphatic C-H stretching vibration decreased markedly and almost disappeared. This indicates that labile aliphatic compounds decreased when demethoxylation, demethylation, and dehydration of lignin occurred (Kleen and Gellerstedt, 1995; Jakab *et al*., 1997; Sharma *et al*., 2004).
- The loss of OH and aliphatic groups due to a concurrent development of fused-ring structures, especially in biochars produced at higher pyrolysis temperatures. Bagreev *et al*., (2001) and Rutherford *et al*., (2004) found that pyrolysis temperatures above 400°C enhance dehydroxylation.
- Lignin lost its aliphatic carbon more slowly than cellulose because lignin contains substantial aromatic character to begin with.

Biochar is widely accepted as a stable material with a long residence time in soils ranging from centuries to millennia (Lehmann *et al*., 2007). At the same time, it is still vulnerable to biotic and abiotic decomposition as a function of time. The stability of biochar depends on the type of biomass feedstock and pyrolytic conditions. Cheng *et al.,* (2006) reported early mass loss due to the action of abiotic factors on the labile fraction of biochar such as carbohydrates and volatiles in short-term decomposition studies.

After biochar application to the soil, the outer surfaces were exposed to surface oxidation (Lehmann, 2007), but due to its recalcitrant nature for complete oxidation and release of nutrients, million years required. In short period of time as proved in present study, the degree of disintegration and decomposition was minimal or negligible. In soils where plants were present with biochar, a decrease was observed in functional group wavelength intensity as compared to the unplanted treatments.

The basic pattern of functional group on specific wave numbers remains unchanged for almost all of the treatments, showing the aromaticity of high temperature biochar. However the big shift, either positive or negative in spectral intensities for particular functional group bands reflects activity of the plant and microbial community.

Understanding of the alterations in functional groups on biochar is important since this information is needed to optimize the properties of biochar for specific purposes, such as modifying soil pH, CEC, nutrient retention, or carbon sequestration. By using this information, the relationship between biochar properties and functional groups could be established. It is quite possible to design the desired biochars for a given application for set purposes. For example, one could produce high pH and EC biochars for applying to acidic soils by controlling the high percentage of aromatic carbons. Moreover, sequencing of functional groups could be changed.

If biochar contains more oxygen-containing functional groups, such as carboxyl, carbonyl and phenols, it would be favourable for the degradation of organic matter and give a boost to microbial activity in soil-biochar complexes. At higher degree of aromatization, biochar is difficult to mineralize so could easily be stored in the environment for millennia.

7.11 How biochar surface chemistry affects plant growth and soil properties

Functional groups present on biochar surface determine its reactivity, solubility, degradation and adsorption capacity but it could be variable under specific conditions (Akca and Nimla, 2015; Li *et al*., 2020; Jiang *et al*., 2021; Liao *et al*., 2022). These functional groups include carboxylic, phenolic groups, Hydroxyl, aldehyde and ketones (Janu *et al*., 2021). Functional groups at the biochar surface determine lot of characteristics of biochar which are directly affecting plant growth and soil properties. Some of them are as follows:

1. Functional group present at biochar surface determine the binding of trace elements, contaminants, organic pollutants and could be improved or modified to enhance the required sorption characteristics (Rashid *et al*., 2019; Yaashikaa *et al*., 2020; Amalina *et al*., 2022)
2. The Knowledge of existence or new functional groups mostly determined through biochar surface study may exhibit the exact forecasts of biochar recalcitrance against degradation when applied to soil (Xiang *et al*., 2020; Samsami *et al*., 2020; Singh *et al*., 2021)
3. Thermal stability of biochar is the result of high pyrolysis temperature with increased resistance against microbial decomposition and degradation, is again the reflection of surface composition of biochar dominated by more aromatic functional groups (Papurello *et al*., 2019; Leng *et al*., 2021;Senthil and Le,2021)
4. High lignin component of pyrolyzed biochar, characteristic of C=O stretching of ketones , enriched with more micro-localized sites for the mobilization of contaminant and potentially toxic elements (Esteres *et al*., 2020;Shukla *et al*., 2020; Gopinath *et al*., 2021)

5. The variety of acidic (carboxylic and phenolic) and basic (heterocyclic nitrogen groups) functional groups present on biochar surface interact extensively with metal cations and anions and provide pH-dependent exchange sites on soil minerals which lead to the precipitation of metals (Ahmad *et al*., 2013; Almaroai *et al*., 2013;Han *et al*., 2020; Alkharabsheh *et al*., 2021)
6. Due to the presence of some specific functional groups, biochar increases soil alkalinity, which lead to the precipitation and mobilization of organic matter complex forming metal ions like Copper, Arsenic and Antimony in low solubility soils (Uchimiya *et al*., 2012; Zhang *et al*., 2013; Aziz *et al*., 2020; Dai *et al*., 2020)
7. Adsorption of CO_2 on biochar surface at different temperatures was due to variety of functional groups on biochar surface, high surface area and micropores. At low temperature CO_2 sequestration on biochar surface is due to the micropores and at high temperature CO_2 adsorption is due to Nitrogen containing functional groups at biochar surface (Reza *et al*., 2020; Bolan *et al*., 2021; Gale *et al*., 2021)
8. The adsorption capacity of biochar is mainly due to the presence of pores of accurate size. High temperature biochar have large pore sizes and low temperature biochar are characterized with small pore sizes (Ahmed *et al*., 2020 ; Dai *et al*., 2020)
9. The interaction of biochar with soil organic matter, minerals and microorganisms have significant effects on soil properties, soil aggregation and soil fertility determined by the surface charge characteristics and their development over the time when, biochar is applied to the soil (Tomezyk *et al*., 2020; Li *et al*., 2020; . Ahmed *et al*.,2020)
10. Soil moisture retention is the direct result of alteration in soil physical framework and mineral-organic complex formation after the addition of biochar (Van Zwieten *et al*., 2009; Ahmed *et al*.,2020)

7.12 Conclusion

The study was planned to determine the effect of changes in surface chemistry of biochar and biochar amended soils over the period of 370 days. Biochar and biochar amended soils samples were analyzed through FTIR before the start of experiment and at completion at 370 days to determine the changes in functional group chemistry of biochar surface as a function of time and presence or absence of strawberry plants.

The FTIR spectrum showed the presence of wood characteristic functional groups such as ketones, carboxylic acids esters, anhydrides, aliphatic, CH_3 , CH_2 , C=C, aromatic and carbonyls. During pyrolysis at high temperature (1100°C), cellulose and lignin aliphatic compounds are predominantly converted into aromatic carbon compounds. When this high temperature wood biochar was applied to soil, due to microbial and plant mediated transformation, it becomes more aromatic due to the loss of aliphatic and labile compounds. Broadening of aromatic bands over the period of 370 days also observed in the present study. The results of this study could help to get overview about the usability of high temperature wood biochar for the improvement of soil properties, plant growth and for long-term carbon sequestration.

Chapter 8 General Discussion

8.1 Overview

The results individually presented and discussed in previous research chapters are summarised here to draw the whole picture of the potential use of biochar derived from waste materials. This biochar differs from those used in previos studies because of the origin of the feedstock.

8.2 General findings

The key findings from each experimental chapter with respect to the aims and objectives are summarized in the table below:

Table 8.1 Key findings from chapter 3, 4, 5, 6 and 7

Chapter	Aims and Objectives	Main Findings/Results
Chapter 3 **Biochar characterization.**	• To determine whether the biochar produced (in tonne quantities) meets the biochar standards. • To assess whether application of high temperature wood char to soil is detrimental to ecosystem functioning.	• The biochar used in this study originated from demolition wood and is predominantly composed of all typical wood characters like high organic C with macro- and micro-nutrients in addition to toxic elements and contaminants retained from the starting feedstock. • The nutrients (Ca, K, Na and Mg) were present in the highest concentrations whilst the trace metals, including Fe, Ba, Mo and Mn, were relatively low. Concentrations of toxic metals in biochars, including Pb, Cd, Co, Cr, Cu, and Ni, were present in excess of recommended concentrations (EBR, IBI standards). • Among the ammonium nitrate extracted elements from different size fractions of biochar, major cations were presented (Figure 3.1) due to their importance in the soil-plant system. Ca and K were extracted in the highest concentrations, while Mg was the lowest.

		• Moisture content of the biochar was 11.4 % and is possibly a reflection of the storage method prior to collection.
Chapter 4 **Effects of wood biochar application on strawberry biomass production, nutrient concentration and ecosystem gas exchange.**	• Identify the best rate of biochar in terms of plant growth parameters. • Identify the worst rate of biochar, potentially harmful for the plant growth (due to the release of toxic elements or due to the dissolution of toxic elements up to the levels where it hinders plant growth and instead of any improvement, it causes reduction in plant growth). • Determine how biochar and time both influence ecosystem respiration and photosynthesis.	• Among all strawberry growth parameters, only root dry weight and total biomass were statistically significant. In all other parameters like shoot dry weight, number of leaves, number of stems, root/shoot ratio, total N in shoot and root, and total C in root and shoot, biochar failed to produce any significant difference. • In strawberry shoot, K, P, Zn, Cu, and As content increased with increasing biochar concentration, while Pb content decreased with increasing level of biochar as compared to control. None of the macro- and micro-nutrients exhibited a clear trend with or without biochar in strawberry shoots. In strawberry roots, all macro- and micro-nutrients were non-significant. • The effect of biochar was proved statistically non-significant for leaf temperature and leaf conductance in all biochar treatments. • Biochar ($F_{4,16}$ = 26.67; P< 0.001) and time ($F_{3,60}$ = 35.08; P<0.001) producing differences in ecosystem respiration (Re) for all biochar treatments, but there was no interaction. • The effect of biochar ($F_{4,16}$ = 15.78; P<0.001) and time ($F_{3,60}$ = 39.03; P<0.001) was proved significant in producing difference in net ecosystem exchange (NEE). • Data for gross ecosystem exchange (GEE) showed the significant effects of biochar ($F_{4,16}$ = 5.87; P= 0.004) and time ($F_{3,60}$ = 11.75; P< 0.001).
Chapter 5 **Effects of wood biochar application on soil microbial, biochemical properties and soil respiration.**	• Quantify the effects of biochar and plants on soil enzyme activities. • Determine how biochar influences microbial biomass carbon and nitrogen and how it was affected by presence or absence of	• The effect of biochar, (planted: $F_{4,16}$ = 58.51, P <0.001; unplanted: $F_{2,8}$ = 65.85; P<0.001), time (planted: $F_{3,60}$ = 24.04; P<0.001; unplanted: $F_{3,36}$= 16.28; P<0.001) and plant ($F_{1,20}$ = 4.83; P=0.040) were proved statistically significant for dehydrogenase activity in all treatments. The interaction of time x biochar was also significant for dehydrogenase activity, however time x plant and time x BC x plant interactions were proved statistically non-significant in producing any difference in dehydrogenase activity for all the treatments.

	strawberry plants. • Quantify how different biochar application rates affected rates of CO_2 and CH_4 fluxes.	• The effect of biochar (planted: $F_{4,16}$ = 54.47; *P* <0.001; unplanted : $F_{2,8}$ = 28.41; *P* <0.001), and time (planted: $F_{3,60}$ = 21.88; *P* <0.001; unplanted: $F_{3,36}$= 5.11; *P*=0.013) were proved statistically significant in affecting betaglucosidase activity in soil. Presence of strawberry plant was proved non-significant. The interaction of time x biochar was also proved significant, However time x plant, plant x biochar and time x BC x plant interactions were proved statistically non-significant in producing any difference in beta-glucosidase activity for all the treatments. • Data obtained for phosphatase activity in soil showed that biochar (planted: $F_{4,16}$ = 10.64; *P* <0.001; unplanted: $F_{2,8}$ = 42.20; *P* <0.001) was proved statistically significant as an individual factor but Time was proved significant only in planted treatments, however the effect of time was non-significant in unplanted treatments. • The interaction of time x biochar, time x plant and time x BC x plant interactions, were proved statistically non-significant in producing any difference in phosphatase activity for all the treatments. • Microbial biomass carbon in soil showed that biochar and plant was proved non-significant as a single factor. The effect of time (planted: $F_{3,60}$ = 22.3; *P* <0.001; unplanted: $F_{3,36}$ = 15.29; *P* <0.001) was proved statistically significant as an individual factor. The interactions of time x biochar, time x plant, plant x biochar and time x BC x plant, were also proved non-significant. • Data obtained for microbial biomass nitrogen in soil showed that biochar was proved statistically non-significant as a single factor. The effect of time and plant ($F_{1, 20}$ = 5.23; *P* 0.033) was proved significant for microbial biomass nitrogen in all treatment. The interaction of time x plant, plant x biochar and time x BC x plant, were proved statistically non-significant in producing any difference in soil microbial biomass carbon. • Data obtained for CO_2 flux in soil showed that biochar (planted: $F_{4,16}$ = 12.52; *P* <0.001; unplanted:

		$F_{2,8}$ = 40.79; P<0.001) and time (planted: $F_{6,120}$ = 28.32, P <0.001; unplanted: $F_{6,72}$= 27.5, P <0.001) were proved statistically significant. Presence of strawberry plant was non-significant for CO_2 flux. The interaction of time x plant, plant x biochar and time x BC x plant interactions, were proved statistically non-significant in producing any difference in CO_2 flux for all the treatments. • In case of CH_4 flux in soil showed that time (planted: $F_{3,60}$ = 24.04; P <0.001; unplanted: $F_{3,36}$= 16.28; P <0.001) was proved statistically significant. The effect of biochar as a factor proved to be significant only in unplanted treatments ($F_{2,8}$ = 7; P=0.018); however it has failed to produce any difference in CH_4 flux in presence of strawberry plants. Plant ($F_{1,20}$ = 4.83, P=0.040) as a factor also proved non-significant for CH_4 flux. The interaction of time x biochar, biochar x plant, time x plant and time x BC x plant, were proved statistically non-significant..
Chapter 6 **Effects of wood biochar application on soil physical and chemical properties.**	• Determine the effects of biochar on soil chemistry. • Quantify effects of biochar on physicochemical properties of soil, either positive or negative.	• Biochar has significant effect on gravimetric water content, soil bulk density and water filled pore space. Gravimetric water content and water filled pore space increases with increasing levels of biochar while soil bulk density decreases with increase in biochar. • Soil bulk density was significantly affected by the presence or absence of strawberry plant. High bulk density was recorded in unplanted treatments. • Soil pH and organic matter content increases with increase in biochar application rates however the effect of time was inconsistent in producing any visible difference in pH and organic matter over the period of 370 days. • Biochar has induced accountable difference in NO_3-N and NH_4-N in biochar amended soils in both planted and unplanted soils but again time has no obvious trend although its statistically significant. • Olsen-P was significantly affected by biochar and time. Available P content in soil was increased with increasing rates of biochar application but time has again no obvious trend of increase or decrease. At different sampling time we recorded different P content. • Biochar and time both are statistically significant for

		total C, S and N. Total C content increases with increase in biochar but decreases with number of days. Total S content in soil increases with number of days but have inconsistent effect of biochar. Total N content declined with increase in biochar but its content increased with time. • The effect of time was more obvious and significant in available soil nutrients, although the effect was inconsistent. Effect of biochar is non-significant for most of available nutrients except available calcium. • Cation exchange capacity is significantly affected by biochar but the difference is very slight or negligible among different biochar treatments • Total nutrients are more affected by biochar presence or absence and there are significant differences in nutrients concentration among different biochar treatment. • Some potentially toxic heavy metals were reportedly high in concentration in biochar amended soils with time. • Copper and molybdenum are not affected by biochar, time or plant.
Chapter 7 **Plant mediated changes in surface chemistry of biochar.**	• Identify the dominant functional groups present in the biochar, topsoil and biochar-amended soils. • Determine if the presence of plants (in this case strawberry) affects the nature and type of surface chemistry of biochar amended-soil.	• The maxima of all the functional groups (aliphatic, aromatic and carbohydrates) were recorded in the control soil (0 % biochar) with or without plants. • Major shifts in the spectra for all wave numbers were observed in soils amended with biochar at 2.5, 5, 10 and 15 % w/w as compared to the control (0 % biochar) in the presence of strawberry plant. • After 12 months, a marked decrease in spectral bands between 500 and 4000 cm^{-1} was noted in treatments with 2.5 %, 10 % and 15 % biochar. • There was a shift in the amount of the functional groups due to Interconversions of aliphatic, aromatics and hydrocarbons, but no new functional groups were reported over time. • For oxygen-containing groups, there could have been a rearrangement of molecules in the aliphatics and carbohydrates.

		• For aromatic functional groups, some of the biochar-associated aliphatic carbon (largely from cellulose) was vulnerable and lost rapidly. • Carbohydrate decomposition could have occurred, and utilization by plants and microbes could result in an increase in some carboxylic and carbonyl groups being formed over time.

8.3 General Discussion

It was concluded that biochar originating from chemically processed demolition wood, has very few positive effects on plant growth and soil properties. After the application of biochar, initially it had a limited positive boost in microbial activities, enzyme functions, change in soil pH, availability of some nutrients and improvement in soil physical and chemical properties like low bulk density and greater water holding capacity. This initial boost in biochemical properties was due to the availability of easily digestible carbon which acts as a substrate for microorganisms, increasing availability of nutrients in the rhizosphere due to dissolution of insoluble mineral complexes. However, with the prolonged exposure of biochar, soil microorganisms and enzymes were likely deactivated due to lack of energy and substrates. Some of the highly available nutrients are adsorbed and physically blocked in biochar pores which significantly reduce their availability.

There was either no effect or an inconsistent effect of biochar on most of the available or total soil nutrients, however some of the heavy metals were increased with the time. Furthermore, the heavy metal content of the biochar was exceptionally high and metals were present in the plants at harvest. Whilst the biochar could not be used in agricultural settings, it was hypothesised that it might be useful for reclamation of contaminated land. This could still be

possible, but only if it did not exacerbate plant metal uptake, since this could result in transfer of metals through food webs. Nevertheless, metal/biochar interactions are complex and it is possible that in contaminated soil, metals could be adsorbed onto the biochar rendering them immobile, thereby benefiting the soil. It is, however, clear that biochar originating from anything other than virgin wood (or agricultural residues) should not be used in agricultural or horticultural soils.

The most positive effects of this high temperature biochar were reported for soil respiration where the decrease in CO_2 flux with increasing rate of biochar was observed. Similarly there was no obvious trend in either CO_2 or CH_4 flux over the period of 370 days. Biochar is an entity which requires at least 40-50 years in soil to exhibit its desirable signature benefits like increase in total carbon and its sequestration in soil.

From the literature it was determined that this kind of biochar could be beneficial for purposes other than agricultural, especially with additional post-production processing:

- Coal mine restoration with biochar application can help restore the lost carbon sink by promoting plant growth and enriching the mine spoil, not only helps to sequester the atmospheric carbon but also reduces the extent of afforestation, agriculture manipulation and grassland deterioration by industrialization.
- Biochar plays a crucial role in protecting the plants from unnatural and imposed abiotic stresses like soil salinity and drought.
- Biochar application has been proved as cost-efficient and environment friendly approach to minimize the adverse effects of traces metal contaminants.

- Designer biochar with specific properties are popular in remediation of soil pollutants by restricting the absorption of trace metals in plants.
- The biochar addition increases soil water retention and aeration by adding more efficient pore space that further decreases bulk density and improves microbial proliferation.
- Biochar application affects the permeability of cells, metals absorption, and uptake in the plant by interacting with the electrochemical properties of the enzyme and membrane.
- Biochar stimulates and initiates the metabolic mechanisms in plant and reduced the metal toxicity reduced the metal toxicity in plants.
- Post-processing of biochar to enhance carbon content and reduce metal concentrations in high-carbon partially pyrolyzed or gasified high temperature biochar would greatly improve the potential range of applications.
- Post-processing of biochars like heating, activation, grinding, sieving, granulation and leaching can strongly modify their effectiveness in promoting plant growth, human health and potential climatic benefits.

8.4 General conclusions and future work

The increasing number of global threats like climate change, water shortage, fertility decline, soil contamination, and degradation and scarcity of food security always creates a demand for a new action or strategies to secure the future. One of the approaches in order to acquire practical solution is the introduction of new technologies or restoration of primitive technologies, which have practical utilities with the current and available resources along with implementation of proper regulations. Use of biochar as discussed and assessed in the present study is one of the primitive technologies which mankind has practiced for centuries

with the latest approaches nowadays. New biochar is highly recommended for carbon sequestration, waste management, and soil contaminant remover other than agricultural and environmental uses. However in depth understanding of biochar technology is required for its utilization on a broader level.

The understanding of good or bad properties of biochar and the knowledge of possible changes in biochar structure and surface chemistry when subjected to natural environments in presence of topsoil and indigenous microbes over time was important. All this information is needed to optimize the properties of biochar for specific purposes, such as pH, CEC, nutrient retention, or carbon sequestration. The utility of this research would be highly effective under special circumstances and unusual environments. I want to utilize my valuable findings for the improvement of my motherland soils which are subjected to severe environmental stresses in the last few years like earthquakes, erosion and floods. The extent of soil deterioration is so severe that now soil depth is critically minimal which makes it impossible to grow anything.

I want to introduce use of good quality biochar locally produced from high agronomic crop origin or animal manures for restoration of soil physical chemical and biological properties; by the introduction of biochar we will improve biological microbes and biological animals as biochar is favourite food for lot of biological entities, which will construct and improve physical structure, and properties that will in turn improve the soil chemical framework and properties backed by a strong microbial population which uses biochar as a favourite carbon source.

Biochar is known for its properties which enable soil engineers like earthworms and microbes which are characteristics of healthy soil; locally produced biochar from local feedstock is

reasonable to afford and in the first instance I want to start with the crops and plants which requires less efforts and compatible to the environment. The most preferable crops are leguminous plants, grasses and some crops like wheat and maize which requires fewer efforts in term of getting response and in turn add beneficial additives to soil.

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