CRANFIELD UNIVERSITY

James Ulyett

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SCHOOL OF APPLIED SCIENCES

DOCTOR OF PHILOSOPHY

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ABSTRACT

A loss of soil organic matter (SOM), whether through natural means or management practices, results in soil degradation. Biochar as a soil amendment can alter soil properties, ultimately affecting the availability of nitrogen and water to plants and thus crop growth. The effects of biochar are not definitive, and often dependent on both the soil type and the biochar applied. Biochar properties can change according to the feedstock and production parameters, thus for their effective use further investigation is required to link biochar properties to its effects in soil. A high-temperature (600˚ C) biochar from a mixed-hardwood feedstock was investigated. The biochar increased the soil water retention, as demonstrated by a water release curve and field trials. This retention was predominant at higher water potentials, which was attributed to the greater number of meso (storage) pores in the biochar. Biochar did not affect the soil's saturated hydraulic conductivity; this is thought to be due to the low number of macro (transmission) pores in the biochar. Thus there was no effect on the transmission rate in the soil. Biochar reduced gross ammonium levels in the soil via adsorption, but resulted in increased non-exchangeable ammonium levels, possibly due to physical entrapment. Where carbon was already abundant in the organically managed soil, the adsorbed ammonium reduced nitrification through lower substrate availability. The range of carbon fractions added as a result of the biochar amendment increased the total organic carbon (TOC) content of the soil, but this supplementary carbon was released by the microorganisms as carbon dioxide. Microorganisms in the relatively carbon poor conventionally managed soil (with lower TOC), assimilated the additional labile carbon increasing microbial biomass. The higher microbial biomass, combined with improvements in pH and the higher ammonium levels (as a result of the ammoniacal fertiliser) increased nitrification. These changes in water and nitrogen availability did not alter crop yields as measured in the glasshouse and field trials. The effects of this biochar in a sandy agricultural soil depended on the type and level of carbon and nitrogen present in the soil, thus consideration of these factors should be taken when applying.

Keywords: Biochar; High-temperature Pyrolysis; Soil Organic Matter; Nitrification; Mineralisation; Ammonium Adsorption; Microbial Activity; Water Retention; Porosity.

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CEC	Cation exchange capacity
DWT	Dry Weight
FC	Field Capacity
LSD	Least Significant Difference
MBC	Microbial Biomass Carbon
mS	Micro-Siemens
NEA	Non-Exchangeable Ammonium
pHBC	pH Buffering Capacity
PSD	Pore Size Distribution
PSE	Pore size equivalent
PWP	Permanent wilting point
SOM	Soil Organic Matter
SWC	Soil water content
TC	Total Carbon
TN	Total Nitrogen
TOC	Total Organic Carbon
TON	Total Oxidisable Nitrogen
WRC	Water Release Curve

1 INTRODUCTION AND LITERATURE REVIEW

1.1 Food Security and Land Management: A contradiction?

Demand for higher crop yields is becoming a more urgent global issue (Godfray et al., 2010). The rate of population growth is higher now than in the past centuries, increasing by 1.1% each year (Lee, 2011). Long term projections (Bloom, 2011) anticipate that the global population will reach 9.3 billion people by 2050 and 10.1 billion by 2100.

Population increases are supported by the higher productivity achieved through agricultural intensification, which increased in frequency after the development of the Haber-Bosch process that allowed the artificial production of ammonium. These high-input, high-output systems rely on artificial fertilisers as the major source of nutrients for crop growth. Their effectiveness has seen the global use of nitrogenous fertilisers increasing seven-fold from 11.6 Mt to around 80 Mt between 1960 and 2002 (Pretty, 2008).

Degradation of a soil could be described as a reduction in the soil's ability to provide the environment for effective functioning. The functionality varies across soils and the usage of that soil, a well-functioning and a high quality soil for one purpose may not translate to another (Nortcliff, 2002). For an agricultural soil, the function could be described as the provision of the environment for optimal crop production, such as sufficient, but not excessive, nutrients, water and stability with minimal resistance to plant growth.

Increased agricultural intensification may present a risk of soil degradation, leading to reduced productive capacity in the future (Lal, 2009). Stockdale et al. (2002) noted that some practices within intensive conventional agriculture (such as over-cropping and over-tillage) can lead to losses in soil organic matter (SOM) and thus the degradation of soil structure, lowering the retention of water and nutrients. Without sufficient replenishment of SOM via the addition of organic amendments, organic carbon levels could continue to decrease, further degrading the soil (Figure 1-1).

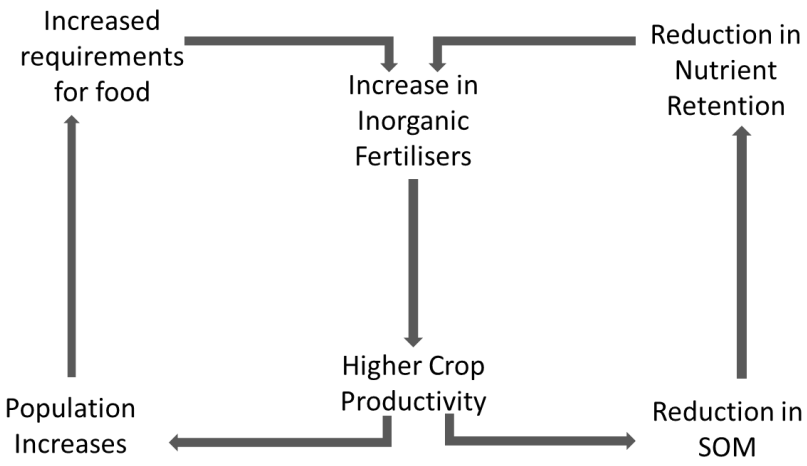


Figure 1-1: 'Two sides of the same coin' – The interaction between fertiliser usage and impact on crop productivity and soil quality

Maintaining soil carbon levels and the fertility of the soil reaches beyond the impacts of food production. Currently, the pool of carbon stored in the soil is 3.3 times that in the atmosphere. Carbon losses from the soil can enter the atmosphere and exacerbate global warming and vice-versa (Lal, 2004, 2010). With higher global temperatures and changing local climates, mitigating the degradation of the soil and increasing the efficiency of crop production will play an important role when supporting global populations

Given the issues surrounding the intensification of agriculture, there is interest in a more sustainable approach to managing soil reserves, to provide the essential function for crop growth and prevention of degradation. Sustainable agriculture can encompass a wide variety of practices and philosophies, and although generally well understood, consensus and specific definitions remains elusive. It is agreed however that increasing the production of food whilst lowering the impact of this on the environment is an important issue (Godfray et al., 2010).

Nitrogen is often considered, agriculturally, one of the most important nutrients as the primary limiting factor to crop growth (Hofman & Cleemput, 2004). As such, nitrogen additions are often required to increase crop productivity. With recent price increases of inorganic nitrogen fertilisers, farmers are making moves towards more cost effective methods of nitrogen application such as supplementary organic fertilisers (Williamson, 2011). One alternative to the high input conventional systems is through organic

management. The aims of growing crops organically vary from improvements in biodiversity to soil fertility, however are usually centered around reducing the reliance on inorganic fertilisers as the main source of nutrients to create a more sustainable approach to farming (Hole et al., 2005).

Studies of organic farming practices have suggested improvements of soil fertility in indicators such as SOM, aggregate stability and pH. Depending on the growing conditions however, yields are typically lower (between 5 and 34%) than for conventional practices due to nitrogen limitations (Mäder et al., 2002; Seufert et al., 2012). Organic practices can produce yields that are equal to or greater than conventional under conditions such as higher phosphorous (Oehl et al., 2002; Seufert et al., 2012).

From the supply of nutrients and water, to the provision of structural stability, the soil and its unique set of physical and bio-chemical properties influence the growth of the plants. The current and historical soil management techniques can have wide impacts on a soil's physical and biological status thus influencing the crop's productivity. Recent agricultural research in the UK has focused on the development of optimising crop production and mitigation of environmental damage, particularly regarding nutrient efficiency. This refers to the balance between agricultural production and the sustainability of the system used to produce the crops (Dungait et al., 2012).

To limit the definition of organic farming, the term will abide by legislation within the UK. Organic farming has been considered a prototype to a more sustainable form of agriculture (Nowak et al., 2013). Even within the term 'organic farming' there is a variety of philosophies and practices that may or may not be undertaken. Gomiero et al. (2011) refers to organic production as that which eliminates the use of not just artificial fertilisers and pesticides, but also that of genetically modified organisms and certain preservatives. As the focus of the current study is on nitrogen cycling and water management, the differences in organic and conventional farming will be highlighted regarding the supply of nitrogen.

Organic certification comes with strict guidelines regarding the use of artificial fertilisers, and therefore tend to utilise a variety of organic materials such as manure, compost and sludge (Bengtsson et al., 2005). Although land converted to organic status

has been decreasing since 2008, as of 2011, the current land area registered as organic in the UK stands at 656,000 ha, around 3.8% of the total agricultural land area (National Statistics & DEFRA, 2012).

Consumer demand plays a part in the drive to farm organically, the global market for organically produced food and drink products has tripled between 1999 and 2007, with revenues increasing from 15 to 46 billion USD, with Europe representing over half (25 billion USD) of the market (Sahota, 2009).

1.2 The Effects of Organic Matter Introduction on Soil Properties

A major influence on soil properties and fertility following a change in management is the soil organic carbon content (Powlson et al., 2011), as reductions in SOM levels is associated with the process of soil degradation and fertility reduction (Mariangela & Francesco, 2010). The addition of organic amendments to agricultural soils increases the SOM levels.

With soil quality and properties being a complex issue, it was argued by Dexter (2004) that many of the soil quality indicators such as water infiltration, aeration and rootability have a main cause in the soil's physical properties. It is also said that the soil's physical attributes have recurring impacts on other aspects of the soil quality (biological and chemical). Indeed these three components are often referred to together due to the complex interactions and feedback systems operating between them.

The physical properties of a soil determine the environment that microbes and plants must survive in. The texture of the soil (proportion of sand, silt and clay particles) is influential to the soil physics due to the change in pore distribution. Soil texture is a product of the regional parent geological material and is not influenced by agricultural management practices (Gomiero et al., 2011). This micro-environment provided by the soil for microbes and plants is highly influenced by the soil's structure. This arrangement of the soil particles affects the available moisture content, gas diffusion and biological functions which in turn can impact on the plant uptake of nutrients.

The addition of organic matter into the system can increase the cohesion between soil particles improving aggregate stability allowing a more stable soil structure resistant to

external physical pressures such as tillage and compaction. An increase in soil biological activity with organic matter additions can improve aggregation via microbial exudates and binding together soil particles (Mariangela & Francesco, 2010). Organic matter can decrease the bulk density of the soil through dilution of the denser mineral fractions (Shepherd et al., 2002; Khaleel et al., 1981). Lowering the bulk density increases the pores between particles and therefore can improve the water/ air mixture within the soil matrix.

Unlike conventionally managed farms, organically managed systems cannot rely on artificial fertilisers to overcome the restrictions in plant growth caused by nitrogen as a limiting factor. The ability to provide a suitable environment for growth is much more dependent on the quality of the soil (Stockdale et al., 2002).

Soil organic matter (SOM) is a highly complex mixture of organic detritus across a range of molecular complexity in varying stages of decomposition (Hofman & Cleemput, 2004). The SOM equilibrium is dependent on the input and the rate of mineralisation. Stockdale et al. (2002) stated that there is no fundamental difference between the soil processes of an organically managed and a conventionally managed soil, such as nutrient cycling and biological processes. However, the pools of each form and their relative importance to crop growth can vary.

1.3 Biochar Characterisation

Biochar, as a black-carbon solid residue produced by the pyrolysis of organic materials (Lehmann et al., 2006) is often distinguished from charcoal by its intended use as a specific soil amendment (Sohi et al., 2009). During high temperature pyrolysis of biomass, carbon atoms form conjugated planar ring systems within crystalline structures, that resist biological decomposition (Downie et al., 2009). This recalcitrance confers long-term stability on biochar in soil, potentially lasting centuries (Lehmann et al., 2006). There has been increasing interest in the use of biochar as a soil amendment due to its reported potentials for amelioration of soil degradation and supporting higher crop yields including higher nutrient availability for plant and improved water storage potential (Woolf, 2008; Lehmann et al., 2006).

The feedstock and even the production parameters can be highly variable altering the properties of the biochar produced (Mclaughlin et al., 2009). Although key characteristics of the biochar selected for research will be assessed, approximate character ranges can be established from the literature to be used for the development of hypotheses. A table of various physical and chemical properties from different feedstocks and production temperatures can be found in Table S1-1.

1.3.1 Physical Characteristics

Biochars can exhibit high specific surface areas, and can range between 100 and 500 m^2 g^{-1}, increasing with peak pyrolysis temperature (Vanderslice & Marrero, 2009; Antal & Grønli, 2003; Yao et al., 2012). This is a similar range to that of clays and so in soil with a high percentage of sand (which exhibit lower surface areas); the addition of biochar therefore could increase the surface area within a soil.

The physical properties of a soil determine its ability to retain water effectively. Amending the soil with applications of biochar is therefore interesting from a research perspective to expand on the existing evidence that it positively influences the water retention of certain soils with the potential to increase crop yields. Tyron (1948), as cited by Woolf (2008), showed that the effects of charcoal on soil moisture differed with soil type; particularly that the greatest increase was on sandy soil with a negative impact on clay soils. This indicated that biochar may be inappropriate for use on a clay soil and so sandy soils were chosen for this research.

Some biochar has a notably low bulk density compared to other feed stocks, Karaosmanoğlu et al. (2000) noted that a feedstock from the stalk of a rape seed plant produced a biochar with a bulk density as low as 0.14 g cm^{-3}. How biochar affects the bulk density of a soil has been tested at field scale at the sites of old charcoal production facilities which had higher levels of charcoal in the soil, compared to off-site locations and showed a 9% reduction in bulk density (Oguntunde et al., 2008). Laird et al. (2010) set up columns of clay-loam soils with a hardwood-feedstock biochar against controls without, these showed that although the bulk density of the soils increased over time due to consolidation, amending the soil with biochar reduced bulk densities compared to the control (1.1 compared to 1.2 g cm^{-3}). There was no significant difference found between biochar treatments ranging between 5 and 20 g kg^{-1}.

1.3.2 Chemical Characteristics

During pyrolysis, high temperatures release volatile compounds from the feedstock as gases and condensates, reducing such elements as nitrogen, phosphorous and sulphur. This can be affected by the feedstock which determines the initial level of such elements, but is influenced greater by the production temperature; as temperature increases, the volatile matter content decreases. This is also affected by the length of time the feedstock is held at the peak temperature, the longer the residence time, the greater the loss of volatile matter. Nitrogen content within biochar tends to be low; < 0.6% according to Antal & Grønli (2003). Volatilisation of nitrogen begins at 200° C and increases with peak production temperature (DeLuca et al., 2009).

The H:C and O:C ratios of a biochar are an indication of the extent of its carbonisation; it has been suggested that values below 0.6 and 0.4 respectively are required for the biochar to be a useful soil amendment (Schimmelpfennig & Glaser, 2012).

Ion exchange is the substitution of one ion for another between a solid surface and an electrolyte solution. A negatively charged surface attracts positively charged ions (cations), and the capacity of this surface to reversibly hold cations is known as the cation exchange capacity (CEC).

The potential for biochar to increase the CEC and the resultant adsorption properties of a soil is well documented (Atkinson et al., 2010; Collison et al., 2009). Indeed biochar has been shown to have between 1 and 3 orders of magnitude higher sorption properties than native organic matter (Durenkamp et al., 2010).

1.4 Soil Water Dynamics

Due to the relationship between SOM reduction and the degradation of soil physical properties (Celik et al., 2010), intensive management practices such as over-tillage and over reliance on artificial fertilisers can exacerbate plant stress caused by water deficit (Smith & Elliott, 1990).

According to Cary & Hayden (1973), there are two major requirements when managing the soil water regime:

1. Retention of an adequate water supply in the root zone to support optimal plant growth.
2. Rapid infiltration and drainage of surface water during periods of high water input, to prevent saturation of the root zone which would reduce oxygen availability.

The movement and thus non-movement (retention) of water in the soil is determined by its energy state (Bouma et al., 2003b). The energy state is affected by the surrounding forces, the total potential energy is the sum of these forces and is the amount of work that plants must apply to move the water (Steudle, 2000).

The ability of the plant to take up the water and the associated dissolved nutrient ions is of significant relevance when considering changes in water management regimes. The hygroscopy of a surface is its ability to attract and hold water molecules; demonstrated by Kuron (1930, as cited by Bachmann & Ploeg, 2002), and is more pronounced in drier soils.

Although research on the implications of biochar on soil physical properties have been studied (Atkinson et al., 2010) these only include brief aspects of the water cycle as a property of the soils physical characteristics. There is currently a dearth of research on the effects of biochar on the water dynamics of a soil and the soil properties that affect it. The proposed research will add to the knowledge in this area and help to fill this gap.

The water regime is complex with many interrelated processes within the soil itself and the water properties. All these factors affect a plant's ability to extract water and nutrients and hence have an impact the yield of a crop. The effect biochar has on the water dynamics and leaching of a soil is variable and the conditions under which biochar might be beneficial are not fully understood (Woolf, 2008).

1.4.1 Soil Water Retention

Water is held in the soil matrix by two major processes (1) adsorption to the particle surface and (2) capillary action in the soil pores (Bachmann & Ploeg, 2002).

To understand the mechanisms of water retention in the soil it is useful to separate adsorption from capillarity processes. It is emphasised however, that the two are

inextricably linked; the process of capillarity would not occur without the initial electrostatic attraction between the liquid and solid interface.

Adsorption

Adsorption is the ability of water to maintain contact with a solid surface, also known as wetting. The strength of adsorption is dependent on the adhesive force of the water spreading across the surface and the cohesive force between water molecules attempting to pull the water drop back into a state of minimum surface area (Sophocleous, 2010).

The adsorption of water is influenced by the soil's surface of the soil (Franz et al., 2000); the specific surface (surface area per unit of mass) is an important characteristic within a soil for the retention of water and ion exchange. Petersen et al. (1996) noted the importance of specific surface and the strength of adsorption and desorption of water molecules at different matric potentials. It was found that in dry soils (low matric potential: -1500 kPa) specific surface area was more influential at retaining water than in wetter soils (high matric potential: -10 kPa). The frequency distribution of particle size has significance on water retention as smaller particles have larger specific surfaces (Petersen et al., 1996). The soil's texture therefore has a large influence on the ability of a soil to retain water, which classifies soils according the proportion of sand (2000 µm – 60 µm), silt (60 µm – 2 µm) and clay (< 2 µm) particles (as defined by the European Classification system).

Clays, with the highest specific surface have the highest potential for adsorption. Specific surface areas can vary with the type of mineral; montmorillonites, with complex internal structures can have up to 810 m^2 g^{-1} depending upon the surface exposed by expansion (Carter et al., 1986).

The majority of recent research carried out does show positive trends, linking biochar additions with higher soil water retention. For instance, Dugan et al. (2010) showed that the addition of a maize stover biochar increased the water holding capacity (WHC) of three types of soil (sandy loam, silt loam and loamy sand) but showed the greatest increase in the loamy sand (80% sand fraction). For each of these treatments however, there was no difference in the WHC due to changing the rate of biochar application. This was suggested partly to be due to the water repellence of the biochar and possible

negative impacts on soil structure. However, only a limited range of application rates of biochar were used (0, 10 & 15 t ha^{-1}). This could also indicate that there is a limited difference in the WHC at these rates and a larger range of application rates may be required to demonstrate a detectable effect on water retention. Some research, (Busscher et al., 2010) did not show any difference with the addition of biochar on the inferred WHC of a soil, (by measurement of the volume of water needed to maintain 10% water content), although it did show a reduction (~240 g to ~215 g) in the amount of water leached at the highest biochar treatment (20 g kg^{-1}). This inconsistency was attributed to the larger pores retaining some water when leached but not when indirectly measuring the WHC.

Capillarity

The phenomenon of water rising in a thin tube is well known and is due to the physical properties exhibited by the water molecules as a result of the adhesion of water to the sides of the tube and the cohesion of water molecules causing the bulk of the water to follow. The source of the rise is due to the curvature of the water surface in the vapour-liquid interface, the height at which water is held and the pressure required to remove this water is determined by the radius of the capillary; the smaller the radius, the lower the contact angle and the greater the adsorption therefore a larger rise in water level.

Traditionally capillarity was the predominant factor considered when modelling soil water retention with little distinction between capillarity and adsorption (Bachmann & Ploeg, 2002). This assumed that the naturally occurring pores in a soil reacted similarly to bundles of tubes with high connectivity, highlighting the importance of pore size to the ability to retain water. Because of the capillary process, the pore size as well as the substrate surface is an important aspect in water retention; as smaller pores retain water more strongly.

When assessing the pore size distribution within a soil, it is useful to classify pores according to their functionality with regards to availability of water to plants. The range of equivalent pore sizes (pore size equivalent: PSE) that hold water available for plant uptake are between 0.2 and 50 µm (Abel et al., 2013). Pores over 50 µm diameter therefore cannot retain water by capillarity under gravitational force and are known as

macro pores (also transmission pores).Once gravitational force has drained water from the macropores, the soil is said to be at field capacity (interchangeably known as the water holding capacity).

Laird et al. (2010) showed that the WHC increased by 10, 12 & 15% with the addition of 5, 10 & 20 g [biochar] kg^{-1} dry soil, compared to the control (0 g kg^{-1}) although this research was expanded beyond evaluating just the WHC by examining the retention of water under various pressure heads (-33, -100, -500 & -1500 kPa). This approach showed a higher retention of water under -100 and -500 kPa soil matric potential, but only at the highest rate of biochar application (20 g kg^{-1}). This indicated that the soils ability to retain water under higher stress levels increased at high levels of biochar application.

Brockhoff (2010) showed that, in comparison to a control, the available water holding capacity (AWHC) increased by 170% and 370%, with 10% and 20% biochar additions respectively in a soil of approximately 80% sand.

Water retained in the soil requires energy from the plants for extraction; as the pore size decreases more work is required to extract the water. Not all this water is available for plant use however; pores smaller than 0.2 µm hold water with forces in excess of what plant roots can supply. These are called residual pores. Between 0.2 and 50 µm therefore, the pores are known as storage pores and contain the plant available water.

1.4.2 Soil Water Movement

The ability of a soil to retain water is not the only factor that can affect a plants ability to take up water, the movement of water through the soil must also be considered. Water will move, or be retained, according to relative differences in energy states (Bouma et al., 2003a); the retention and movement of water are thereby negatively correlated.

Water will move from higher energy status to a relatively lower energy status. The energy status of water in the soil is dependent upon two major factors; gravitational energy (g) and pressure head (h). Above the water table, the pressure head is a negative force resulting in suction (hygroscopy) on the water. The hydraulic head (H) is the sum of the gravitational and the pressure heads ($g + h$).

Water movement cannot be considered a separate issue from water retention as the two processes are connected; the greater the retention force in a soil, the greater the force required for movement, such as plant uptake, and the less that will be drained under gravitational pressure. Thus if the addition of biochar to a soil could increase the water retention by increase in porosity (Mukherjee & Lal, 2013); surface area (Vanderslice & Marrero, 2009) and ion exchange capacities (Liang et al., 2006) then this could also indicate a reduction in the movement of water.

For the movement of water in the soil, important factors include the distance between the two points of movement (L), the difference in hydraulic head (ΔH), and the ease that the water can move through the porous material: the hydraulic conductivity (K).

The rate of water flow (Flux) within saturated soils is predicted using Darcy's law which states that $q = (\Delta H / L) * K$. Where 'q' denotes the flux.

The hydraulic conductivity (K) relates to the porous medium itself; as K increases, the greater the movement of water for a given hydraulic gradient. The hydraulic conductivity is highly affected by the soil's physical properties, primarily the pore size distribution which is in turn affected by the texture of the soil. Just as smaller pores retain water more strongly, larger pores provide greater flow as the energy required to desorb the water is less. Assuming a capillary tube, a doubling of a pore diameter can provide a flow of water 16 times greater (Bouma et al., 2003a). This has implications for the availability of water to plants and the drainage of the soil.

After periods of heavy rainfall the soil is at risk of saturation, under these conditions, the oxygen levels are reduced which can be detrimental to plants over sufficient time. The larger macropores quickly drain the gravitational water to field capacity where the ability for the soil to retain water is important for plant production. As such, the greater the macro-pore volume of a soil, the quicker drainage occurs.

Sandy soils, when compared to clay or silt soils, have a greater relative number of macropores and a greater macro-pore volume, therefore under saturated conditions (when macropores are full) water within these soils will move with greater ease and show higher hydraulic conductivities (Brady & Weil, 2002). The pore size distribution of the biochar is therefore an important characteristic for the water dynamics and could

impact on the saturated hydraulic conductivity. However, if as suggested by Tseng & Tseng (2006) that up to 95% of the biochar pores are micro-pores, it could be proposed that the addition of biochar will not affect the number of the macropores in the soil, therefore the volume of macropores in the soil will remain unchanged resulting in no change to the movement of water through the macropores.

In a field scale experiment with a biochar application rate increase from 0 - 16 t ha^{-1}, (Asai et al., 2009) showed both an increase in WHC and saturated hydraulic conductivity of undisturbed cores. It was suggested therefore that the higher applications of biochar increased the soil water availability to plants. A Finnish field trial also noted an 11% increase in the WHC of an organically managed field with a biochar application rate of 9 t ha^{-1} (Karhu et al., 2011).

Similar effects have been shown by the addition of organic matter into soil. The increase in meso-pores (particle size 2 nm to 50 nm) and micro-pores, and the higher electrostatic attraction of the organic matter increases retention, but does not decrease the movement of water through the macro-pores or the hydraulic conductivity. Indeed, soil porosity and hydraulic conductivity have been shown to increase as a result of organic matter addition (Wong et al., 1999). This is attributed to an improvement in structure such as stability and strength of aggregates, and a decrease in bulk density (Soane, 1990).

Despite increases in water retention after the addition of organic matter to soils, increases in hydraulic conductivity have also been seen. Organic matter addition can improve the structure of a soil, namely through increased soil aggregation, and stability of aggregates (Lal, 2009).

Biochar has been proposed to increase the aggregation of soil (Verheijen et al., 2010). Higher microbial activity and root growth have been suggested to increase binding of aggregates physically (root structure) and chemically by root and microbial exudates.

The known effects of biochar on the hydraulic conductivity are less clear. The majority of results appear to be in accordance with initial expectations whereby biochar increases conductivity. The saturated hydraulic conductivity of sites with charcoal amendments in Terra Preta soils in Ghana was found to be higher (11.4 and 6.1 cm h^{-1} respectively)

than in the adjacent soils (Oguntunde et al., 2008) supporting results by Asai et al. (2009). According to Laird et al. (2010) there was no effect of biochar on the soil's saturated hydraulic conductivity. Though bulk density of the soil did increase over time due to gravitational consolidation however the bulk density of the soils amended with biochar were significantly lower than the controls.

Brockhoff, (2010) however, detected a decrease in the saturated hydraulic conductivity of a sports-turf soil's with biochar application from 84.8 cm $hour^{-1}$ (control) to 55.9, 29.2 and 6.6 cm h^{-1} for bio char application rates of 5%, 15% and 25% respectively. This could be due to the unusually high percentage of sand (80%) not normally found in agricultural systems.

1.4.3 Soil Water Dynamics Summary

The management of water within agricultural systems is important; maintaining an adequate level of water is essential to maintain optimum crop growth; too little water is detrimental to crop growth as this reduces nutrient uptake and structural support, whereas too much water reduces oxygen diffusion into root cells, halting respiration. To optimise the soil water regime for plant uptake requires a combination of maximising water storage at times of low water input, and rapid drainage under saturated conditions.

The retention, and therefore drainage, of water is dependent upon the energy state of the water in the soil; if the matric potential of the soil is greater than the gravitational potential then water is retained in the soil matrix. This is reliant on the physical characteristics of the soil. Similar to soil, biochar is a porous medium and so has the potential to alter the soils physical properties, influencing soil water dynamics. The effects this has will play a vital role in managing water inputs to an agricultural system and as such the effects and mechanisms of biochar amendments must be quantified. Biochar is incorporated into the topsoil, and as such the focus of the research will be within the top 0.15m (Zhang et al., 2012).

Although the effects of biochar addition on the water regime of soils have been considered, studies show much variability. The majority of studies on the effects of biochar on the soil water dynamics indicate that there is a positive correlation with the addition of biochar and higher retention of water. This correlation is less clear for soil

drainage, as measured by hydraulic conductivity. The water regime of soils can be described by the physical properties of the soil; the majority of studies however do not associate these differences with quantitative changes of soil specific surfaces or porosity with biochar addition. This, with the varying use of biochar feedstocks and production parameters leads to a lack of comparability between studies.

The management of the soil could also significantly affect how biochar will influence the soil, however little research has been considered comparing organic and conventional agricultural systems.

There also lacks an agreement on the rate of biochar required to gain optimum agronomic advantage. This could again be due to the variability of biochar types, but it could be noted that biochar application rates below 20 t ha^{-1} show little or no significant difference from control plots.

The effect of time often remains unconsidered, with the potential for chemical changes in the soil, the effects and longevity of this is important when considering biochar as a long term solution to improve soil productivity.

1.5 Soil Nitrogen Dynamics

Nitrogen can be released into the soil by the decomposition of organic materials in the soil. This can be native soil organic matter or from an anthropogenic source. Depending upon the type of agricultural systems, the input of nitrogen can take many forms. Organically managed systems utilise materials of a biological origin (e.g. compost and farm-yard manure), while, conventional systems, tend to rely upon artificial fertilisers (such as ammonium nitrate and urea) for the major nutrient supply, though it is not uncommon practice to utilise organic matter also. The nitrogen cycle, shown in Figure 1-2, is a representation of the major forms of nitrogen found in the soil and the potential transformations between them. The relative amount of each form (pool) is dependent upon the transformations rate and the level of substrate.

Soil organic matter, where the nitrogen is bound with carbon, is a heterogeneous mixture comprised of living matter (microbial biomass) and non-living matter (humus and partially decomposed residues) such as proteins, nucleic acids, chitin, peptidoglycan

and amino sugars (Deenik, 2006). The microbial activity involved in the degradation of organic matter is equally heterogeneous, involving a variety of bacterial species (Kuenen & Robertson, 1988) that release extracellular enzymes to break down specific substrates.

Mineralisation is the transformation of organic nitrogen to inorganic (mineral) nitrogen, and is a multistep process that breaks down macromolecules into subunits (such as amino acids and urea) then into ammonium ions (NH_4^+). As such this requires a combination of microbial species and enzymes. Two sub-reactions make up the process of nitrogen mineralisation; organic matter is hydrolysed to ammonia (NH_3) which is then converted to ammonium (ammonification). It is at this point in the cycle that the nitrogen becomes available to plants.

The fate of ammonium can follow several pathways (Figure 1-2):

1. Nitrification into nitrate, via nitrite
2. Adsorption onto cation exchange sites
3. Immobilisation by uptake into microbial or plant biomass

Agricultural systems are not closed systems; although part of the total plant biomass will return to the SOM pool as residue (stalks; senescent leaves), the majority can be removed for external uses. Nitrogen is also at risk to leave the system as a result of denitrification, volatilisation of ammonia (particularly at a high pH), or leaching. As a result, nitrogen can be lost from agricultural systems over time and must be replaced either by the addition of organic amendments or artificial fertilisers to mitigate the detrimental effects on plant productivity.

The nitrogen cycle can be interpreted, not as the determined fate of a nitrogen atom, but as pools of different nitrogen forms, where the sizes are constantly changing to achieve chemical equilibrium. The individual nitrogen atoms transform to achieve this balance. Therefore, if the size of one pool is altered, the equilibrium will shift altering all pools, potentially affecting the amount of nitrogen available to plants.

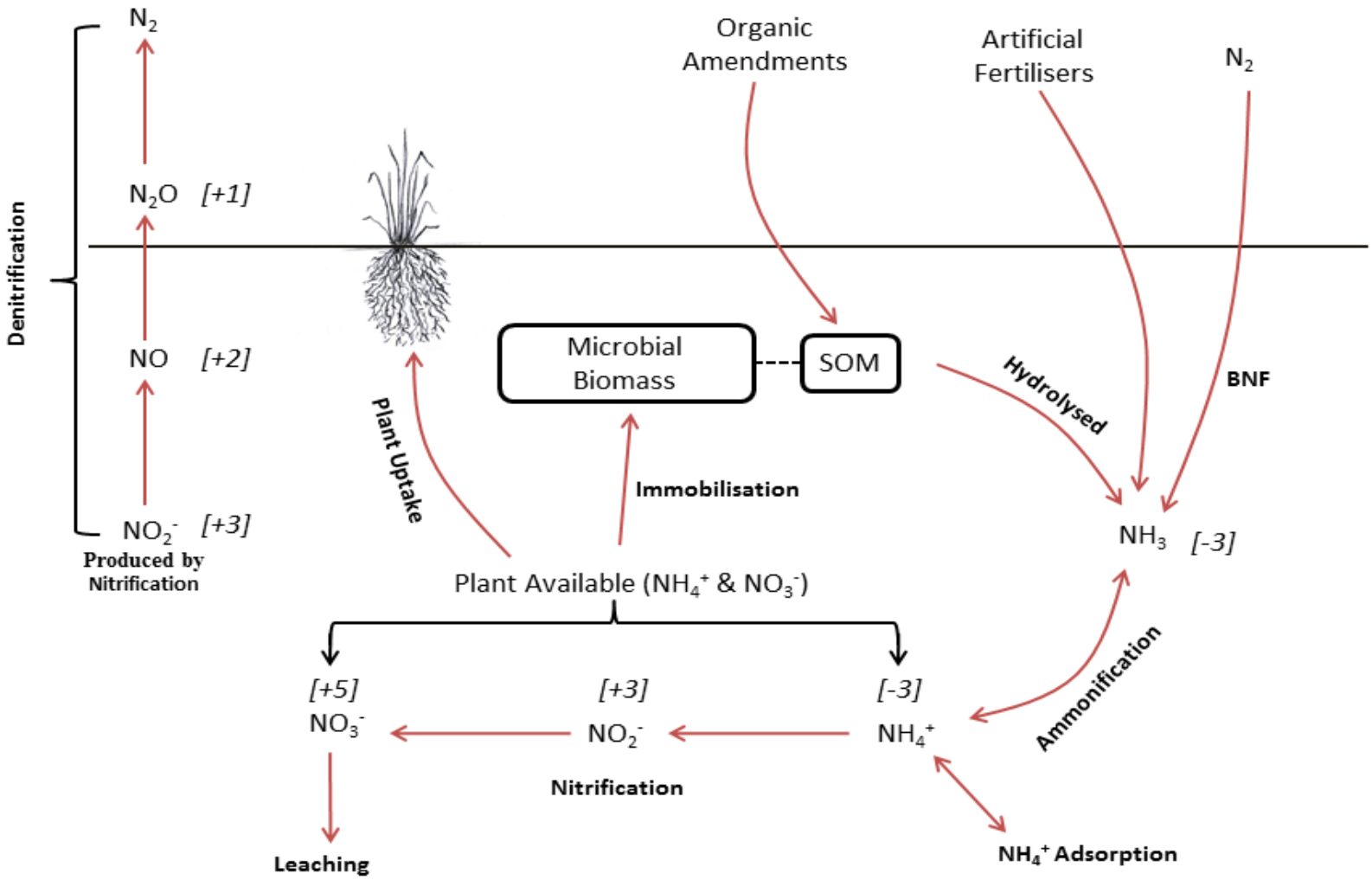


Figure 1-2: 'The nitrogen cycle': Forms and transformations of nitrogen in an arable soil with relevant *[oxidation states]*. SOM: Soil organic matter; BNF: Biological nitrogen fixation.

1.5.1 Nitrogen Availability

The classical paradigm of mineral nutrition theory, proposed by Liebig in 1842, suggests that plants only use nitrogen in the form of inorganic (mineral) ions for nutrition (Schimel & Bennett, 2004). These ions can have a positive or negative charge (cations and anions respectively). The most common forms taken up by plants include ammonium (NH_4^+) and nitrate (NO_3^-). Research however has shown that plants can utilise organic nitrogen for nutrition. The relative importance of organic nitrogen on plant growth is unclear (Schimel & Bennett, 2004) due to a paucity of evidence (Jones et al., 2005), though it is suggested to be only of significance in nitrogen poor sites (Nordin et al., 2001).

The majority of ion uptake into plants is with the mass flow of soil water into roots; the greater the concentration of ions in the solution the greater the uptake into plant roots. As such, availability of nitrogen to plants and microbes is affected by the water content of the soil but also other abiotic factors including pH and temperature.

Mineralisation and nitrification are some of the most important processes affecting availability of nitrogen to microbes and plants (Owen et al., 2010).

1.5.2 Biochar and the Nitrogen Cycle

The alteration of a soil's nitrogen equilibriums is widespread (Fields, 2004), especially for agricultural gain. It is important to manage the nitrogen levels in the soil; as with water, enough must be maintained within the root zone for plant availability. For biochar to be an effective amendment the change in equilibrium must be beneficial. In this context, beneficial equates to an increased uptake of plant nitrogen from the soil. This could be from an increase in available nitrogen or an improvement in the nitrogen use efficiency of the plant.

It has been suggested that key areas where biochar may impact on the nitrogen pools, includes the nitrification of organic matter and the retention of nitrogen by ion exchange (Verheijen et al., 2010; Clough & Condron, 2010). Whether biochar acts simply as an additional medium for these processes to occur, or whether biochar can provide significantly improved micro-site conditions is unclear.

The increase in retention to the biochar surface has been attributed to a high cation exchange capacity. In the short term this could reduce the availability of nitrogen to plants, however it could also be interpreted that the greater retention will increase the uptake over longer

periods due to a reduction in leaching. Nitrification is a biological process; how biochar alters the fundamental soil properties that can in turn affects microbial activity is highly significant.

While the addition of biochar to a soil could have the potential to impact on plant growth by altering both the water and nitrogen dynamics individually, the interaction of water on nitrogen pools must be considered.

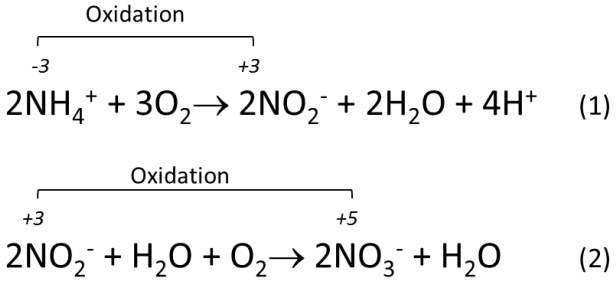
1.5.3 Nitrification

Nitrification Overview

Ammonium (NH_4^+) is converted to nitrate (NO_3^-) via the intermediate, nitrite (NO_2^-). Although nitrification is often associated with the oxidation of an ammonium molecule by chemoautotrophic bacteria; this is not the sole definition of nitrification. It is known that certain types of heterotrophic bacteria and fungi can also form nitrate; though a much slower process, this form of nitrification can dominate acidic soils but it is rarely the case in temperate agricultural systems where extremes in pH are controlled. Thus a more accurate definition of nitrification is the biologically mediated oxidation of a reduced organic or inorganic compound (Cheng et al., 2011), though focus within the literature review will be upon nitrification controlled by chemoautotrophic bacteria found in temperate soil systems.

Ammonium and nitrate are both plant available, but differ in the rate of plant uptake. Ions have the potential to adsorb to particle surfaces thereby reducing mobility; the relatively fewer number of anion exchange sites to cation exchange sites asserts that nitrate is more mobile than ammonium (This mechanism is discussed in greater detail in **Chapter 1.5.4: Ion Adsorption**). Thus the rate of transformation from ammonium to nitrate is influential to plant uptake. An increase in nitrification rate may increase nitrogen uptake by plants, but the greater mobility could also increase the potential for leaching.

Ammonium is nitrified in two oxidation reactions, the first (nitritation) in Equation 1 and the second (nitratation) in Equation 2 by two types of nitrifiers (Buday et al., 1999).



Ammonium oxidisers Equation 1: Genera include *Nitrosomonas* and *Nitrosolobus*.

Nitrite oxidisers Equation 2: Genera include *Nitrobacter* and *Nitrococcus* (Peng & Zhu, 2006).

The family of *Nitrobacteriaceae*, to which these bacteria belong, are aerobic (Paul et al., 2003), and as autotrophic bacteria, obtain carbon via photosynthesis from carbon dioxide but obtain energy for metabolism from the oxidation of ammonium and nitrite. These bacteria are prevalently found in alkaline soils.

Biochar Effects on Nitrification

As a biologically driven process, nitrification can be limited by many key variables; those that could reasonably be affected by the addition of biochar will be given greater detail, as these are more likely to be the controlling mechanisms. However, much of the literature has focussed on forest soils as a result of char input from forest fires with no studies showing changes in the rate of nitrification with the addition of biochar to managed grassland or agricultural land. Clough & Condron (2010) suggested that the lack of biochar influence on nitrification in agricultural land as opposed to forest soils was due to an already active bacterial presence and high mineralisation rate, as such the addition of biochar has little effect. A study by Ball et al. (2010) on boreal areas historically active with forest fires showed gross nitrification rates increased from 0.288 to 0.477 $\mu g\ g^{-1}\ d^{-1}$ along with a fourfold increase in ammonia oxidising bacteria. According to (Warnock et al., 2007) evidence indicates that an alteration of the soils physical and chemical properties such as nutrient availability and a more appropriate pH level could influence the microbial communities.

Soil Water Content and Oxygen Concentration

The provision of sufficient potential gas-exchange for micro-organisms is dependent on the water content as the pores can be filled with either water or air. The primary mechanism for the gas exchange in undisturbed soils is by diffusion. This can therefore be limited by the depth and density of the porous substrate; resulting in a reduction in gas diffusion with increasing depth. As such the majority of nitrification occurs in the topsoil; this could have significance as typically the top 15 cm of soil will contain the biochar amendment.

Gas diffusion is a passive mechanism and can be influenced by the type of gas, pressure, temperature and the physical properties of the soil. As diffusion is through the soil pores, the porosity is clearly a primary parameter affecting oxygen diffusion. As porosity increases, as does the connectivity between pores; this reduces the tortuosity in the soil increasing diffusion of gases (Marshall, 1959). A key parameter for the oxygen diffusion through the soil is the air filled porosity; the volume of pores filled with air for a known water content. Moldrup et al. (2000), accurately predicted gas diffusivity from soil water characteristics from a range of soil textures, thus demonstrating the close relationship between gas diffusion and soil physical properties. Barnard & Leadley (2005) found that increases of soil water content, as a result of increasing carbon dioxide levels causing plant stomatal closure, resulted in a decrease in nitrifying enzyme activity. It could therefore be understood that the moisture content will affect the availability of oxygen and the nitrification rate.

Smaller pores hold water at higher matric potentials, reducing the flow of water and causing blockages for oxygen movement. This can decrease the connectivity and increase the tortuosity of the soil, reducing the rate of diffusion. Biochar with a high micro-porosity (Tseng & Tseng, 2006) could decrease oxygen diffusion by increasing the water retention. Biochar, with a characteristically low bulk density (Mukherjee & Lal, 2013) has been shown to decrease the bulk density of soils (Soane, 1990; Oguntunde et al., 2008; Laird et al., 2010). The decrease in bulk density due to biochar addition could influence the oxygen diffusion rate. If, as suggested that the addition of biochar could increase the aggregate stability (important attribute of structure) of a sandy soil and increase drainage of water then this will decrease the time that the soil is in saturated conditions.

Temperature

Temperature is an important variable affecting microbial activity. Microbes grow at an optimum temperature and only within a limited range according to the species. The largest determinate of soil temperature however, is the ambient air temperature which will remain unaffected as a result of biochar application.

There has been some interest on the effects of biochar on the albedo (diffuse reflectivity of a surface) of the soil. As a charred material, the matt black surface of biochar could darken the soil, reducing reflected light. Oguntunde et al. (2008) showed that under disused charcoal production sites, the reduction in albedo resulted in an increase in soil mean temperature. This could increase the temperature of the soil, but may require large amounts of biochar to achieve this effect. Sohi et al. (2009) considered the potential in large spatial scales for biochar to decrease albedo; and determined the effect was likely be evident on a global scale rather than a local effect.

pH

Nitrifying bacteria show maximal growth in alkaline conditions (Kuenen & Robertson, 1988). Increases and decreases in pH of a forest floor resulted in respective increase and decreases of net nitrification rate (Ste-Marie & Paré, 1999). Nitrification however is an acidifying process due to the release of hydrogen ions during the oxidation of hydroxylamine to nitrate and could reduce the activity of nitrifying bacteria (Han et al., 2004).

There is evidence to suggest that the addition of biochar could increase the pH of the soil (Lehmann et al., 2006), due to a high pH of the biochar itself. The pH of biochar is influenced by the production process; higher temperatures can result in a biochar that has higher pH (Gundale & DeLuca, 2006). This was also examined by Rutherford et al. (2008) who showed that as temperature increased in the pyrolysis of pine wood, the pH increased from ~4 to ~9. Changing the pH of the soil can affect the bioavailability of nutrients to plants; in agricultural soils, which have a tendency to be between neutral and alkaline, there is the risk of increasing the pH to a detrimental effect on the plants (Mikan & Abrams, 1995).

Substrate Availability

Mineralisation and nitrification are enzymatic reactions, and as such the level of substrate is a key factor influencing the rate of reaction. As substrate concentration increases there is greater contact between substrate and the enzyme's active site. Many enzyme kinetic curves give a relationship, whereby the enzyme activity is inhibited by an upper threshold substrate level (substrate inhibition curve) (Reed et al., 2010). Both nitritation and nitratation are inhibited by excesses of their substrate (Carrera et al., 2004).

For nitritation the primary substrate is ammonium. For agricultural systems, where there is potential for nitrogen to be lost via leaching, biochar has been proposed to increase the level of ammonium in sandy soils by increasing the cation exchange capacity (Verheijen et al., 2010). This mechanism is discussed in greater detail in **Chapter 1.5.4: Ion Adsorption**. However, an accumulation of ammonium will also increase the pool of ammonia, as these reversible reactions are in equilibrium. An inhibition of nitrification has been shown with increases in ammonia (Buday et al., 1999).

1.5.4 Ion Adsorption

Nitrogen availability for uptake by plants is dependent upon the adsorption to soil particles and the ease of dissolution as in addition to nitrification, ammonium can be adsorbed onto soil particles (Wang & Alva, 2000). Factors influencing the adsorption of ammonium to the soil's surface include available surface area and the surface chemistry (Waters et al., 2010). The importance of a particle's specific surface to adsorption and the potential impacts as a result of biochar additions is discussed in **Chapter 1.4.1: Soil Water Retention** and is as relevant for the retention of dissolved ions as it is for water molecules. The soil's CEC is linked with the specific surface area, cation exchange capacity increases with surface area due to a greater availability of area for the reactions to occur (Kabata-Pendias, 2004).

Cations are retained in the soil by cation exchange sites which form from the excess of negative charges on the soil's surface (Franzmeier & Steinhardt, 1990) and is measured by the cation exchange capacity (CEC). Ions are present as either anions or cations; the dominant charge present will influence the ion that is retained.

There are two major sources of charge in soil (Sollins et al., 1988):

1. Variable charges: these are pH dependant due to functional groups on the particle surface such as hydroxyls

2. Permanent (constant) charges: caused by an imbalance by isomorphous substitution of one cation by another of similar size but differing charge. These are not affected by changes in pH.

Permanently charged soils are more common in temperate regions, deriving their predominant negative charge from the clay content of the soil (Sollins et al., 1988; Xiong et al., 2010). As such the clay content of the soil can significantly affect the retention of nitrogen in a soil. Soils with high sand contents are thus more susceptible to nitrogen leaching than soils of higher clay content (Fraters et al., 1998).It has been shown that in solution, both ammonium and nitrates can be utilised by plants, (Camberato, 2001b). Nitrates though are often quoted as being the major nutrient ion relevant to plants in soils. This is typically due to the higher CEC levels in temperate soils causing a higher retention of ammonium but not anionic nitrogen such as the total oxidisable nitrogen (TON: nitrites and nitrates) consequently, the movement of ammonium is relatively lower than that of TONs (Camberato, 2001b).

An increase in CEC was noted by Steiner et al. (2008) when charcoal was added to tropical plantations. Eldridge et al. (2010), applied two rates of biochar (168 and 335 t ha^{-1}) to two types of soil (sandy loam and silty clay loam). It was found that both application rates increased ammonium sorption in both soil types, but the highest increase was seen in the sandy loam with a lower CEC. The clay loam showed increases in ammonium retention of 20 and 40% for each respective biochar application rate but the sandy soil showed a 90 and 149% increase respectively.

It has been suggested that the CEC of the biochar will increase over time in the soil due to surface oxidation and/or the adsorption of organic matter (Cheng et al., 2006; Liang et al., 2006; Zimmerman, 2010). Biochar has been shown to have a high capacity to adsorb cations due to a high surface area and charge density which increases with peak production temperature; increasing from ~50 $mmol_c$ kg^{-1} to ~250 $mmol_c$ kg^{-1} within the production range of 300° C to 800° C (Lehmann, 2007). Liang et al. (2006) showed that the charge density (CEC per surface area unit) was up to 1.9 times higher in a biochar amended soil than an adjacent control soil. This was attributed to the oxidation of the biochar particles and the adsorption of organic matter to the biochar surface. The surface area of biochar is an

important parameter to evaluate adsorption especially for organic molecules (Shinogi & Kanri, 2003). Eldridge et al. (2010) performed a Langmuir isotherm on pure green-waste biochar. This found that over 90% of the ammonium adsorbed to the biochar was exchangeable and therefore available for plant uptake. It was noted by Kabata-Pendias (2004) that SOM can contribute between 25 and 90% of the CEC of an arable soil. This could indicate that an organically managed soil would show higher CEC levels and so show a lower relative increase in CEC with biochar addition.

Nitrogen dynamics are significantly affected by the water status of the soil. The effects of water content on the nitrogen dynamics are well known; during a pot trial, Quaye et al. (2009), found an interaction between the percentage of soil field capacity and nitrogen uptake into a maize variety at 80 kg ha^{-1} equivalent. This showed that as the moisture content of the soil increased (30, 50 & 100% field capacity), nitrogen uptake and resultant yield increased significantly.

Increased ion adsorption, due to the addition of biochar, could indicate a reduction in plant availability, however as shown by Eldridge et al. (2010) that the majority of this is extractable, it could be postulated that the greater adsorption will increase the supply in soil due to a reduction in leaching.

1.6 Research Aims

This project looks at the application of a biochar to two similar soils from farms that have had historically different management approaches; one was certified organic for 14 years whilst the other conventional for over 20 years. The thesis also aims to evaluate how a high temperature biochar can impact on the soil's nitrogen and water dynamics with interest in the availability for plant uptake.

In order to investigate and elucidate the effects of biochar application to the soil, one must understand the nature of biochar itself. The effects of adding biochar to the soil are a product of the direct and indirect parameters of the biochar. Thus cataloging these characters is paramount to understanding results in the soil. This is of particular importance as biochar can exhibit a wide variety of characteristics.

1.7 Knowledge Gaps

The definition of biochar is imprecise and covers a wide range of a number of qualities. The intensity of these qualities depends on the feedstock and the combination of production parameters. Because of this and the range of soils that biochar has been applied to, a consensus of how biochar affects the soil's properties after application and the wider impacts this can have has not been reached.

Although this research does not intend to formulate such a consensus, to reach one will require consolidating a biochar's specific characteristics with the effects observed once applied which is often overlooked. As the state of the biochar to be used is unique the relevant characteristics are also unknown.

The effect of biochar on various aspects of the water dynamics in the soil has been studied. This is primarily on individual but important measurements such as the ability of a soil to retain water at field capacity or the available water capacity. Less tested, is the effects of biochar application on a number of aspects of the water dynamics, and over a range of water potentials comparing retention at each range with the specific pore size distribution of the biochar. Biochar is often just reported to be highly porous, the detailed distribution of the pores within however, is important to establish the conditions that a biochar will have a beneficial effect.

Nitrogen is often considered one of the most important nutrients in an agricultural system, it is unsurprising therefore that the effects of biochar on the nitrogen dynamics has often been studied. It was suggested by Clough & Condron (2010) that biochar may impact on nitrification due to research on boreal ecosystems after forest fire incidents, but this has yet to be observed in agricultural systems.

This project will also attempt to integrate the effect biochar has on the water dynamics with the nitrogen transformations. From these gaps, the following objectives have been formulated.

1.8 Objectives

1. To characterise the physical and chemical properties of the biochar and note how this compares to biochar used in previous research

2. To investigate how the addition of biochar can affect the water regime of a sandy soil

3. To explore the interaction of soil water on nitrogen transformation due to the addition of biochar to a sandy soil and its implications on plant nitrogen uptake

1.9 Hypotheses

1: Increasing the rate of biochar application will increase the retention of water in a sandy soil.

2: The drainage rate of sandy soil at saturation will increase with increasing application rates of biochar.

3: Increasing the biochar application rate to a sandy soil will increase the adsorption of both ammonium and nitrate but decrease the availability to plants at a given matric potential.

4: Increasing the biochar application rate will increase nitrification rate of ammonium, releasing more nitrates.

1.10 Thesis Layout and Overview

Figure 1-3, shows the overall methodological approach to fulfilment of the objectives, including how these methods integrate with one another to answer the overall research question.

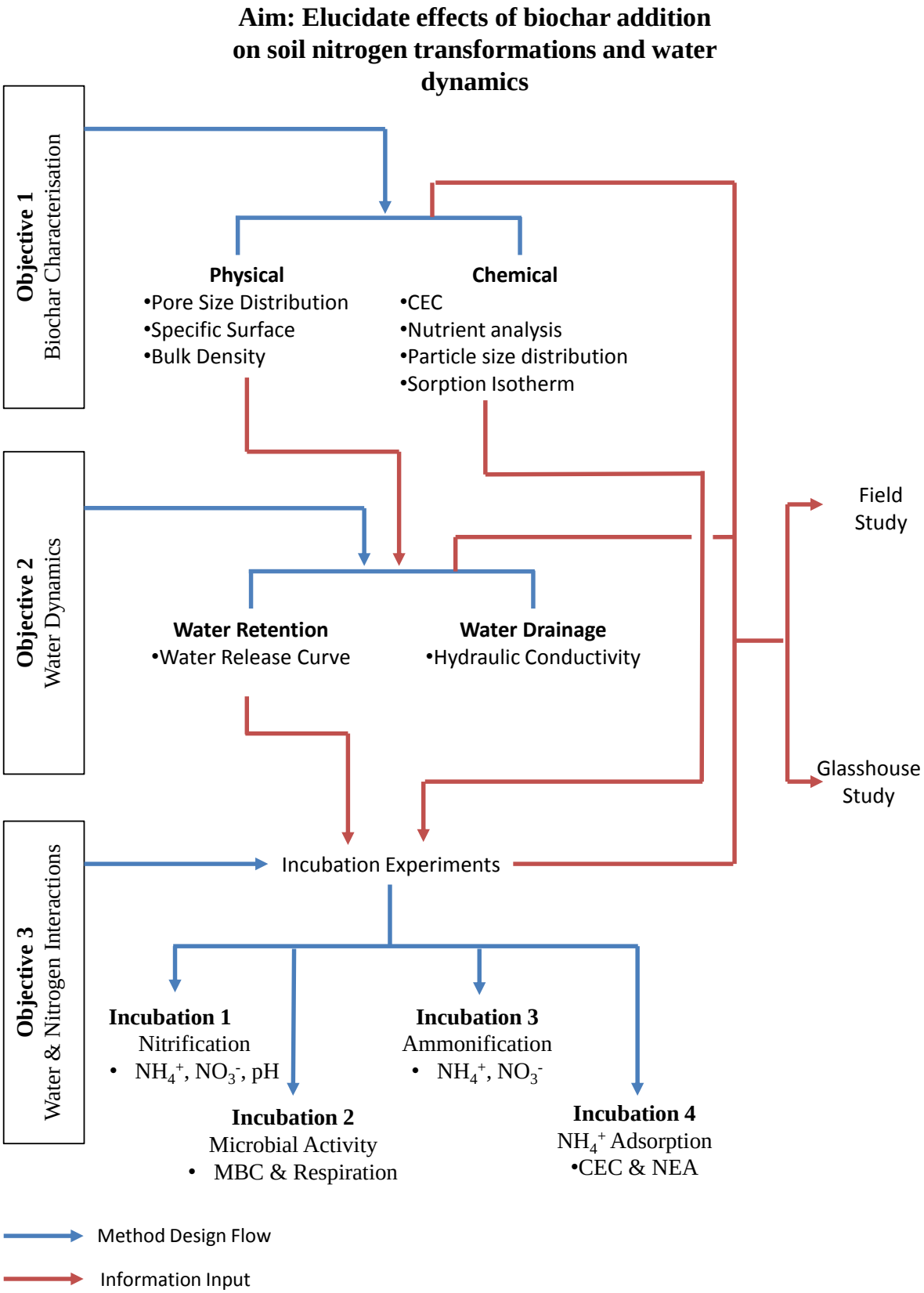


Figure 1-3: Overview portraying the three objectives of the project and the experimental work that will consider these.

2 MATERIAL CHARACTERISATION

This chapter focuses on quantifying specific variables of the biochar, soils and fertilisers used within the study. Details of the production and preparation process are provided as necessary.

The objectives of this chapter were to determine the characteristics, identified within the literature review, to allow an appropriate evaluation of experimental results. This will then place the materials in context with previous research efforts and allow more accurate comparisons with the wider research community. Laboratory analyses were performed to characterise the biochar, soils and fertilisers.

2.1 The Biochar

As noted within **Chapter 1: Introduction and Literature Review**, the definition of biochar is imprecise and, depending on the feedstock and production parameters, can cover a wide variety of characteristics (Mclaughlin et al., 2009). This makes the outcomes of applying biochar to soil, difficult to predict and generalise (Schimmelpfennig & Glaser, 2012) as a wide range of both positive and negative effects can be found, depending on the characteristics of the biochar and the soil, and the type of crops grown (Jeffery et al., 2011).

Research into biochar is prompted by the potential for a positive impact on soil amelioration and crop productivity; soil improvements with the addition of biochar have been attributed to increased water retention, cation exchange capacities and the creation of more amenable conditions for micro-organisms (Mclaughlin et al., 2009). The addition of biochar could affect soil properties directly, through the nutrient content of the biochar itself, or indirectly, whereby the biochar impacts on the soil's physical and chemical properties.

2.1.1 Biochar Production

The biochar was sourced via the Charcoal Foundation (Scarborough, North Yorkshire). A deciduous mixed wood feedstock of sycamore (*Acer pseudoplatanus* L.), Oak (*Quercus sp.*), Beech (*Fagus sylvatica* L.) and Bird Cherry (*Prunus padus* L.), was pyrolysed in a Tropical Products Institute (TPI, David Hutchinson, UK) Metal Ring Kiln (Figure 2-1).

Figure 2-1: Tropical Products Institute metal ring kiln used to produce biochar. (Photo courtesy of David Hutchinson, received 2009)

Pyrolysis, including the initial combustion of sacrificial material, lasted 16 hours, peaking at 600º C to produce the biochar (Figure 2-2). The particles produced were crushed to pass through a 15 mm sieve.

Figure 2-2: Biochar crushed to a diameter less than 15 mm.

2.1.2 Biochar Analysis

Prior to analysis, the biochar was dried at 40º C to remove surface moisture attained during storage and prepared as appropriate for the analysis. Analyses of the biochar was performed between 3 and 5 samples, the number of replications (*N)* are mentioned as required. Unless specified, analysis was performed on biochar sieved to particle sizes between 1 and 2 mm, to

be consistent with future experimental design (see **Chapter 3.4.2 Biochar Preparation** for further explanation of this decision).

2.1.2.1 Chemical and Nutrient Characteristics

The inherent nutrient content of the biochar was analysed by a variety of techniques. Total nitrogen (N), carbon (C) and hydrogen (H) were measured by dry combustion ($N = 5$). Biochar samples were dried at 105˚ C to remove residual moisture and finely ground to encourage complete incineration using the catalytic tube analyser (Vario EL III, CHNOS elemental analyser, Hanau, Germany, British Standards Institute, 1995).

Mineral nitrogen compounds ($N = 3$) were extracted from 10 g biochar using 50 ml 2 mol L^{-1} Potassium chloride (KCl) solution over two hours before filtering using Whatman No. 4 filter paper (MAFF, 1986). Ammonium and the total oxidisable nitrogen (TON) compounds (nitrite and nitrate) were detected using a segmented flow analyser (Burkard Scientific Series 2000, Uxbridge, UK). Biochar pH ($N = 5$) was determined using a glass electrode in a 5:1 ratio of de-ionised water and biochar after shaking for one hour (British Standards Institute, 2005a).

The cation exchange capacity (CEC) of the biochar ($N = 3$) was measured with 1 mol L^{-1} ammonium acetate and 10% (w/v) acidified potassium chloride solution (MAFF RB427, 1986). Biochar (0.5 g) was covered with 20 ml ammonium acetate for 5 hours to ensure filling of the pores and saturate the biochar's surface with ammonium ions. The biochar was then filtered (Whatman No. 2 filter paper) and leached with successive 25 ml volumes of ammonium acetate (totaling 250 ml) to strip away exchangeable cations from the biochar's surfaces and replace with ammonium ions. After removal of excess ammonium acetate with 25 ml volumes of ethanol (totaling 125 ml), the ammonium was extracted from the biochar with successive 25 ml volumes of KCl (to collect a total volume of 100 ml extract). By measuring the ammonium levels within the KCl extract (Burkard Scientific Series 2000 segmental flow analyser, Uxbridge, UK) a measure of how strongly the biochar adsorbs cations (CEC) can be determined using the formula:

$$CEC\ (cmol+\ kg^{-1}) = \frac{(N_s - N_b)}{140} \times \frac{0.25}{m} \times 1000$$

N_s = Ammonium detected in KCl extract of sample (mg L^{-1})

N_b = Ammonium detected in KCl extract of blank (mg L^{-1})

m = Mass soil (g)

An adsorption isotherm (N = 10) was performed with a parallel sampling method (to ensure no pseudo-replication) at 20˚ C (OECD, 2000) using 50 ml ammonium chloride solution at concentrations of 1, 10, 100 and 1000 mg L^{-1} with 0.5 g biochar sieved between 1 and 2 mm.

Biochar was added to 50 ml centrifuge tubes with 45 ml de-ionised water. This was shaken for 72 hours to allow the water to saturate the pores. Five-ml of an ammonium chloride stock solution was then added to produce the desired final concentration. This was then shaken for a further 72 hours to allow adsorption to equilibrate before filtering through an inert (Whatman x) 0.2 µm filter paper as centrifuging did not sufficiently remove biochar particles down to 0.2 µm (OECD, 2000).

Controls included:

1. Without biochar to determine the extent that ammonium could be adsorbed to the surface of the tube during shaking
2. Without ammonium to determine the amount of ammonium the biochar added to the solution.

A preliminary study was used to determine the appropriate mass of biochar, and time for equilibration. This was also used to determine whether filtering adsorbed significant amounts of ammonium before analysis.

2.1.2.2 Physical Characteristics

Although the majority of analysis and all the laboratory studies used a fraction of the biochar particle sizes (see **Chapter 3.4.2: Biochar Preparation**), the field trials used the biochar in the state after production (crushed to less than 15 mm). This contained a variety of particle sizes and was characterised by sieving using a vibratory sieve shaker (Retsch® AS200 Basic). The amplitude was adjusted to achieve a vibration height of 3 mm.

The porous structure of the biochar (Figure 2-3) and the characteristics associated with this (such as surface area) governs many of the physical effects exhibited in the soil (Atkinson et al., 2010).

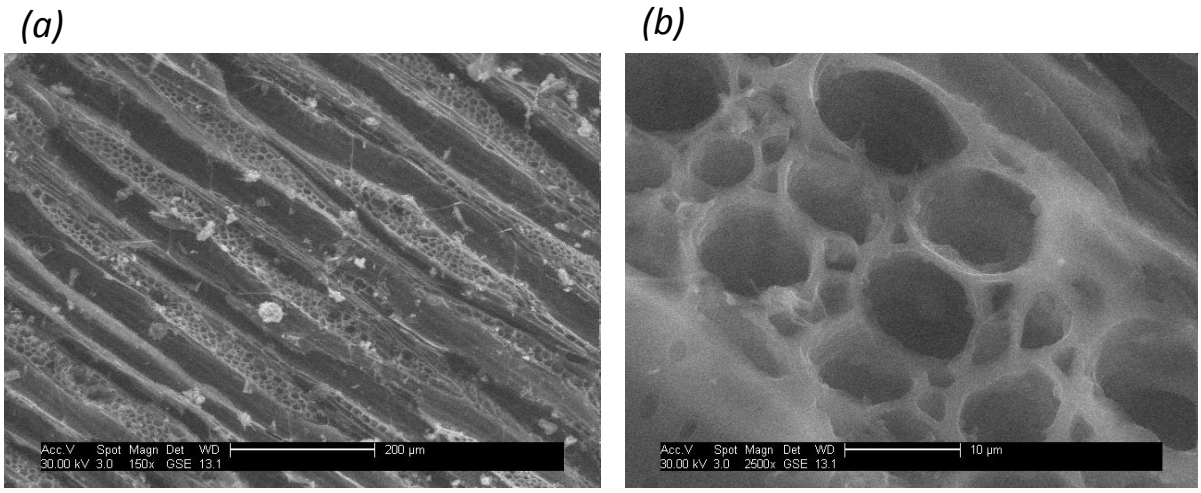


Figure 2-3: The porous nature of the biochar as illustrated by Scanning Electron Micrographs (SEM). Figure magnifications: *(a)* 150x and *(b)* 2500x.

A combination of mercury porosimetry by equilibration and Brunauer, Emmett and Teller (BET) nitrogen adsorption (British Standards Institute, 2005b) was used to characterise the pores. Mercury was used due to its non-wetting properties (contact angle of ~140˚), as such the mercury will not spontaneously enter a pore without external pressure (Giesche, 2006). Mercury was forced into the pores of the biochar (approximately 0.3 g) under incremental pressures ranging from 0 to 237,870 kPa (Quantachrome Poremaster 60-GT), filling pores between 200 µm to 0.0036 µm (3.6 nm) in diameter. Mercury porosimetry was not used to analyse the biochar and soil mixtures. The intrusion of mercury under pressure can affect porosity by either opening up pores that would otherwise not be there or by compression (Smith & Schentrup, 1987; Johnston et al., 1990). This is much more prevalent in materials where the porous material is comprised of separate particles such as powders or soil. The solid fraction of biochar on the other hand is connected and less likely to be contorted or altered by pressure.

2.1.3 Results and Discussion

2.1.3.1 Chemical Characteristics

The summary of the biochar's chemical and nutrient analyses can be found in Table 2-1.

Table 2-1: Chemical and nutrient properties of the biochar. Means and standard errors (SE) calculated from $N = 5$. TN = total nitrogen. TC = total carbon. NH_4^+: Ammonium. TON: Total Oxides of Nitrogen. U = undetectable: lower than the instrument's detection limit of 0.1 mg-N L^{-1}.

TN	TC	C:N ratio	NH_4^+	TON	pH	CEC	H:C ratio
%			mg kg^{-1}			cmol+ kg^{-1}	
0.64	75.1	117.46	U	0.40	10.02	66.33	0.041
(0.03)	(2.03)	(4.61)	(U)	(0.22)	(0.14)	(1.72)	(0.001)

As biomass is heated in the absence/reduction of oxygen, the carbon atoms within the material cannot fully oxidise into gaseous carbon dioxide. A fundamental feature of biochar, as suggested by its other name: black carbon, is a high proportion of carbon. As expected, the biochar used in the current study had a high ratio of C:N (117.46). This is typical, particularly of biochar produced at high temperatures where more volatiles are released as nitrogen, oxygen and hydrogen compounds (Verheijen et al., 2010; Table S1-1). This suggests that, with regards to this characteristic, the biochar is favourable for use as an agricultural amendment due to the potential for enhanced stability (Schimmelpfennig & Glaser, 2012). During high temperature pyrolysis of biomass, carbon atoms form conjugated planar ring systems within crystalline structures that resist biological decomposition (Downie et al., 2009). This recalcitrance can confer long-term stability on biochar in soil, potentially lasting centuries (Lehmann et al., 2006).

Highly carbonised biochar contains less chemically active material (Schimmelpfennig & Glaser, 2012) which could impact directly on soil processes. The biochar used in this study exhibited a large degree of carbonisation with a H:C ratio of 0.04 and is considered to be appropriate as a soil amendment. It is comparable to similarly produced biochars which have H:C ratios ranging from 0.01 (an oak biochar produced at 550º C) to 0.05 (wood-waste biochar produced at 400º C) (Spokas et al., 2011). Biochars of a similar production temperature however (as seen in Table S1-1) still show volatile matter levels between ~10 and 40%.

As with total nitrogen, the level of extractable nitrogen (ammonium and TON) is low. This is attributed to the high carbonisation temperature driving off nitrogen compounds as volatiles.

This would indicate that any effect on the nitrogen cycle is indirect as there is little inherent nitrogen that can add to the soil.

Biochars can exhibit a wide range of pH, however was noted by Verheijen et al. (2010), that biochars typically range from 6.2 - 9.6. With a pH of 10.02, the biochar used in the current study is at the high end of this range. Changes in the soil's pH can influence the soil microbial activity (Schimmelpfennig & Glaser, 2012). A study of various biochars and their characteristics (Spokas et al., 2011) showed that biochar's of similar specifications had a range of pH from 5 to 10.5.

The CEC of the soil is influential on the adsorption and retention of ammonium. As such, the addition of biochar with a high CEC could affect the retention and availability of ammonium to microbes for nitrification. The method of CEC testing can vary between laboratories (Mclaughlin et al., 2009), as such values may differ when comparing to different studies. In the current study, the ammonium acetate method of CEC determination was used as it did not require centrifugation, but filtering. This was an advantage with biochar, which, due to a low density, would float in the supernatant. The CEC of the biochar is useful as an initial starting value, but it has been noted that the CEC can change, thus altering the adsorption properties, over time (Cheng et al., 2006).

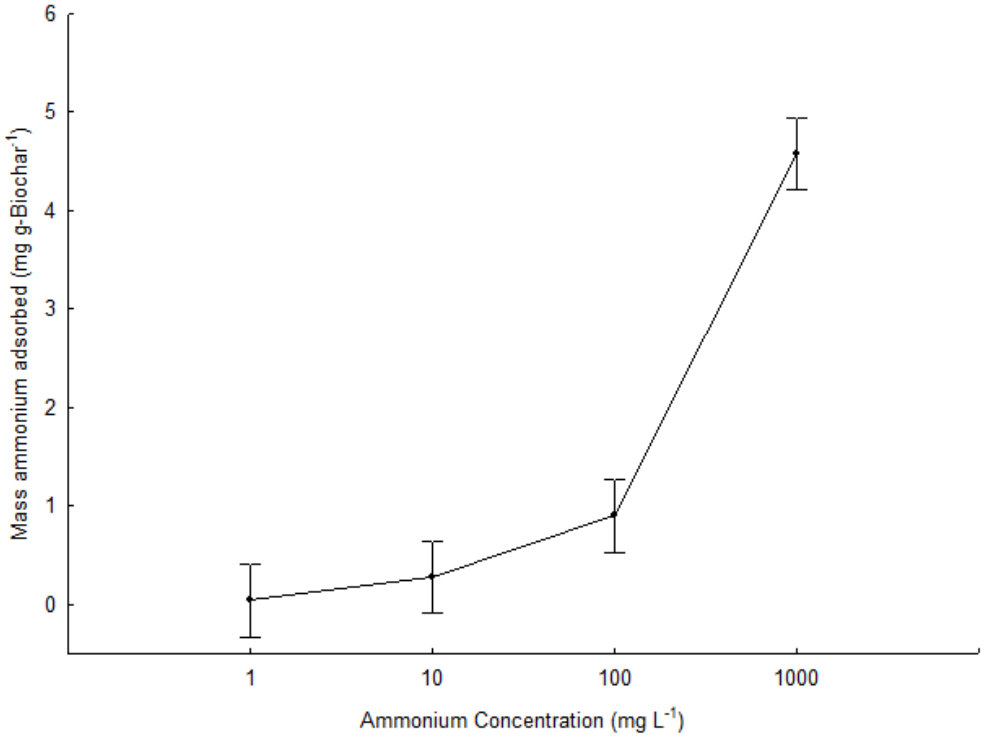


Figure 2-4: Adsorption of ammonium to biochar with increasing ammonium concentration (N = 10).

Figure 2-4 showed that biochar adsorbed up to 4.6 mg-ammonium g^{-1} but also indicates that biochar could adsorb more at greater concentrations. The detection of TON in the extracts indicated that the ammonium solutions were not completely stable and some of the ammonium was converted, impacting on the ammonium concentration. The extent of this conversion was consistently below 1 mg L^{-1} and could be considered minimal, except in the lower concentrations where the conversion to TON accounted for 31% of the ammonium added. Sterilisation by autoclaving could help eliminate the impact of biological nitrification.

A One-way analysis of variance (ANOVA) on the controls (without biochar, $N = 3$) showed that filtering did not remove significant amounts of ammonium from the solution ($P = 0.20$).

Sources of error include instrumental error, particularly at lower concentrations close to the tolerance level of the machine. It is also possible that ammonium could sorb to the surface of the storage containers which could not be quantified. Indeed, it can only be assumed that the initial stock solutions were as calculated as ammonium could be adsorbed during storage.

2.1.3.2 Physical Characteristics

Figure 2-5 shows the results of sieve fractionation of the biochar after manufacture. The majority of the biochar particles produced had a diameter between 2 and 15 mm (~ 68%). It is noted here that a substantial fraction of the biochar are small particulates under 106 µm (> 10%). This biochar was produced by a slow pyrolysis (16 hours), this was chosen as fast pyrolysis results in large amounts of dust and small particulates (Laird et al., 2009; Shrestha et al., 2010) that can pose health risks and losses in biochar during application.

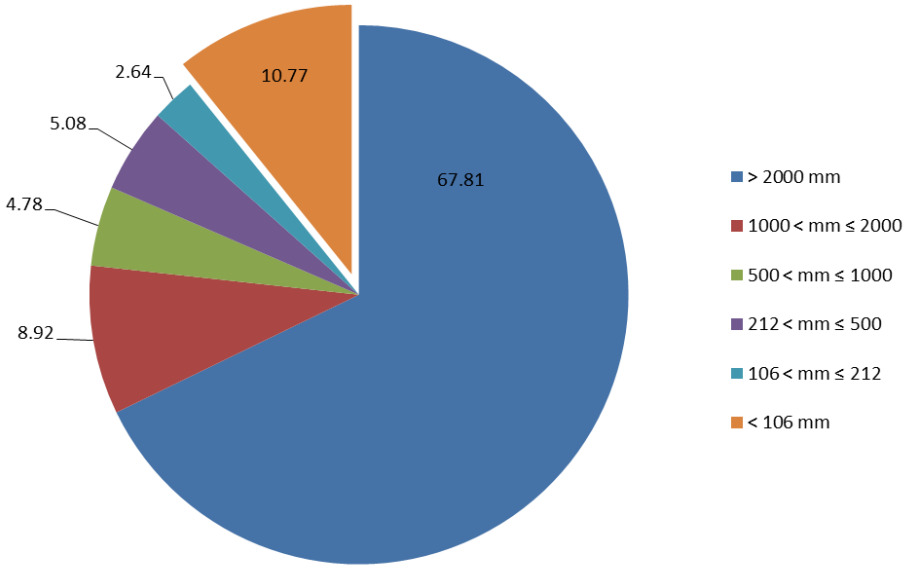


Figure 2-5: Chart showing the particle size fractions of the biochar after pyrolysis with the percentage of each fraction.

Mercury intrusion porosimetry analysis revealed that the biochar's specific pore volume was calculated to be 1.31 cm^3 g^{-1} (standard error of 0.03). However as pore characteristics are complex, they cannot be reduced to an effective single value; the distribution of pore sizes (Figure 2-6) shows that the biochar exhibited a bi-modal pore size characteristic. There was a high frequency of pores found at 20 µm and a second, higher peak, at 1.5 µm. The mean specific surface area of the biochar was 39.5 m^2 g^{-1} with a standard error of 1.5 m^2 g^{-1}. BET nitrogen adsorption resulted in a surface area between 14 and 30 m^2 g^{-1}. The results also indicated that the presence of volatile compounds on the biochar's surface was inhibiting the effectiveness of the analysis, and could explain the low values measured for a biochar of this type. Expressing porosity and surface area as a single value can be problematic, this is shown in Table S1-1, which shows a large range of surface areas with biochar of similar production temperature, ranging from ~5 to 400 m^2 g^{-1}. The biochar used in this study is lower than the mean of 167 m^2 g^{-1} referenced in Table S1-1.

Figure 2-6 utilises the log differential intrusion (dV/dlogD). This is the derivative of intruded mercury volume (V) with respect to the pore diameter (D) (Batten & Lafayette, 2008). This aids the identification of where the ranges of common pore sizes occur.

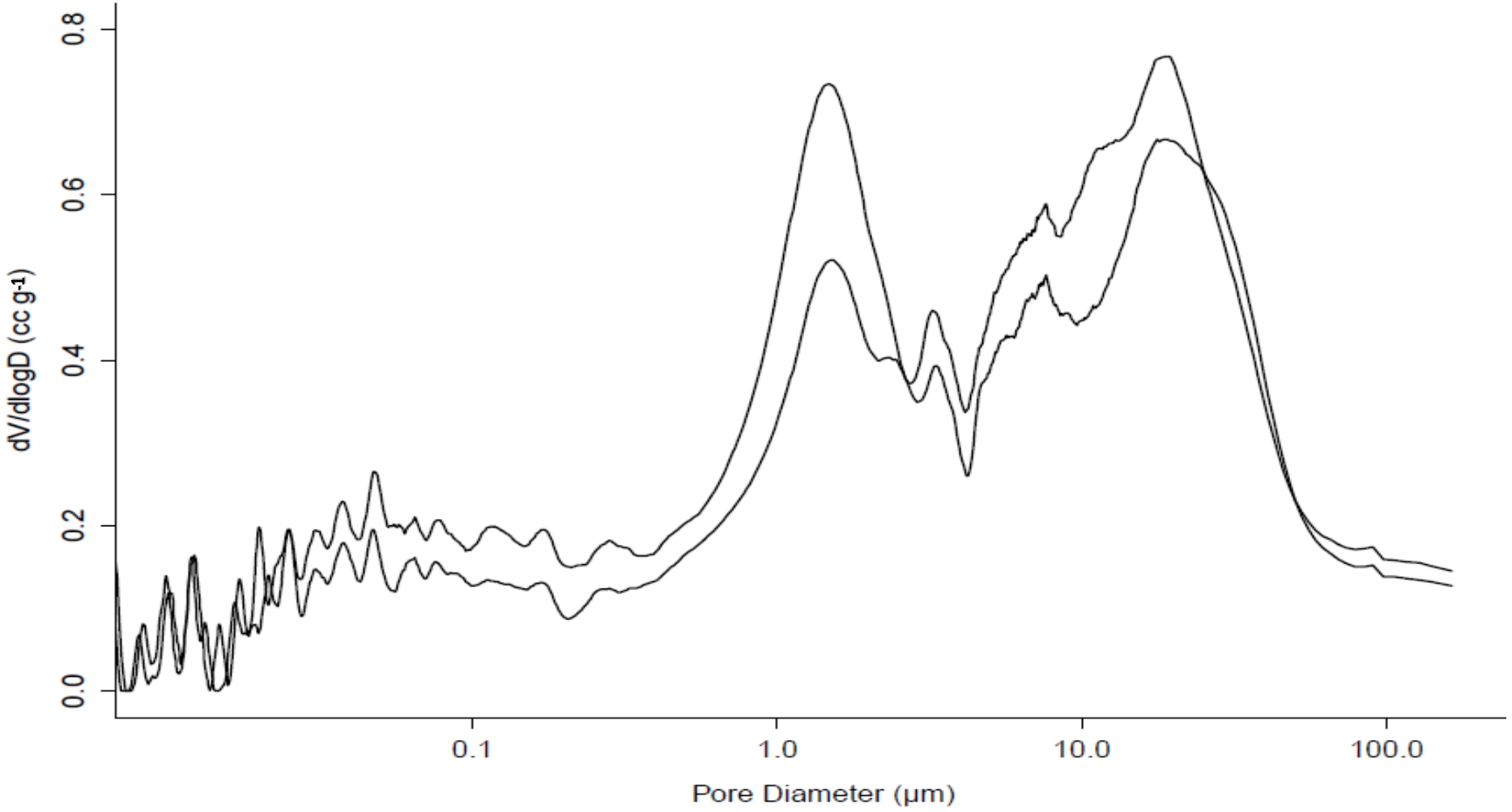


Figure 2-6: Distribution of different pore size diameters within the biochar. Each line represents a different replicate (N = 2). V: volume of mercury intruded; D: pore diameter.

2.2 The Soils

The impact biochar may have upon the physical and chemical properties of the soil, is not only dependant on the biochar, but also the initial soil type and characteristics. It has been suggested that the greatest benefit of adding biochar to the soil could occur in sandy soils as opposed to a clay dominant soil (Woolf, 2008). This could be interpreted to the greater impact of the biochar's chemical and physical properties on a more inert and coarse textured soils such as sand (Mukherjee & Lal, 2013). As such, the soils used for all laboratory (**Chapters 3 and 4**) and glasshouse based experiments (**Chapter 5**) were selected for their sandy textures. Soil used in the field experiments is discussed separately in **Chapter 6: Field Trials**.

For analysis, unless specified, soils underwent preparation identical to that prior to use in laboratory and glasshouse experimentation. This included air-drying at 40˚ C before grinding to pass through a 2 mm sieve and homogenisation.

2.2.1 Soil Analysis

The soils were collected from two sites in the East Anglia region of the UK; these sites were managed under organic and conventional farming systems (Rushbrooke Farm, Suffolk and Silsoe Farm, Bedfordshire respectively). At the time of collection, in 2010, the site at Rushbrooke Farm had been organically managed for 14 years and Silsoe Farm managed conventionally for 35 years.

Soils were collected between 0 and 0.15 m from a trench covering a wide area of the field where there was no vegetation. This bulk soil collection was homogenised and used for all experimental procedures except for the Field Trials. The textural analysis by particle size distribution of each soil is shown in Table 2-2 as measured using the sieving and sedimentation methods (British Standards Institute, 1998a).

Table 2-2: Textural analysis of soil (used in laboratory experiments) using particle size distribution (British Standards Institute, 1998a). Texture is determined using the UK classification system.

	Soil Management System	
	Organic	**Conventional**
% Sand	63.18	76.24
% Silt	22.51	14.71
% Clay	14.31	9.05
Textural Analysis	**Sandy Loam**	**Sandy Loam**

Classifications are based upon UK (England & Wales) soil classification schemes. The positions of the soils on the textural diagrams are shown in Figure 2-7. This shows the texture triangle of both the organically and conventionally managed soils.

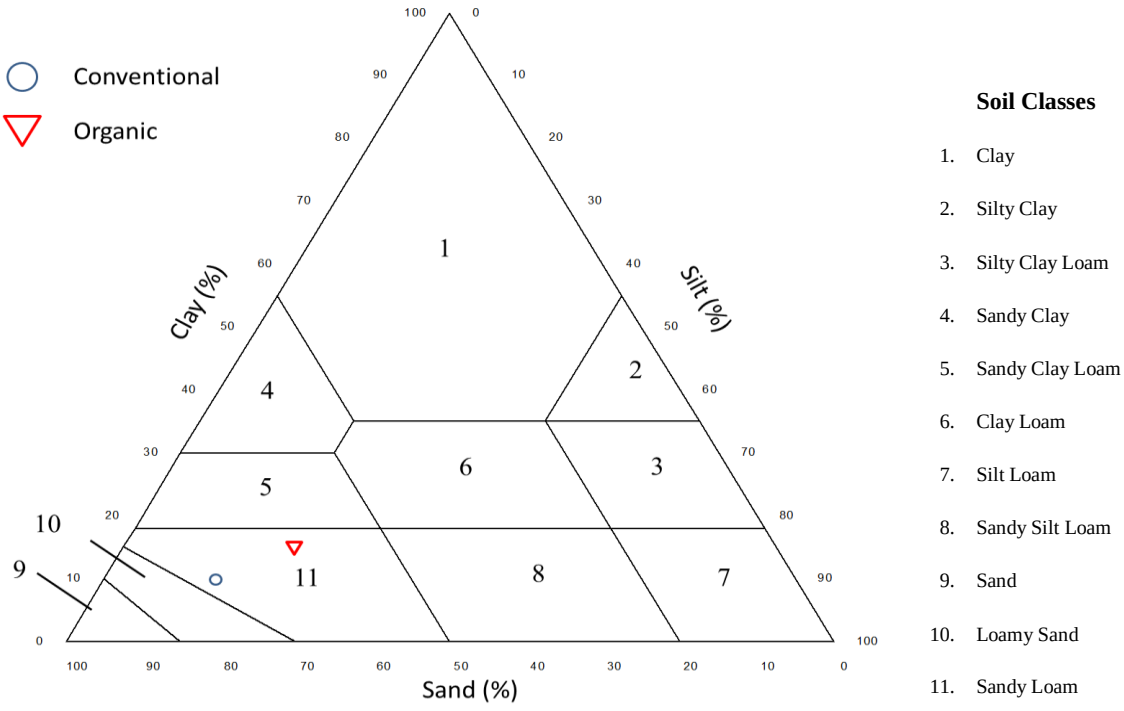


Figure 2-7: Textural triangle showing the experimental soils on the UK based soil classification system. Diagram created using Texture AutoLookup (TAL) (for Windows) Version 4.2 (Teh, 2002).

The relevant nutrient levels and chemical characteristics of the two soils used within the study can be found in Table 2-3. This highlights the differences between the soils that were historically under different management regimes.

Table 2-3: Chemical and nutrient attributes of the organically and conventionally managed soils used in the laboratory experiments, showing means and (standard errors). Calculated from *N* = 3. TN = total nitrogen; TC = total carbon; TOC = total organic carbon. Org. :Organically managed; Con.: Conventionally managed

	TN	TC	TOC	C:N	NH_4^+	NO_3^-	pH	CEC
	%				mg kg^{-1}			cmol+ kg^{-1}
Org.	0.173	1.654	1.54	9.57	0.37	1.61	7.154	33.45
	(0.002)	(0.006)	(0.01)	(0.09)	(0.12)	(0.12)	(0.008)	(0.58)
Con.	0.095	0.887	0.83	9.30	0.68	6.68	6.831	23.60
	(0.003)	(0.021)	(0.01)	(0.08)	(0.12)	(0.88)	(0.003)	(0.65)

2.3 Fertilisers

Commercially available fertilisers were added to the soils (pot and laboratory experiments) as a source of nitrogen. Fertiliser nutrients were matched to those typical for the different (organic and conventional) management systems; PAS-100 accredited green waste compost (GWC) was added to the organically managed soil and a commercial 8-12-8 NPK fertiliser (Scotts Sportsmaster Pre-Seeder fertiliser to the conventional one. The nitrogen in the fertiliser was ammoniacally based.

The GWC (Table 2-4) was obtained from MEC recycling in Lincolnshire. Upon arrival, the compost was dried at 40º C and ground until the compost could pass through a 1 mm sieve. This allowed for homogenisation of the mixture for accurate nitrogen application and ensuring the consistency of the parameters throughout the duration of experiments.

Table 2-4: Chemical characteristics of the green-waste compost. Mean values and standard errors (SE) are calculated using *N* = 4. OM = Organic matter (measured using loss-on-ignition).

TN	TC	TOC	OM	C/N Ratio	NH_4^+	TON	pH
%					mg kg^{-1}		
1.65	21.50	20.48	38	13.00	476	359	8.1
(0.03)	(0.54)	(0.15)	(1.16)	(0.46)	(93)	(76)	(0.02)

2.4 Chapter Conclusions

Based upon the analysis, the biochar used in the current study is deemed suitable for use as a biochar amendment that, under suitable conditions, exhibit high stability and potential for improvements in soil quality and crop productivity. The feedstocks used are appropriate for use in the UK and Europe.

This study is limited to the use of just one biochar. As biochars can be produced using a variety of feedstocks and production conditions, this study attempted to asses a biochar that could feasibly be used on a wide scale in the UK and Europe.

3 SOIL WATER DYNAMICS

3.1 Introduction

The moisture content of a soil plays an important role within an agricultural system and can determine the ability of the soil to function by directly and indirectly affecting the biological and chemical interactions. These include structural stability for plants; the decomposition and release of nutrients; and the movement and uptake of these nutrients into plants. As such, a change in the water status of the soil has great agricultural implications on crop growth and productivity.

The regulation of water levels in the soil is therefore essential when maximising plant growth and soil fertility. In water limited environments, plant growth is restricted by the reduction of nutrient uptake and photosynthesis. There are also effects on the soil, such as reduction in mineralisation and microbial activity.

According to Cary & Hayden (1973), there are two considerations when optimising the soil water regime, (1) rapid infiltration and drainage of surface water during periods of high water input, (heavy rains etc.) to prevent water-logging of the root zone which would limit oxygen levels and (2) retention of an adequate supply of water in the root zone to support optimal plant growth. Determining the availability of water additions to a system can also be alluded by the fate of the water. Stoof et al. (2010) also suggested that the combination of water retention (water storage capabilities) and the infiltration (rate of water flow) can determine the fate of precipitation on a given area and therefore whether it is available for plant uptake and utilisation within the soil.

Stress caused by water deficit can be exacerbated by a reduction in soil organic matter (SOM) and the resultant indicators in soil degradation such as aggregation, affected by certain intensive land management practices such as over-tilling and a reliance on commercial fertilisers (Franzluebbers, 2002). Bronick & Lal (2005) concluded that management practices that increase attributes such as aggregation and soil structure increase productivity. Equally however, plants and microbes in the soil require gas exchange, which cannot take place in too much water.

Water retention capabilities are affected by several factors, but primarily physical characteristics of the soil such as texture, structure and bulk density (Stoof et al., 2010). The

effects these have on the soil can all be linked to the pore characteristics such as size distribution and continuity of the pathways which affect water movement in the soil (Lipiec et al., 2006).

3.2 Chapter Objectives

This chapter is to achieve Objective 2: To investigate how the addition of biochar can affect the water regime of a sandy soil (**Chapter 1**). Two laboratory experiments were set up to test the hypotheses. A water release curve (WRC) measured changes in water retention with biochar amendment. The saturated hydraulic conductivity method measured the ease with which water flowed through the saturated soils.

Specifically therefore, this chapter investigates how the addition of biochar could affect water retention and movement within organically and conventionally managed soils.

3.3 Chapter Hypotheses

Increasing the application rate of biochar in the soil will increase water retention and the available water stores.

The porous attribute of biochar will reduce the bulk density of the soil and thus the rate of water flow through the soil under saturated conditions will increase with biochar application rate.

3.4 Water Release Curve Methodology

3.4.1 Experimental Set-Up

The WRC considered three independent variables in a 2 x 3 x 2 factorial designed experiment, conducted in triplicate. The two soils (collected from the respective organically and conventionally managed farms as described in **Chapter 2.2**) were amended with three rates of biochar application including 0, 30 & 60 t ha^{-1} (0, 1.52 and 2.99% by mass respectively). The percentage of biochar by mass was calculated by assuming a depth of 0.15 m (depth of field biochar application – **Chapter 6: Field Trials**) and a bulk density of 1.3 g cm^{-3}.

The final factor was to consider how water retention with biochar may change over time due to the aging of biochar; a comparison was made between unaged soil/biochar mixtures and after incubating the above soils for a period of 3 months at 25º C. Soil moisture content (SMC), during the incubation period, was maintained at 50% field capacity. Field capacity was determined from a preliminary trial by saturating triplicate samples of each treatment and equilibrating to a soil water potential of -5 kPa (Nemes et al., 2011).

3.4.2 Biochar Preparation

The biochar was dried at 40º C and sieved to achieve a particle size between 1 and 2 mm. The removal of particles larger than 2 mm from this experiment ensured that the biochar added to the rings was representative of the mixed feedstock used to produce the biochar.

The production and crushing of the biochar yielded over 10%, by mass, of particulate matter less than 106 µm (Figure 2-6: **Chapter 2**). These have the potential to alter the soil's pore size distribution, through mechanisms that are independent to the porosity of the biochar, by blocking larger pores in the soil (Figure 3-1). This will reduce the reliability of scaling these laboratory experiments to the field, where such preparations will not take place.

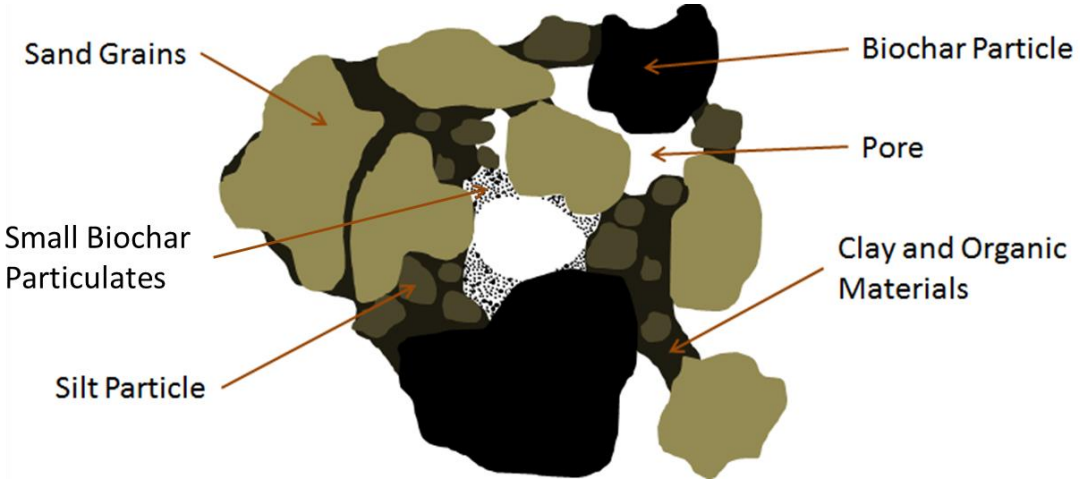


Figure 3-1: Diagram showing how small biochar particulates could reduce the pores sizes in the surrounding soil. To counter this effect, these were removed by sieving between 1 and 2 mm.

3.4.3 Packing the Rings

The soil was prepared by air-drying at 40˚ C and grinding to pass through a 2 mm sieve, as for soil analysis preparation described in **Chapter 2.2**. The soil and biochar was then hand-mixed to achieve the required biochar application rate.

Water retention is dependent upon pore characteristics, thus soil bulk density is influential in soil water storage capacities (Bouma et al., 2003c, 2003b; Stoof et al., 2010). For differences in the WRC to be attributed to the biochar, it was important that the surrounding soil had comparable pore characteristics.

Biochar has a low bulk density but is more resistant to external compression than soil. This is derived from the higher molecular order of the turbostratic carbon within the biochar as production temperature increases (Tsai et al., 2012). Had each ring been compacted to equal bulk densities, the lower density biochar would raise the packing density of the surrounding soil, altering the pore characteristics leading to incomparable treatments.

The rings were packed to achieve a comparable surrounding soil matrix. Metal rings, of approximately 20 mm depth and 52 mm diameter, were secured with a mesh base. These were packed by adding the prepared soils and repeatedly tapping the sides until no further consolidation occurred, before measuring bulk density.

3.4.4 Experimental Procedure

The WRC protocol is based upon the procedure of the (British Standards Institute, 1998b). Metal rings, with a mesh base (Figure 3-2), were packed with known quantities of the prepared soils, and placed on a sponge water bath to saturate the pores. The rings were weighed twice each week until the mass of the soil peaked and fell. The largest mass measured was then used to calculate the SMC of the soil (% by mass) at saturation.

Figure 3-2: Metal ring used for the water release curve. Mesh base secured in place with cable-tie which would not degrade during the experiment.

Saturated samples were then subjected to various pressures to create specific soil water potentials within the samples, using sand tables and pressure membrane cells (Figure 3-3), which cause the emptying of specific pore size equivalents (PSEs), and left to equilibrate. Equilibration was deemed complete when changes in sample mass did not exceed 0.1 g over a 7 day period as suggested in the protocol (British Standards Institute, 1998b). The increments in pressure used are summarised in Table 3-1. The discrepancy between the pressures selected was due to practical problem when conducting the experiment, such as the desiccation of the cell membranes that allow the flow of water out of the samples but not the flow of air.

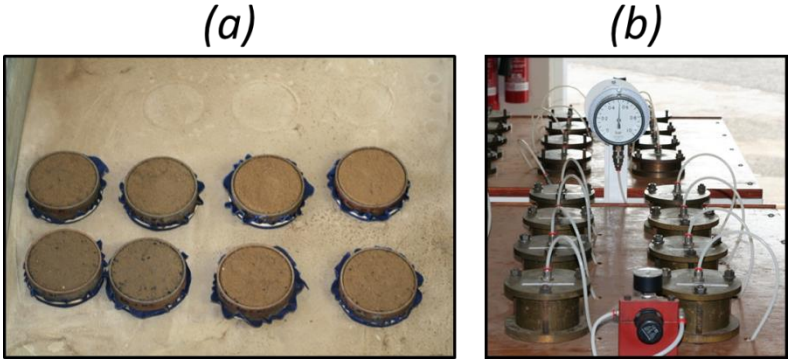


Figure 3-3: Subjecting water release curve samples to increasing pressures from sand tables-*(a)* to pressure membrane cells-*(b)*.

Table 3-1: Summary of the pressures used to produce the water release curve, and the relevance to the plant's ability to take up water.

Soil Water Potential (kPa)	Significance to Plants	Without Incubation	After 3 Months Incubation
0	Saturation	✓	✓
-1		✓	✓
-5	Field Capacity	✓	✓
-7.5		✓	
-10			✓
-50		✓	✓
-1500	Permanent Wilting Point		✓

3.5 Hydraulic Conductivity Methodology

The hydraulic conductivity is a measure of the permeability of the soil, which affects the rate of water flow as demonstrated by Darcy's Law. Measurement of the hydraulic conductivity was designed to be in tandem with the WRC.

3.5.1 Experimental Set-Up

The study of biochar on hydraulic conductivity considered two independent variables in a 2 x 3 factorial designed experiment conducted in triplicate. The soils from the organically and conventionally managed sites were amended with three rates of biochar application including 0, 30 & 60 t ha^{-1}. The experiment did not consider the effect of aging on hydraulic conductivity and thus the data collected was compared only to the WRC on un-aged biochar and soil mixtures.

Hydraulic conductivity however uses larger columns of soil than the WRC. The columns were packed to the same bulk density as found in the WRC rings to achieve comparable physical conditions.

Following the methodology for the WRC, the biochar was air-dried at 40˚ C and sieved between 1 and 2 mm to preserve the surrounding soil's pore size distribution and for biochar representation. To compare the results of the hydraulic conductivity to the WRC, the columns used in the former were packed to the same bulk density as in the rings of the latter, and can be found in Table 3-2.

Rigid plastic columns, approximately 60 mm depth and 68 mm diameter, were placed on a metal mesh base with 1 mm openings. These were packed to the required bulk density by hand, adding the soil incrementally. The soil was wetted to 50% field capacity prior to packing for a more uniform density.

Table 3-2: Bulk densities of the hydraulic conductivity columns as determined by the mean packing densities within the un-aged water release curve rings

Soil Management	Biochar Application Rate (t ha^{-1})	Bulk Density (g cm^{-3})
Organic	0	1.43
	30	1.39
	60	1.30
Conventional	0	1.60
	30	1.53
	60	1.48

3.5.2 Experimental Procedure

Hydraulic conductivity was measured using the falling head method (British Standards Institute, 1990). The packed columns were clamped at each open end, to allow the flow of water through whilst preventing soil losses. The samples were immersed in water to saturate the soil for 24 hours before water was allowed to flow through the samples and the rate of flow measured. The rate of flow was measured by timing a drop in water level of a manometer by 20 cm. Samples were repeated thrice, as suggested (British Standards Institute, 1990), to ensure reliability of the data.

3.6 Statistical Analysis

Changes in the packing density of the soils and in the SMC over incremental soil water potentials was analysed using a repeated measures analysis of variance (ANOVA) (General Linear Model) using STATISTICA V.12 (Statsoft Ltd, 2013). Saturated hydraulic conductivity was analysed using a factorial ANOVA.

The statistical significance level was determined with $\alpha = 0.05$. For multiple comparisons, a Fisher's least significant difference (LSD) analysis was used to compare individual means (Sokal & Rohlf, 1995). Probability plots of residuals were used to determine the normality of the population distributions and anomalous data were occasionally removed prior to analysis, though data were left intact where possible.

3.7 Results

3.7.1 WRC Ring Bulk Densities

With the un-aged biochar and soil mixture, the addition of biochar reduces the bulk density of the soil (Figure 3-4; Table S3-1). Tables of supplementary data (denoted by the prefix 'S') can be found in the section: **Supplementary Material**. In the organically managed soil, this decrease from the control (1.43 g cm^{-3}) was evident only at 60 t ha^{-1} biochar ($P < 0.001$; 1.30 g cm^{-3}); there was no difference at 30 t ha^{-1} biochar ($P > 0.05$; 1.39 g cm^{-3}). Soil that had undergone conventional management however showed a decrease in bulk density with the addition of either 30 or 60 t ha^{-1} biochar from 1.60 g cm^{-3} to 1.53 and 1.48 for the 30 and 60 t ha^{-1} biochar respectively ($P < 0.001$), however no difference was found between the two rates of biochar ($P > 0.05$).

After aging for 3 months, the bulk density of the organic and the conventional soil showed no difference. The addition of biochar decreased the bulk density in the organically managed soil only from the control of 1.28 g cm^{-3} to 1.20 and 1.17 (30 and 60 t ha^{-1} biochar respectively) with no difference between the two rates of biochar. The addition of biochar did not affect the bulk density of the conventionally managed soil (Figure 3-4).

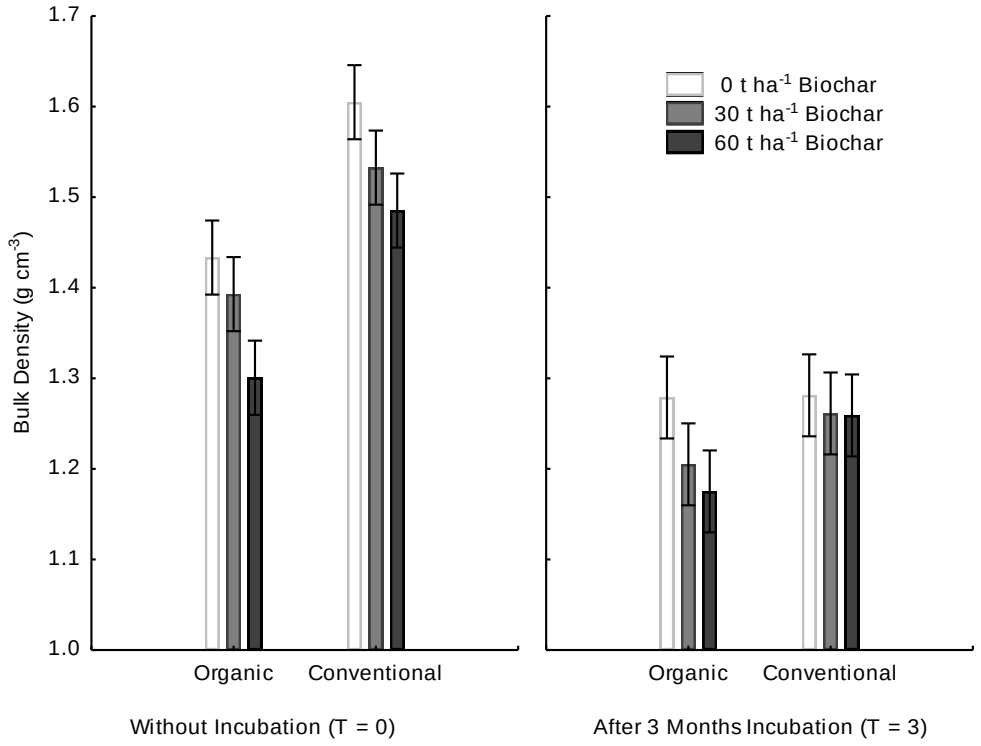


Figure 3-4: Bulk densities of the packed WRC rings before saturation. Organic: Organically Managed Soil; Conventional: Conventionally Managed soil. Bars are standard errors (N = 6).

3.7.2 Water Release Curve

The repeated measures ANOVA (Table S3-2) showed that the organically managed soil retained more water than the conventional over the range of SWPs ($P < 0.001$; Figure 3-5).

The ANOVA only showed that the addition of biochar increased the retention of water in the aged treatment ($P = 0.01$) but not in the un-aged ($P > 0.05$; Table S3-2).

Further analysis with a Fisher's test of least significant difference showed that the addition of biochar did increase the retention of water at specific SWPs in both un-aged and aged samples. These increases in SMC occurred between SWP ranges of -1 kPa to -10 kPa. The addition of biochar did not significantly affect the retention of water at saturation, nor at the lower SWPs (-50 kPa and lower; Figure 3-5).

The increase in SMC with biochar addition is at its maximum (3.9 and 11.3% increases from control of 25.6% by mass) at -5 kPa under organic management without incubation.

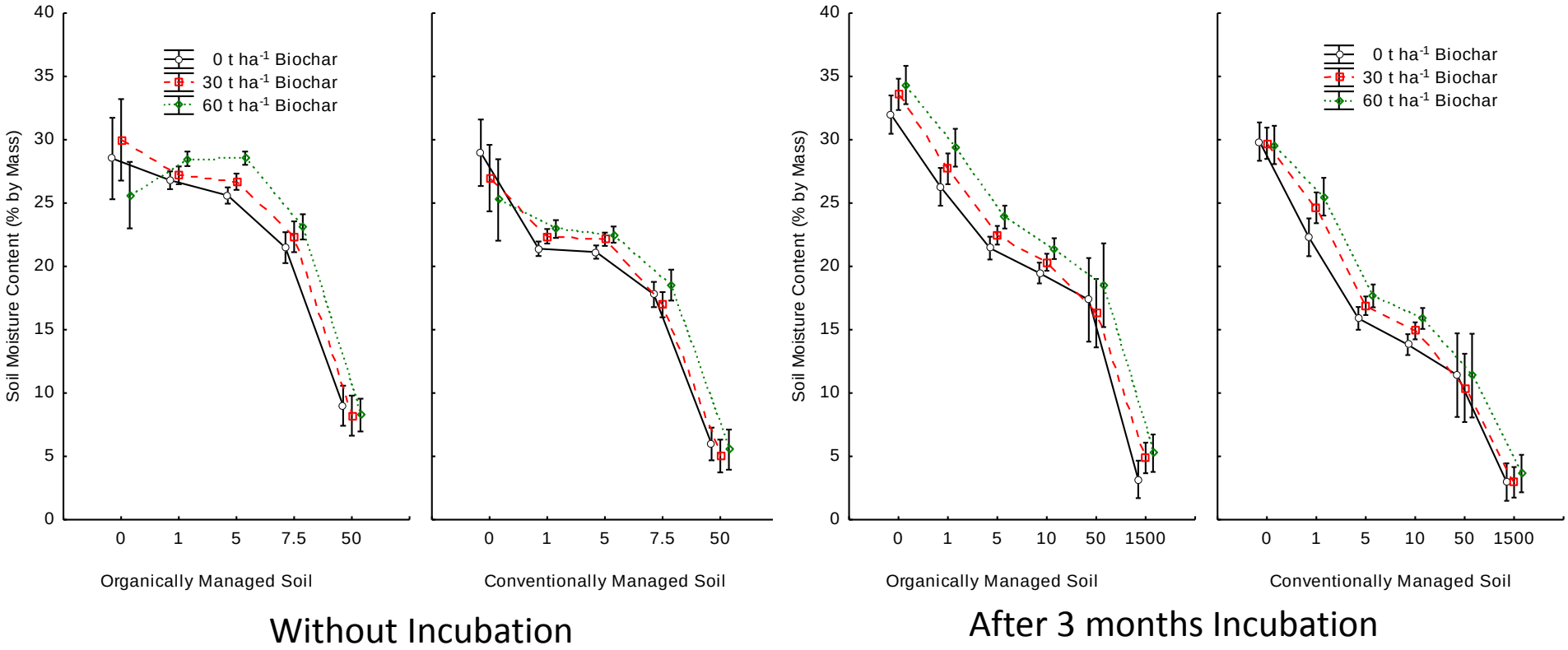


Figure 3-5: Water retention over incremental soil water potentials from 0 kPa (saturation) to a maximum of -1500 kPa (permanent wilting point)

3.7.3 Saturated Hydraulic Conductivity

There was no observable change in hydraulic conductivity between the two soils without the addition of biochar (P = 0.1; Table S3-3). There was also no link between biochar application rate and hydraulic conductivity (Figure 3-6) as no change was detected in the organically managed soil but a small decrease with the application of 60 t ha^{-1} from 0 and 30 t ha^{-1} in the conventionally managed soil (P = 0.05 and 0.03 respectively).

Due to the presence of large standard error bars, there is little evidence to suggest that the addition of biochar altered the permeability of the soil under the experimental conditions.

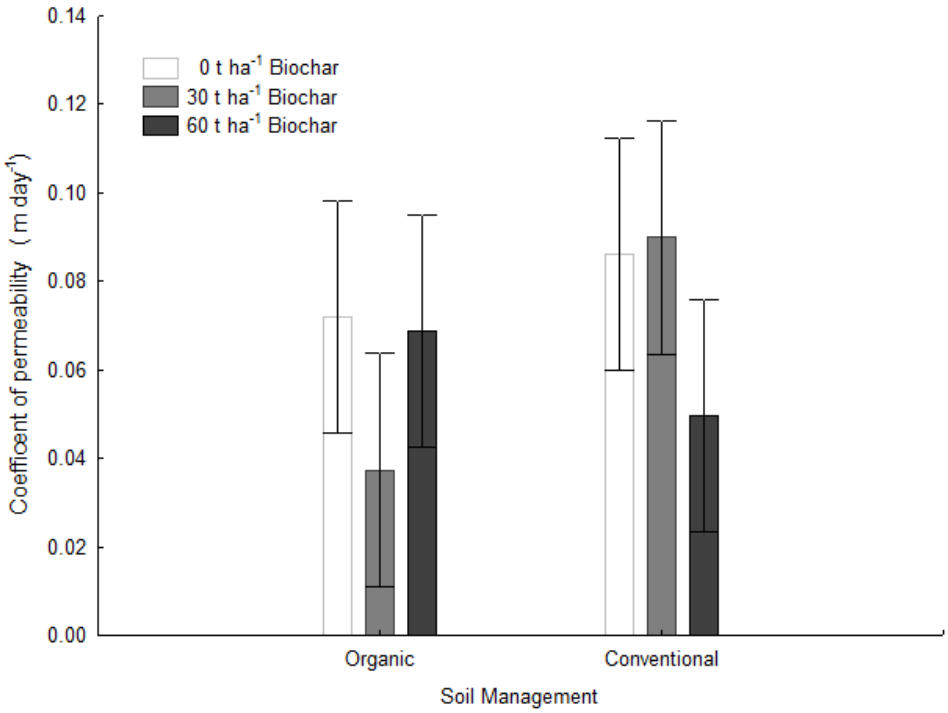


Figure 3-6: Saturated hydraulic conductivity (N = 3). Soil and biochar used did not undergo aging by incubation. Bars are standard errors, each replicate is comprised of 3 pseudo-replicates (British Standards Institute, 1990).

3.8 Chapter Discussion

Determining soil pore size distribution (PSD) with WRCs (British Standards Institute, 1998a) assumes complete wetting with water-to-surface contact angles less than 90º. As biochars may sorb hydrophobic substances (Schimmelpfennig & Glaser, 2012) the WRC could not be used to estimate PSD.

The bulk density of the packed WRC rings was typically lower in the soils from the organically managed site compared to that from the conventionally managed. Bulk density is influenced by the pore size distribution of the soil; increasing the level of SOM is known to decrease the bulk density of a soil, through dilution of denser mineral substances of the soil matrix and increasing porosity (Shepherd et al., 2002). As such, the management system of the soil (depending on the level of organic matter input) can alter the bulk density of the soil (Mariangela & Francesco, 2010; Bronick & Lal, 2005; Bulluck et al., 2002). Indeed the total organic carbon (TOC) levels of the organically managed soil were higher than that found in the conventional (1.54% ± 0.01 compared to 0.83% ± 0.01 respectively).

The addition of biochar decreased the bulk density of the packed soil in the rings for both the organically managed soil and the conventional, within the un-aged treatment. As the bulk density is not an intrinsic property of the soil but a function of the solid particle to inter-particle void ratio, the bulk density is therefore directly proportional to the porosity.

The addition of biochar to a soil is well documented to lower the bulk density. This is dependent on the parameters the biochar underwent during production; wood-based biochars produced at high peak temperatures typically exhibit higher porosity than those produced at lower temperatures (Bagreev et al., 2001). With the close relationship between soil porosity and bulk density, the addition of biochar has been demonstrated to lower the bulk density of a soil (Lei & Zhang, 2013), the hypothesis suggests therefore that the reduction in bulk density with biochar is due to the high porosity of the biochar.

Little research can be found regarding the extent to which oxidation of biochar during aging in the soil can change the surface area and the distribution of pore sizes in the biochar and over what time scale. Hale et al. (2011) aged biochar and soil mixtures, in 2 month incubations at 40% field capacity, by separate biological, chemical and physical means. Hale et al. (2011) showed that the biologically aged biochar (by inoculation with bacterial groups, *Actinobacteria*, *Proteobacteria*, and *Bacteriodetes* extracted from sediment with a carbon and

nutrient source) showed an increase of micropore surface area from 122 to 165 m^2 g^{-1} however concluded this was minimal in comparison to chemical (exposing the biochar to 60 and 110˚ C in airtight containers. Water content was adjusted to 40% WHC. Prior to aging, biochar was sterilised with 1% (by volume) Sodium azide) and physical aging methods (biochar was sterilised with Sodium azide as with chemical aging and exposed to 42 freeze - thaw cycles between -70 ˚ C (5 h) and 20 ˚ C (19 h)).

As such, although it may be a contributing factor, it seems unlikely that the aging of the biochar at 25˚ C with just biological aging over 3 months will have altered the porosity of the biochar to the extent of influencing the bulk density exhibited.

A lowering of bulk density and the resultant increase in porosity has been known to increase water retention (Zhang et al., 2012). The repeated measures ANOVA concluded that the organically managed soil retained more water than the conventional. This corresponds with the lower bulk density shown also, as a result of the higher organic matter in (1.54% ± 0.01 total organic carbon and 0.83% ± 0.01 respectively; **Chapter 2**). Soils with high inputs of organic matter and carbon has been shown to increase the water holding capacity (Mariangela & Francesco, 2010).

Although the repeated measures ANOVA only showed that the addition of biochar increased the retention of water in the aged treatment ($P = 0.01$), a Fisher's test of least significant differences showed that the addition of biochar did increase the retention of water at various SWPs. The retention of water was shown to be significantly higher with biochar predominantly within ranges in SWP from -1 kPa to -10 kPa. The addition of biochar did not significantly affect the retention of water at saturation, nor at high SWP (-50 kPa and higher).

This effect of the biochar on the WRC was surprising as previous research has indicated the prevalence of micro and nano-pores within high temperature biochars which result in high surface areas up to 3000 m2 g^{-1} (Abel et al., 2013; Schimmelpfennig & Glaser, 2012), and could result in the retention of water within these pores. These results therefore indicate that the pores within the biochar show a high proportion of meso-pores (> 0.2 µm) rather than micro-pores.

Analysis of the biochar's pore characteristics by mercury intrusion (**Chapter 2**) suggested that there was a bimodal distribution of pore whereby the majority of the pore sizes were between 0.5 - 2 µm, and 5 - 50 µm. This indicated that the addition of this biochar to the soil

would have an impact on this range of pore sizes in the soil and not to the micro-porosity of the soil.

Despite indications that the addition of biochar to soil can lower bulk density and increase water retention, no difference in saturated hydraulic conductivity was found between management systems and no relationship was observed with biochar application rate. This could be attributed with the limitation of using disturbed soil samples. Although the overall bulk density was selected to match those found in the rings to the WRC, there may have been differences in densities within the ring resulting in stratification. The saturated hydraulic conductivity is also associated with the soil's macro-porosity (Jirků et al., 2013). Given the limited effect of biochar on the retention of water at SMC close to saturation and the low proportion of macro-pores within the biochar itself, this could explain the lack of change.

3.9 Chapter Conclusions

The addition of biochar decreased the bulk density of the packed soil in the rings for both management systems when using the soil that was not incubated. Wood-based biochars produced at high peak temperatures typically exhibit higher porosity than those produced at lower temperatures. With the close relationship between soil porosity and bulk density, the addition of biochar has been demonstrated to lower the bulk density of a soil, it is suggested therefore that the reduction in bulk density with biochar is due to the high porosity of the biochar. It is suggested that the lower bulk density in the packed rings from the soils that were incubated for three months is due to inconsistent packing as it is less likely that the changes were caused by changes in pore sizes over a short period of three months.

Reducing the bulk density increases the percentage of pore volume in the soil and thus can increase the potential for the retention of water. Indeed the retention of water did increase with increasing biochar application rate, this was statistically significant (as shown by ANOVA) for the soils incubated after 3 months however a Fisher's test of least significant difference showed that the addition of biochar increased the soil moisture content for both incubation times. This increase occurred between soil water potentials of -1 and -10 kPa. A significant difference was not found at saturation (0 kPa) or at permanent wilting point (-1500 kPa).

The mercury porosimetry analysis of the biochar suggested that there was a bimodal distribution of pores, with peaks where the majority of pore sizes were between 0.5 µm – 2 µm, and 5 µm – 50 µm. Despite indications that the addition of biochar to soil can lower bulk density and increase water retention, no difference in saturated hydraulic conductivity was found between management system and no relationship was observed with biochar application rate. This could be attributed to the lower frequency of macro (transmission) pores within the biochar, as described by the pore size distribution by mercury porosimetry, that control drainage under saturated conditions, thus reducing transmission of water through the soil.

4 INTERACTION OF NITROGEN AND WATER DYNAMICS

4.1 Introduction

As discussed in **Chapter 1: Introduction and Literature Review**, nitrogen exists in the soil in several different forms which exhibit varying degrees of availability to plants and microbes. The relative pools of each form within the soil and the transformations between them are in constant shifting equilibria, changing according to the local biotic and abiotic parameters.

The physical and chemical properties of the biochar can affect the local conditions that in turn dictate equilibrium between the nitrogen pools and thus their relative sizes. As different nitrogen forms vary in availability, the size and rate of exchange between forms (and how this might be affected by the addition of biochar) is important, impacting on uptake by plants and microbes.

4.1.1 Chapter Objectives

The objective of this chapter is to use a laboratory approach to assess changes in nitrogen transformations of a sandy soil due to the addition of a biochar. This chapter is a series of experiments that will investigate the effect of biochar on nitrification and aspects that may act as mechanisms for this (as shown in Figure 4-1) and with the impact of changing soil moisture content (SMC).

This chapter will centre on the heterotrophic nitrification process (the conversion of ammonium to nitrate via nitrite) as this is highly influential regarding the availability of nitrogen to plants. Subsequent experiments will support this, by examining potential mechanisms influencing nitrification rate such as the microbial activity, ammonium production and ammonium retention through cation exchange processes (Figure 4-1). Hypotheses pertaining to each of the four aspects will be stated at the beginning of the relevant experimental sections.

The question of whether biochar can impact on soil nitrification processes and its causal explanation will be studies here, it was predicted that the biochar would affect nitrification through a change in ammonium availability.

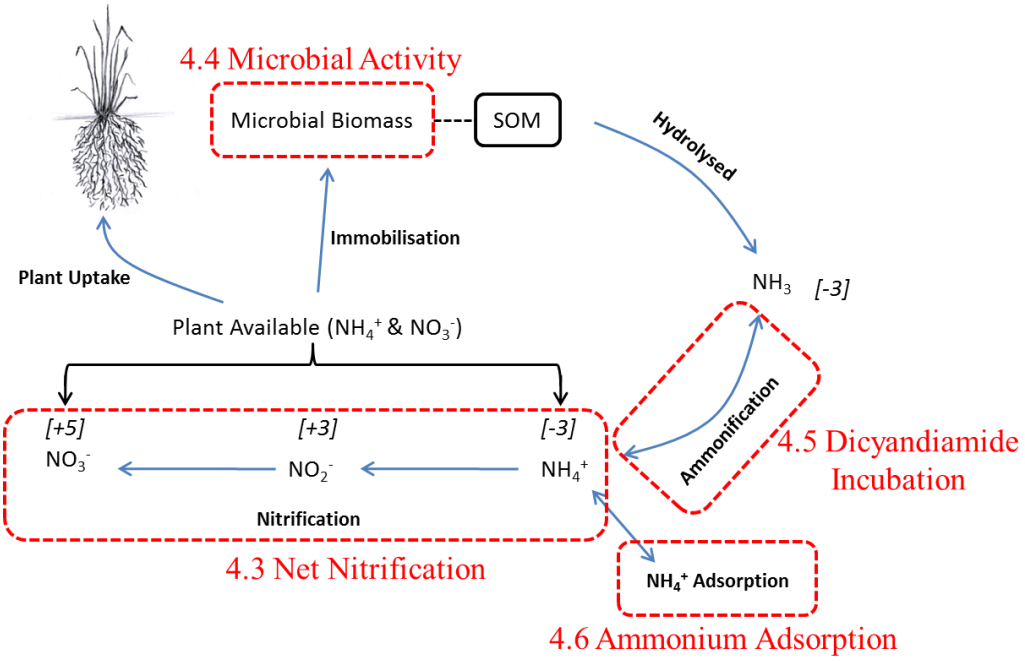


Figure 4-1: Aspects of the nitrogen cycle that will be examined in this chapter with relevant chapter sub-headings.

4.2 Incubation Methodology

A series of laboratory incubations were used to examine the soil's nitrogen transformations with the addition of biochar. The controlled environment allows the isolation and identification of process factors. Equally, this can limit the effectiveness of extrapolating the effects of these mechanisms to larger scales, such as field trials with many more extraneous variables.

4.2.1 Incubation Set-up

Treatments for the incubation experiments included organically and conventionally managed soil with biochar application rates of 0, 30 & 60 t ha^{-1} at SMCs of 25% and 50% field capacity. The soil and biochar was prepared and hand-mixed as described in **Chapter 3.4: Water Release Curve Methodology**.

A nitrogen source, in the form of fertiliser, appropriate to the soil management type (green-waste compost - GWC and an inorganic fertiliser to the organically and conventionally managed soils respectively) was added to investigate the effects of biochar on nitrogen transformation. The amount added was equivalent to an application rate of 140 kg [N] ha^{-1} as determined by the RB209 Fertiliser Recommendation Manual (DEFRA, 2010).

The incubation experiments were set up under identical environmental conditions, to explore the mechanisms surrounding the process of net nitrification within the soil, as portrayed in Figure 4-1.

4.2.2 Incubation and Sampling Procedure

Deionized water was added incrementally (preventing surface ponding) to 350 g of the prepared soil, biochar and fertiliser mixture to reach desired SMCs of 25% and 50% field capacity. Maintaining the SMC at a percentage of field capacity, rather than an absolute value across the treatments, provided an equal soil water potential, which was considered a better indicator of water availability to plants and microbes (Chen et al., 2011).

Triplicate samples were left for 24 hours at 25º C to allow the water to distribute equally through the soil before sampling. The pots were aerated by shaking, and lightly tapped to re-consolidate the soil. The SMCs at field capacity were calculated using WRC rings equilibrating at -5 kPa (British Standards Institute, 2009; Reeve & Carter, 1991), as described in **Chapter 3.4: WRC Methodology**.

The mixtures were incubated, throughout the experiment, at 25˚ C (Figure 4-2) and the SMC was maintained by additional water as required. A perforated lid reduced desiccation.

Soil samples were removed periodically from the pots and prepared according to the specific analysis requirements; the frequency of sampling was dependant on the variable and the length of the incubation experiment in question.

4.2.3 Incubator parameters

The transformation of nitrogen in soil is a biologically mediated process; nitrification occurs within a specific range of temperatures. The containers were maintained at 25º C (Figure 4-2), within the optimal range of 25 – 30º C for nitrification (Norton & Stark, 2011). Nitrifying bacteria require the presence of oxygen; incubation experiments are an artificial system and do not have a regular introduction of gases through cultivation and invertebrates. Shaking the pots after each sampling event assisted with gas exchange.

A tray of water was placed in the incubator to raise humidity and limit water loss by evaporation. This prevented large fluctuations of soil moisture affecting the nitrogen transformations (Yuan et al., 2011).

Figure 4-2: Layout of the incubation experiment. The tray filled with water maintained a raised humidity to reduce water loss by evaporation.

The bulk density of a soil affects the soil's porosity and thus the water availability and gas exchange. Biochar has a low density and is resistant to compression. As discussed in the **Chapter 3.4: WRC methodology**, compressing a soil and biochar mixture to a specific bulk

density, artificially increases the density of the surrounding soil, altering the pore networks in which nitrogen transformations take place. To resolve this, after each sampling event, the pots were re-consolidated by tapping thrice after shaking.

4.3 Net Nitrification

4.3.1 Background

The transformation between nitrogen forms in the soil is an important process, as nitrogen is often considered to be one of the most important nutrients for plant growth and agricultural productivity. As net nitrification does not separate nitrate produced from the nitrate immobilised, this measurement can be used as an indicator for the amount of nitrogen available for supplying plants (Verchot et al., 2001). A net gain therefore indicates that production of nitrogen is greater than the assimilation or loss. How the addition of biochar influences this balance of available and non-available forms can therefore impact on crop growth.

4.3.2 Hypotheses

The addition of biochar to soil will increase nitrification rate and raise the available nitrate pool within the soil.

4.3.3 Soil Measurements and Analysis

Net nitrification was measured by detecting products from the process: ammonium and nitrate. A 2 mol L^{-1} potassium chloride (KCl) solution was used to extract mineral nitrogen (MAFF, 1986). A segmented-flow analyser (Burkard Scientific Series 2000, Uxbridge, UK) was used to detect levels of ammonium; total oxides of nitrogen (TON: nitrate + nitrite); and nitrite. The difference between TON and nitrite determined the nitrate levels. A 1:5 (volume fraction) suspension of soil in deionised water was used to measure pH with the use of a glass electrode (British Standards Institute, 2005a).

This incubation experiment lasted 60 days; periodically, 10 g ± 0.05 g wet soil was removed for extractable nitrogen analysis (Lewis & Kaye, 2011). Nitrogen was extracted from samples taken over 14 sampling events; sampling was more frequent during the first 30 days, when most of the nitrogen changes were anticipated. Less frequently (8 sampling events), 20 g wet-soil was removed; dried at 40º C; sieved to 2 mm; and analysed for water extractable pH.

4.3.4 Data and Statistical Analysis

Changes in ammonium, nitrate and pH were analysed individually using a repeated measures ANOVA (General Linear Model) using STATISTICA V12 (Statsoft Ltd, 2013). Statistical

significance level was determined with $\alpha = 0.05$. For multiple comparisons a post-hoc comparison procedure is necessary to compare individual means (Sokal & Rohlf, 1995). As such a Fisher's least significant difference (LSD) analysis was deemed most suitable.

Probability plots of residuals were used to determine the normality of the population distributions. Anomalous data were identified if outside two standard deviations from the mean and removed prior to analysis if this was the case, though data were left intact where possible.

4.3.5 Results

Initially, ammonium levels were greater ($P < 0.001$; Table S4-1) in the conventionally managed soils compared with the organic soils at both 25% (~ 82 mg kg^{-1} dry soil and ~ 5 mg kg^{-1} dry soil) and 50% field capacity (~ 88 mg kg^{-1} dry soil and ~ 9 mg kg^{-1} dry soil).

Figure 4-3 shows that soil ammonium levels decreased from day 5 of the incubation. Under conventional management, ammonium declined below the instrument's detection limit at 50% FC but was still declining at 25% FC (34 mg kg^{-1}). The already low concentrations of ammonium in the organically managed soil resulted in a quicker decline compared to the conventionally managed soil.

Under both SMCs, increasing biochar application rate reduced ammonium levels in the conventionally managed soil. On day 15, at 25% and 50% FC, ammonium contents were 80, 73 & 67 mg kg^{-1} and 66, 30 & 15 mg kg^{-1} respectively with increasing biochar application (*P*-values < 0.001). No significant changes ($P > 0.05$) were observed from the small initial ammonium contents in the organically managed soil (Figure 4-3).

Initial nitrate contents of 5 mg kg^{-1} increased over the 60-day experiment (Figure 4-3). The total nitrate released was greater ($P < 0.001$; Table S4-1) in the conventionally managed soil, this was particularly noticeable at 50% FC (113 mg kg^{-1} compared with 64 mg kg^{-1}).

At day 60, increasing the biochar application rate from 30 to 60 t ha^{-1} in the organically managed soil resulted in less nitrate (57 and 50 mg kg^{-1}, respectively at 50% FC) compared to the control (*P*-values of 0.01 and < 0.001, respectively) (64 mg kg^{-1} at 50% FC). At 50% FC, for the conventionally managed soils, increasing the biochar application rate initially resulted in greater nitrate levels, but this trend reversed after 30 days (Figure 4-3). At 25% FC, there was a significant drop in nitrate levels after day 50 for the biochar amended conventional soil.

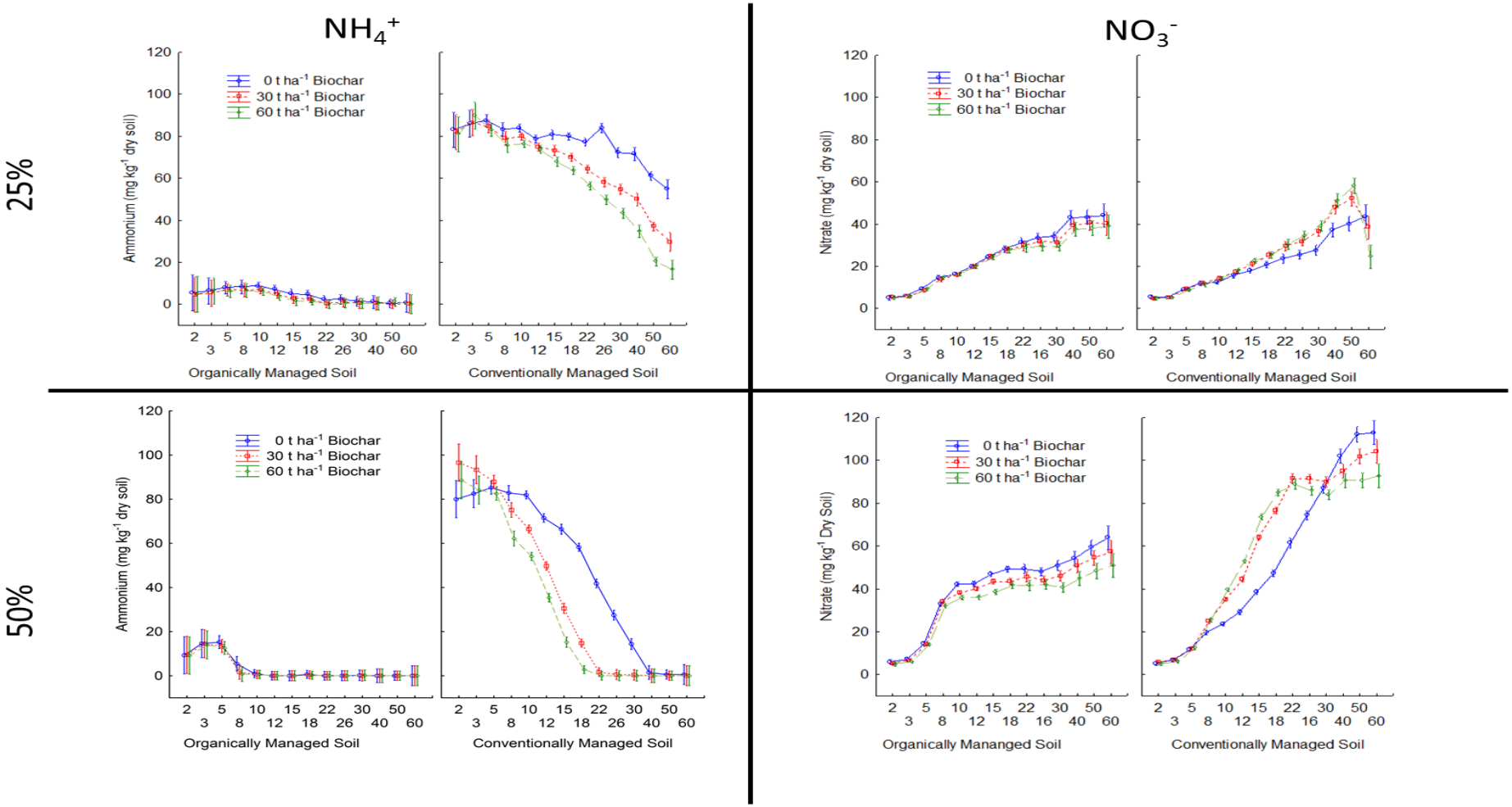
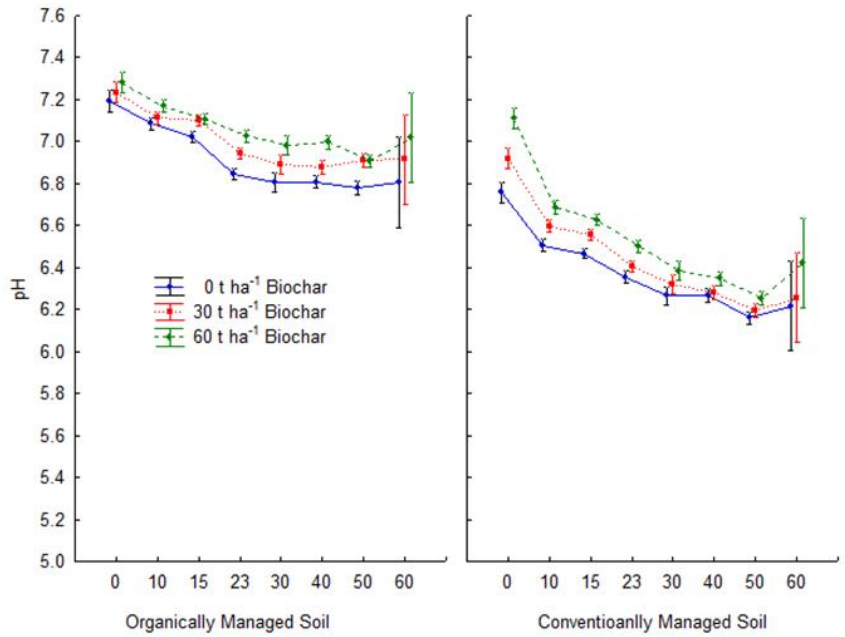


Figure 4-3: Soil ammonium and nitrate concentrations over the 60 day incubation at 25% and 50% field capacity. Bars are standard errors (N = 3)

Soil pH decreased over the 60 days, this decrease was greater in the conventionally managed soil (1.2 pH units) than in the organic soil (0.5 pH units) (Figure 4-4); both decreases had *P*-values < 0.001. The effects of biochar were more pronounced at 50% FC; with biochar additions of 30 and 60 t ha^{-1} the pH in the conventionally managed soil decreased from 6.9 to 5.9 and 7.1 to 6.1, respectively. In the organically managed soil, pH decreases were less pronounced with higher biochar application rates; 7.2 to 6.9 and 7.2 to 7.1 at 30 and 60 t ha^{-1} respectively: all *P*-values were < 0.001 (Table S4-1).

25%



50%

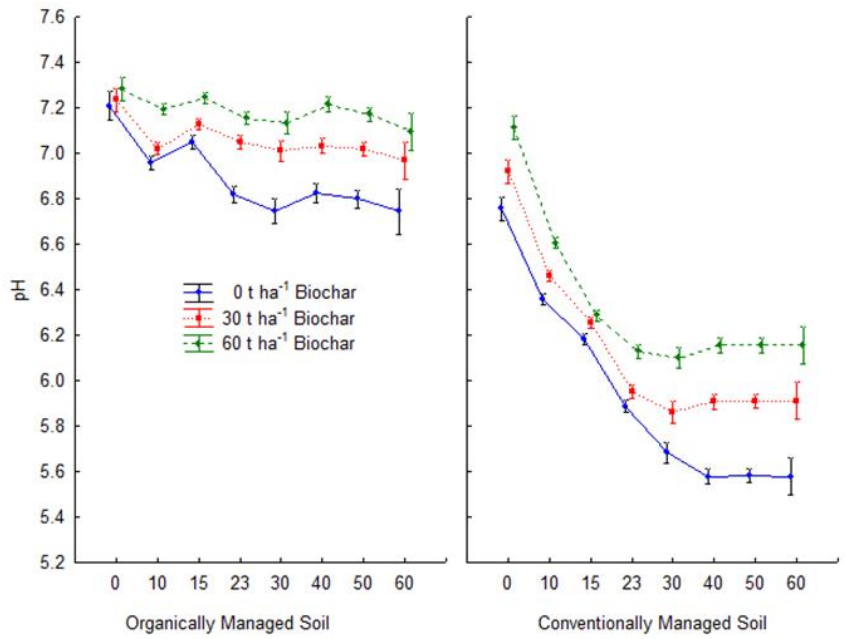


Figure 4-4: Water extractable pH over 60 day incubation at 25% at 50% field capacity. Bars are standard errors (N = 3).

4.3.6 Section Discussion

Over the 60 day incubation, ammonium contents approached zero for all treatments in conjunction with increases in nitrate, indicating nitrification as a cause. This was coupled with reductions in pH over time as nitrification is an acidifying process (Bolan et al., 1991; Hofman & Cleemput, 2004), as shown in Equation 1, **Chapter 1.5.3: Nitrification**.

The pH declined more in the conventionally managed soil relative to the organic. The ability for a soil to resist a change in pH is known as the pH buffering capacity (pHBC) (Wong et al., 2013) and so, the pHBC is a key factor that determines the rate at which pH changes during acidification (Xu et al., 2012). The pHBC of a soil can arise from the protonation/de-protonation reactions of soil minerals and organic matter. As such, important soil properties that influence the pHBC include SOM and CEC; where pHBC increases with both these attributes (Xu et al., 2012; Weaver et al., 2004).

The larger SOM levels and thus CEC in the organic soil than the conventional (33.45 and 23.60 cmol+ kg^{-1} respectively) (Table 2-3) could provide a greater pHBC by higher proton acceptance during the protonation as a result of nitrification, reducing the extent of pH change observed.

The SMC can affect the rate of the nitrogen dynamics, nitrification is a biologically controlled reaction and the process is affected by factors that influence microbial activity. The lower moisture content of the soil can lower microbial activity by restriction of ammonium availability (Stark & Firestone, 1995), due to the isolation of organic matter from microbial mineralisation.

In the conventionally managed soil, the addition of biochar increased the rate at which ammonium decreased. From Figure 4-1, it can be seen that ammonium levels could be affected by a number of potential pathways such as increased adsorption or lower ammonification rates. Given the decrease was coupled with an increase in soil nitrate, this indicates that the addition of biochar raised the nitrification rate.

It is possible that a higher pH resulting from biochar additions (Yuan et al., 2011), created more favourable conditions for the nitrifying bacteria. Nitrification rates are greater when the pH was raised from 7.5 to 9 (Sajuni et al., 2010); and the pH of biochar (10.02) increased the pH in the soil-biochar mixture than in the soil alone. The increase in moisture content at the

larger biochar application rates could also provide more favourable conditions for nitrification (Case et al., 2012).

Biochar is a predominantly carbonaceous material, and it is often stated that the majority of the biochar's carbon is stable and therefore recalcitrant (Schimmelpfennig & Glaser, 2012). Biochar's however can contain volatile matter including labile carbon fractions (Deenik et al., 2010). The content of labile carbon, as with many other nutrient properties, is linked to the biochar's feedstock and production temperatures, indeed as peak production temperature increases the labile carbon content decreases. Despite this, high temperature biochars do contain significant fractions of available labile carbon (Cross & Sohi, 2011; Farrell et al., 2013). The addition of labile carbon to a soil can result in higher microbial activity (Ge et al., 2010; Hue & Sobieszczyk, 1999). The utilisation and thus depletion of labile carbon could also account for the reversal in the effect of biochar on the nitrate levels after 30 days.

In the organically managed soil, the nitrate contents were lower after the application of biochar (Figure 4-3). This however cannot be determined to be caused by changes in nitrification rate, as no effect of biochar was observed on ammonium levels in the organically managed soil. This was due to the low presence of ammonium after Day 8, concealing any effect. Measuring the net ammonium concentration at a given time does not indicate ammonium production, it is suggested that any ammonium produced would be rapidly oxidized to nitrite and nitrate and remain undetectable. To conclude how biochar is influencing the soil to affect the nitrate levels, measuring the gross ammonium levels is required (**Chapter 4.5: Dicyandiamide Incubation**).

The lower levels of ammonium in the organically managed soil compared to the conventional is attributed to greater availability of carbon substrate from GWC which increases microbial demand for nitrogen (Hue & Sobieszczyk, 1999), which subsequently reduces ammonium contents.

The larger CEC of the organically managed soil than in the conventionally managed soil coupled with an addition of biochar could result in greater retention of ammonium ions, reducing conversion to nitrate. Many biochars are noted for their high cation exchange capacity, and addition to soil can increase its CEC directly by the increase in exchange sites associated with the larger surface area (Atkinson et al., 2010). The CEC of biochar may increase with aging because of increasing surface areas and the formation of negative sites as

it oxidizes (Liang et al., 2006). It has been shown that biochar can adsorb ammonium to biochar through cation exchange as a preferential process (Ding et al., 2010).

4.3.7 Section Conclusions

It was concluded that increasing biochar application rate, compared to the control, in the conventionally managed soil increased the nitrification rate resulting in a lower ammonium and higher nitrate. This was attributed to the more amenable conditions to microbial activity provided in the form of labile carbon directly from the biochar, and it is the depletion of this that is suggested to cause the reduction in nitrification rate with biochar, compared to the control after 30 days. Biochar addition could also have indirectly improved soil conditions through an increase in soil pH.

In the organically managed soil however, concluding the mechanism causing the reduction in nitrification rate with increasing biochar application is more complex. Without an observable effect of the biochar on net ammonium, the reduction in nitrate could be attributed to one or more of several mechanisms. One such mechanism was suggested to be due to the higher CEC of the biochar which can adsorb ammonium reducing mobility. It is suggested that the biochar is not directly affecting the nitrate levels due to the limited anion exchange capacity of many biochars.

It is currently unknown whether the biochar is affecting the two soil's nitrification processes differently via separate mechanisms or through a varied response of the same mechanism via an interaction with the soil's properties.

4.4 Microbial Biomass and Activity

4.4.1 Background

Complex interactions exist between the microbiology and the physio-chemical attributes of the soil. Microbes can affect the physical characteristics of the soil through production of exudates (primarily polysaccharides), which bind particles together into aggregates (Le Guillou et al., 2012). This structure within the soil allows roots to grow effectively and take up water and nutrients and affect the functionality of a soil.

As an influence on the decomposition of organic matter and subsequent release of inorganic ions (Smith et al., 2010), the role of microbes on nutrient cycling is of importance to this study. The microbial population and activity directly impacts on the size and availability of various pools of nitrogen in the soil and thus plant uptake. The population and activity of soil microbes are governed by the local environmental factors (Ushio et al., 2010; Wang et al., 2003). Addition of biochar can impact on the soil's properties and influence the microbial populations.

Although a common measure of the microbial presence in the soil is measured in microbial biomass carbon (MBC), the microbial population can also differ in their activity, as many microbes in the soil can be dormant (Wang et al., 2003) and thus do not actively influence nitrogen cycling. Therefore, with regards to the decomposition and transformation of nitrogen, how active the populations of microbes are, is an important variable. A combination of the microbial population (measured by MBC) and microbial respiration (measured through carbon dioxide release) will be used to determine the activity of soil microbes.

4.4.2 Hypothesis

Microbial biomass carbon (MBC) and microbial respiration will increase with the addition of biochar due to the indirect adjustments in the soil's properties including pH and water content. This will correspond with the rate of nitrification found in the nitrification incubation.

4.4.3 Soil Measurements and Analysis

Running in parallel with the nitrification incubation; the status of the soil's microbial populations was also for the duration of 60 days. Sampling events occurred weekly for the first 30 days, then every 10 days following. Soil analysis was performed on fresh (non-dried) soil as required by the methodologies.

Microbial Biomass

Microbial biomass carbon (MBC) was used to estimate the abundance of the microbial population through the chloroform fumigation and direct extraction technique (Brookes et al., 1985; Voroney et al., 2008; British Standards Institute, 1997). Exposure to chloroform causes the microbial cells to lyse (break apart), releasing the cell contents. Organic carbon is extracted then compared to samples where the cells were intact to produce a measure of MBC.

At each sampling event, the fresh-weight equivalent of 25 g air-dried soil was removed for analysis. This sample was then split, half for immediate extraction of carbon, and half for fumigation prior to extraction.

Fumigation was performed with 25 ml ethanol-free chloroform for 24 hours ± 1 hour. Paper towels saturated with de-ionised water retarded desiccation of the soil which could limit the effectiveness of the chloroform. Organic carbon was extracted from the soil by shaking with 50 ml potassium sulphate (0.5 mol L^{-1}) on a side-to-side shaker for 30 minutes ± 1 minute at 300 revolutions min^{-1}. The extracts were filtered (Whatman No. 42 filter paper) and the carbon detected using a segmented-flow analyser (Burkard Scientific Series 2000, Uxbridge, UK).

Microbial Respiration

The prediction that adding biochar to the soil will increase the activity of the soil's microbes (Hypothesis 2) was tested by measuring the release of carbon dioxide. Microbial respiration

is defined as the evolution of carbon dioxide from microbial metabolic processes and as such is a measure of the activity. Carbon dioxide can originate from a variety of sources (Ritz et al., 2006), the combination of these sources was considered a proxy for microbial respiration.

Microbial respiration was measured indirectly using the Rapid Automated Bacterial Impedance Technique (RABIT: Don Whitley Scientific, Bradford, UK; Figure 4-5) (Ritz et al., 2006). Carbon dioxide released via microbial metabolism was absorbed and ionised to carbonate in an alkaline gel (containing 0.5% potassium hydroxide). The absorbance of the carbon dioxide causes a reduction in the conductance of the gel. Conductivity between two gel-embedded electrodes (Figure 4-6) was monitored every 6 minutes for 960 minutes (16 hours). This decrease over time was measured to 10 µS (micro-siemens) and correlated with absorbed carbon dioxide to calculate the respiration rate.

Figure 4-5: Measurement of carbon dioxide release in progress with the Rapid Automated Bacterial Impedance Technique (RABIT). Each unit contains 32 locations for the sealed containment tubes.

At each sampling event, between 1 and 2 g soil (fresh weight) was loosely filled in a glass boat then sealed within a prepared containment tube (Figure 4-6), which limited the risk of sample desiccation throughout the test.

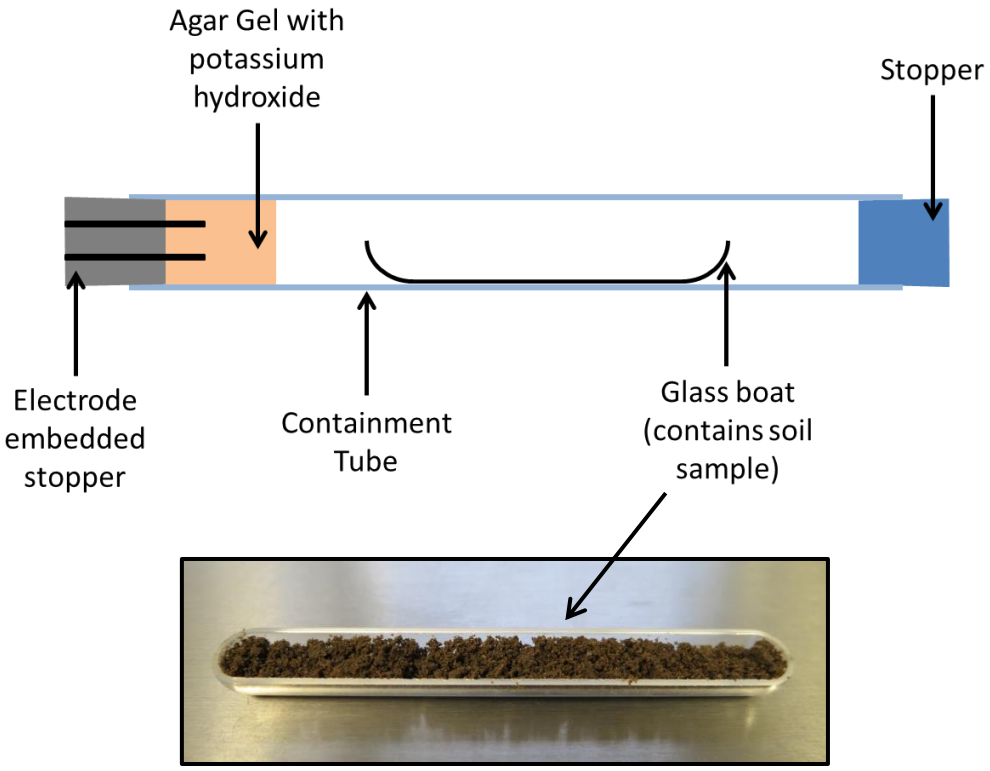


Figure 4-6: Schematic of prepared containment tube for use with the RABIT to measure soil respiration and glass boat filled with 1 – 2 g fresh-weight soil. .

4.4.4 Measurements and Analysis

Microbial biomass carbon was calculated (British Standards Institute, 1997) using:

$$\frac{Mass\ Fumigated\ C_{org} - Mass\ Non\ Fumigated\ C_{org}}{0.45}$$

Where: C_{org} = Organic carbon detected in the potassium sulphate extraction (mg L^{-1}).

Carbon dioxide release was calculated from the reduction in conductance between 120 and 960 minutes. This was to attain the greatest representation. The first 120 minutes were excluded as this included the lag-phase in microbial growth (Butler et al., 2011).

This was calculated using:

$$\left(\frac{\left(\left(\frac{Final\ conductivity - Original\ conductivity}{Time\ (minutes)}\right) \times -1\right) \times 60}{Dry\ Weight\ Soil\ (g)}\right) \times 0.0298$$

Results for both biomass carbon and carbon dioxide release were normalised by multiplying by the total carbon levels of the appropriate soils, 1.654% and 0.887% for the organic and conventional respectively. This is due to the influence soil carbon has on the utilisation and release of carbon by microbes.

4.4.5 Data and Statistical Analysis

Both MBC and carbon dioxide release data were analysed with a repeated measures ANOVA (General Linear Model) using STATISTICA V.12 (Statsoft Ltd, 2013). Statistical significances were determined at $\alpha = 0.05$. For multiple comparisons a post-hoc comparison procedure was necessary to compare individual means (Sokal & Rohlf, 1995). As such a Fisher's least significant difference (LSD) analysis was used. The results of the ANOVAs are provided in the Table S4-2.

Probability plots of residuals were used to determine the normality of the population distributions and anomalous data were occasionally removed prior to analysis, though data were left intact where possible.

4.4.6 Results

Microbial Biomass Carbon

The repeated measures ANOVA revealed that the soil microbial biomass carbon (MBC) was higher in the organically managed soil compared to the conventional ($P < 0.001$; Table S4-2).

Adding 30 and 60 t ha^{-1} biochar to the organically managed soil did not affect the MBC (Figure 4-7), this was true for both soils. There was a significant interaction however between biochar application and SMC in the conventionally managed soil; at 50% field capacity, the addition of 30 and 60 t ha^{-1} biochar increased MBC (91.17 and 90.89 µg g^{-1} [Total Carbon - TC]) ($P = 0.0043$ and 0.0083 respectively) from the control (81.84 µg g^{-1} [TC]). At 25% field capacity the potential for biochar application to reduce MBC was indicated at 30 t ha^{-1} biochar (85.14 µg g^{-1} [TC]; $P = 0.0027$) compare to the control (95.29 µg g^{-1} [TC]). Although the MBC at 60 t ha^{-1} was lower (90.61 µg g^{-1} [TC]) compared to the control this was not significant ($P = 0.1$).

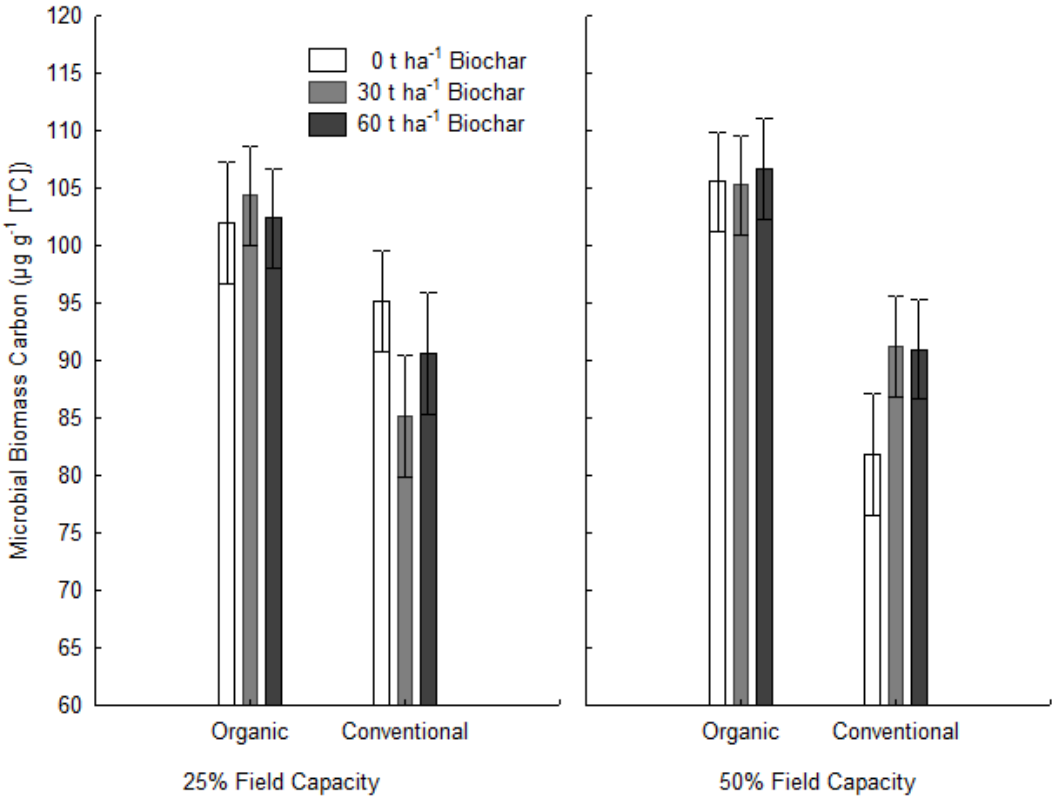


Figure 4-7: Interaction between soil management, biochar application rate and soil moisture content over the 60 day incubation. Bars are standard errors ($N = 3$). TC: Total Carbon

Microbial biomass carbon decreased over the incubation period though fluctuations were observed (Figure 4-8). Despite apparent potential differences in MBC with the application of biochar, as shown in Figure 4-7, the effects of biochar are less distinct over time, with inconsistent significant differences and large variability (Figure 4-8).

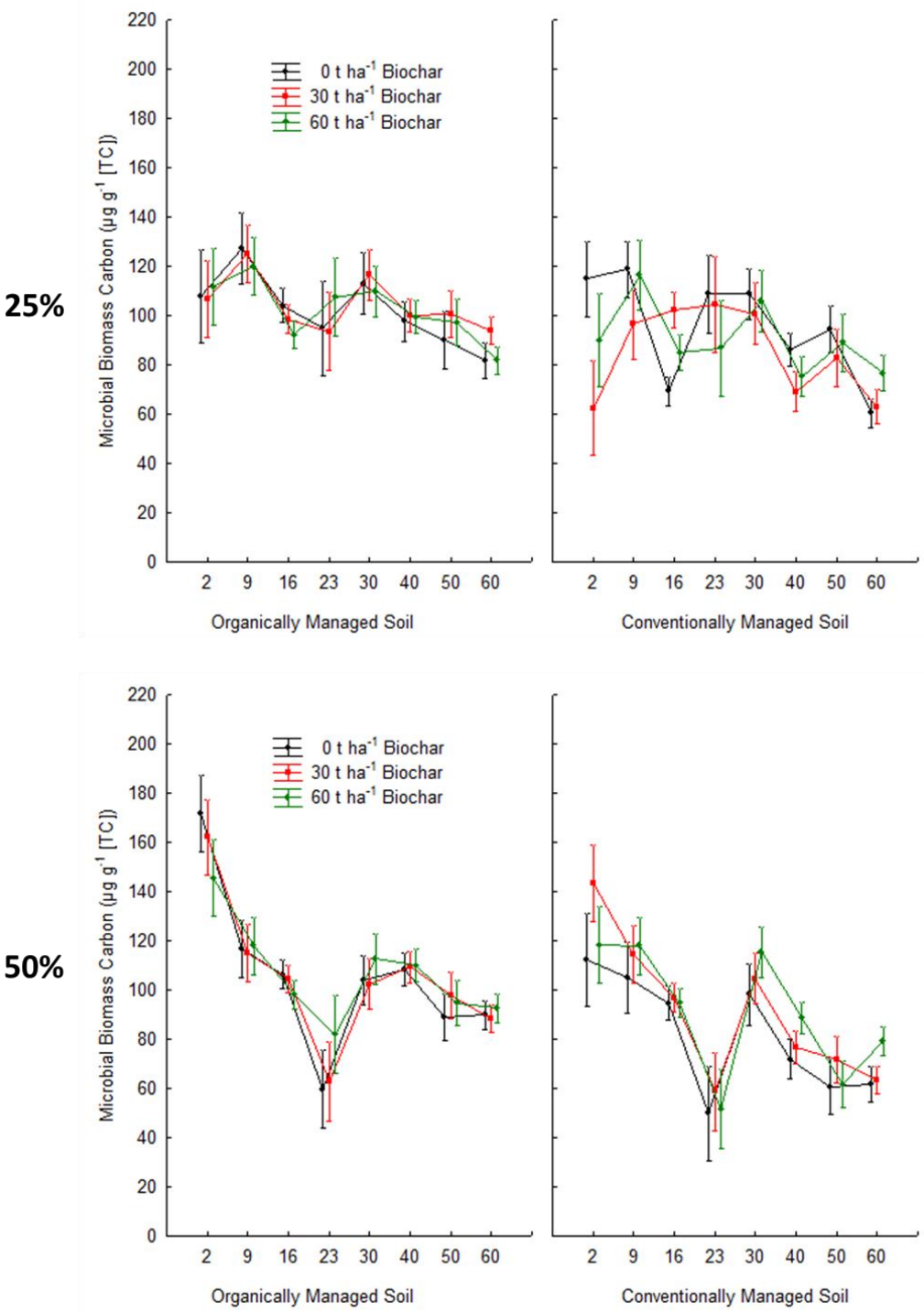


Figure 4-8: Microbial biomass carbon over the 60 day incubation at 25% and 50% field capacity. Bars are standard errors (*N* = 3). TC: Total Carbon

Carbon Dioxide Release

A greater release of carbon dioxide was shown in the organically managed soil compared to the conventional as confirmed by the repeated measures ANOVA (Table S4-2; $P < 0.001$). Carbon dioxide release was also higher at a SMC of 25% field capacity compared to 50% ($P < 0.001$).

The repeated measures ANOVA indicated that the addition of biochar affected the release of carbon dioxide from the soil ($P < 0.001$). Although an interaction between biochar and soil management only approached significance ($P = 0.084$; Table S4-2), the Fisher's test of LSD showed that in the organically managed soil there was a significant increase in carbon dioxide release with 60 t ha^{-1} biochar compared to the control ($P < 0.001$) from 58.7 µg g $[TC]^{-1}$ d^{-1} to 92.2. The application of 30 t ha^{-1} biochar had no effect on the carbon dioxide release compared to the control ($P > 0.05$; Figure 4-9). There was no effect of biochar on carbon dioxide release in the conventionally managed soil.

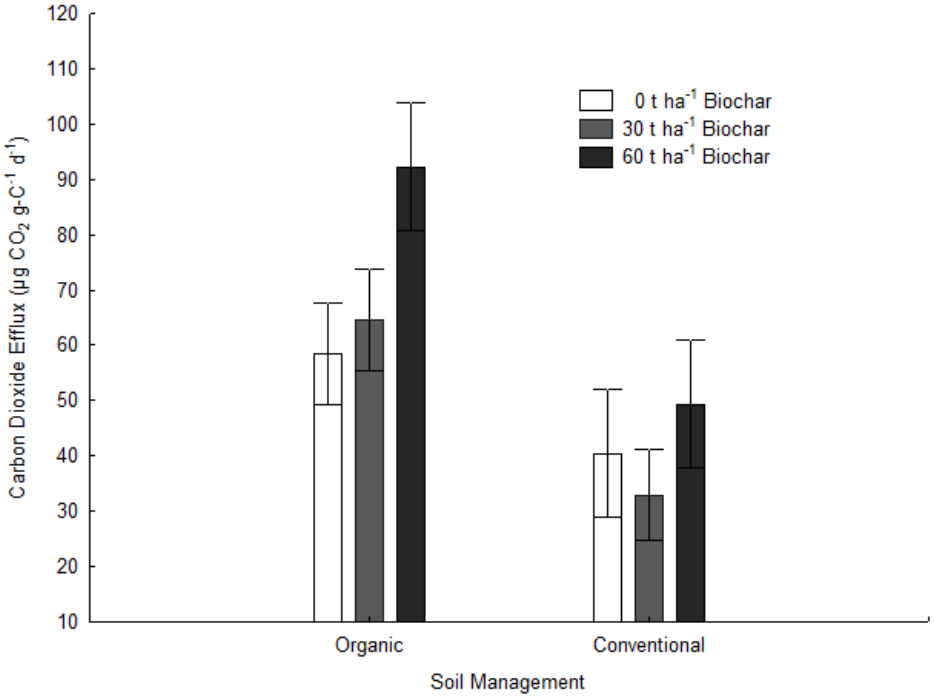


Figure 4-9: Interaction between soil management and biochar application rate. Bars are standard errors ($N = 3$).

As with microbial biomass, the release of carbon dioxide decreases over the incubation. Figure 4-10 shows that the effect of biochar on carbon dioxide release is inconsistent throughout the incubation experiment due to the high variability in the data.

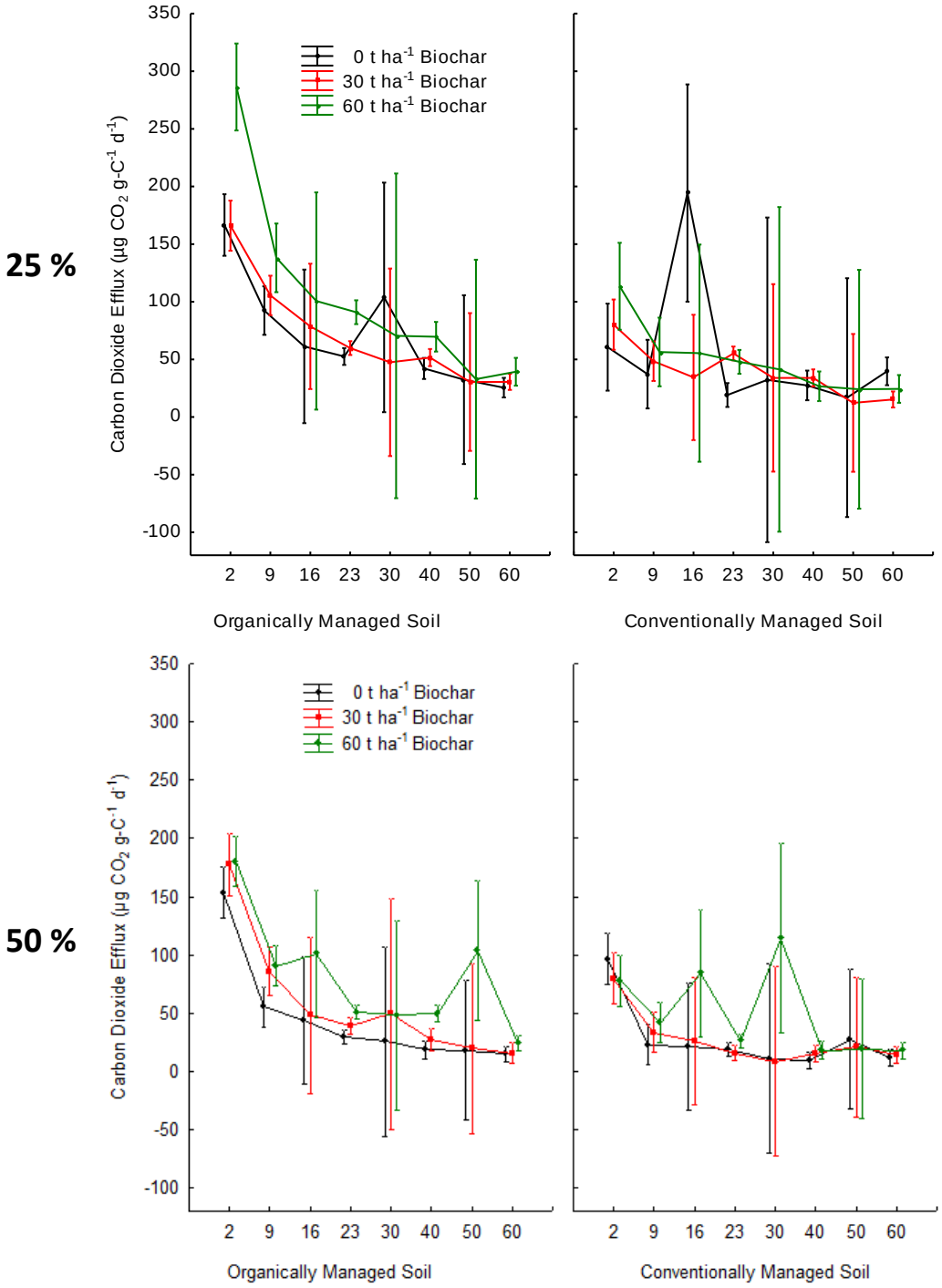


Figure 4-10: Carbon dioxide release over the 60 day incubation period at soil moisture contents of 25 and 50% field capacities. Bars are standard errors (N = 3).

4.4.7 Section Discussion

Higher levels of MBC and carbon dioxide release were observed in the organically managed soils compared to the conventional. Greater levels of both labile carbon and soil moisture within the organically managed soil could have attributed to the greater production of carbon dioxide and MBC. Organic management has been shown to sustain higher organic carbon levels compared to conventional soil management, and the higher microbial activity is indicative of the greater addition of labile carbon, stimulating microbial populations (Ge et al., 2010). A review of organic and conventional farming on biodiversity (Hole et al., 2005) also concluded in a tendency for organically managed soils to have higher abundance of microbial (bacterial and fungal) communities. This was cited to be due to the higher input of organic carbon from animal and green-wastes to the soil.

Organic matter is a complex mixture with a range of structural and functional groups (Christensen, 2001). The soil contains a community of micro-organisms with many populations of microbial species. Soil respiration is a common measure of microbial activity (Anderson & Domsch, 1990) but measures carbon dioxide from a variety of sources and does not specify the activity of the nitrifying bacteria. As such this method did not take into account potential differences between microbial community structures of the management systems, however it has been suggested that differences in nitrifying bacteria between organic and conventional systems are not consistently different (Kong et al., 2010). Hole et al. (2005) also suggested that apart from the increase in abundance, the difference between microbial activities of organically and conventionally managed systems was limited.

A summary of the effects of biochar application on the microbial activity in the conventionally and organically managed soils can be found in Table 4-1.

Table 4-1: Impact of biochar addition on microbial biomass carbon and carbon dioxide production, a summary of effects. N.S: Not significant

Soil Management	Effect of biochar on Microbial Biomass	Effect of biochar on CO_2 Evolution
Organic	N.S	Increased
Conventional	Increased	N.S

Results indicated that biochar did not affect the abundance of soil microbes of the organically managed soil, but did in the conventionally managed soil. With a higher initial MBC, an increase exhibited in the organic soil with biochar addition may be less observable than the conventionally managed soil, which already exhibited a lower MBC.

Under the conventional management, the addition of biochar increased MBC at 50% field capacity but appeared to decrease at 25%. This could be attributed to fractions of labile carbon in the biochar (Cross & Sohi, 2011) impacting more on the conventional system which contains less organic carbon sources, thus the limited observation of this effect in the organically managed soil. This is only seen at 50% field capacity, which is more favourable for microbial activity. The data at 25% field capacity is far less conclusive as the effect is only seen at 30 t ha^{-1} biochar.

Conversely however, increasing the addition of biochar raised the rate of carbon dioxide evolution from the organically managed soil but this effect was not seen in the conventionally managed soil. Whether the addition of biochar or similar substances can produce a priming effect for the decomposition of native organic matter (NOM) (Wardle et al., 2008) in the soil remains contentious (Cross & Sohi, 2011). Other mechanisms have been postulated for greater microbial activity with biochar additions, such as the volatile contents acting as a stimulant (Lehmann et al., 2011).

It has been observed that in soils with higher C:N ratios, or at the addition of such a material, soil microbes utilise the nitrogen present and release the excess carbon as carbon dioxide. This process continues until a state of C:N equilibrium exists (Hue & Sobieszczyk, 1999). The C:N ratio that this occurs at is lower in environments where the substrate contains easily mineralisable (labile) carbon (Hue & Sobieszczyk, 1999). The application of labile carbon in the form of fresh compost could indicate why the effect of higher carbon dioxide release was observed in the organically managed soil but not the conventional.

As noted with the WRC, addition of biochar amendments increased the soils' moisture content. This can be detrimental to microbial activity in soils that have large inherent moisture contents because of reductions in aeration (Case et al., 2012) though in sandy soils such as these, the increase could be beneficial for microbes resulting in more carbon dioxide evolution.

Some of the perceived increase with 60 t ha^{-1} could be attributed to large spikes in carbon dioxide release e.g. at 30 days under conventional management at 25% field capacity (Figure 4-10) and thus these increases are not necessarily indicative of a consistent increase of microbial activity with the addition of biochar. The large variability in carbon dioxide release could be attributed to the range of respiring soil microbes as RABIT does not differentiate between sources of carbon dioxide (Ritz et al., 2006).

4.4.8 Section Conclusions

It was concluded that the addition of biochar affected the microbial activity of the organically and conventionally managed soils differently by increasing carbon dioxide production in the organically managed soil, but not effecting MBC; but increasing MBC in the conventionally managed soil and not affecting respiration (Table 4-1). These effects were attributed to same mechanism: the impact of carbon substrates and resultant changes in C:N ratios and the type of carbon present.

Carbon is a substrate for the growth for soil microbes and a source of energy. In higher C:N environments, microbes utilise and immobilise the nitrogen present and release excess carbon as carbon dioxide (Hue & Sobieszczyk, 1999). With a higher inherent level of organic carbon in the organically managed soil (1.54%) compared to the conventional (0.83%), the addition of further labile carbon from the biochar (but little nitrogen) could result in the excess being released as carbon dioxide. In comparison, the more carbon limiting environment of the conventionally managed soil resulted in less excess carbon and thus no increased release of carbon dioxide. Instead the carbon was utilised by the microbes resulting in the increased MBC.

The activity of the microbes did not correspond with the data from the nitrification incubation however. This could be due to the non-specificity of the respiration measurements, which detected overall soil carbon dioxide release and not directly from the nitrifying bacteria, thus increasing the error in the data.

4.5 Dicyandiamide Incubation

4.5.1 Background

Dicyandiamide (DCD) is a solid crystalline nitrification inhibitor. There are several types of nitrification inhibitors currently on the market including nitrapyrin, ammonium thiosulfate (ATS), and 3,4-dimethylpyrazole phosphate (DMPP). All of these are used to retard or halt the activity of *Nitrosomonas* bacteria and thus the conversion of ammonium to nitrite (Figure 4-11; Camberato, 2001). Nitrite is quickly converted into nitrate, which has higher mobility in the soil than ammonium; using nitrification inhibitors to prolong the residence time of ammoniacal-nitrogen can therefore reduce losses of nitrogen as nitrate, through mechanisms such as reduced leaching or release of nitrous oxides gases (Dennis et al., 2010).

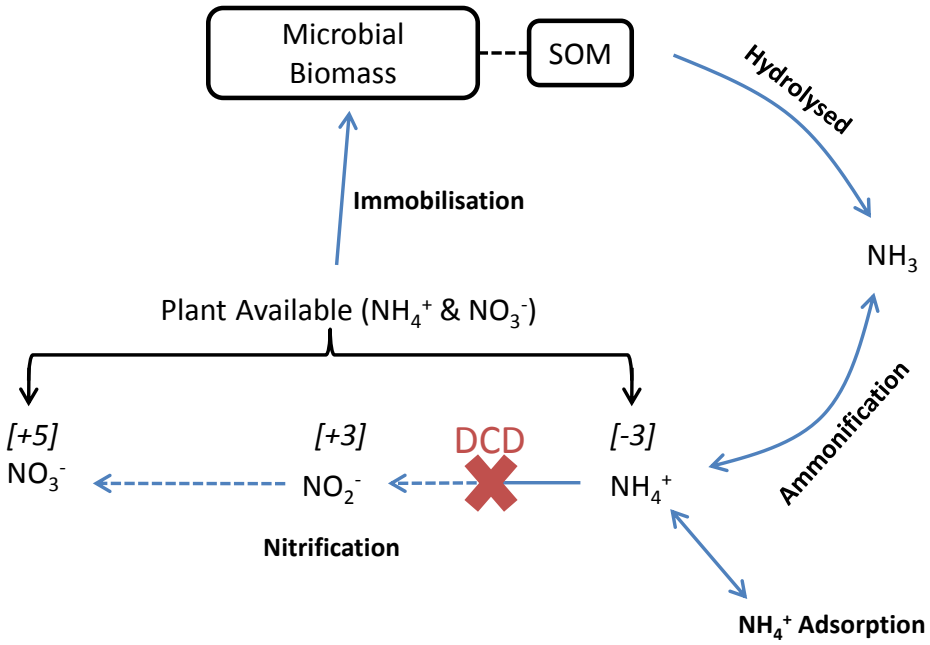


Figure 4-11: A representation of how the application of the nitrification inhibitor DCD impacts soil nitrification.

Nitrosomonous bacteria release ammonia oxygenase enzymes which convert ammonium into nitrite. DCD functions by blocking the enzyme's active site where the conversion takes place (Di et al., 2009).

Within the incubation setting, DCD was used to gain a measure of ammonium production by preventing the conversion of ammonium to nitrate.

Ammonification is the production of ammonium from more complex organic sources of nitrogen. The production of ammonium therefore impacts on the nitrification process. As the level of ammonium in the nitrification incubation (**Chapter 4.3**) was affected by the

conversion to nitrate therefore it could not be concluded whether biochar was impacting on the nitrification rate or the initial level of ammonium, thus reducing substrate availability.

The procedure for incubation setup and sampling events was identical to previous incubations outlined in **Chapter 4.2: Incubation Methodology**. In addition to this procedure however, DCD was applied at a rate of 15% of the total nitrogen (McGeough et al., 2012). As DCD degrades over time (Kelliher et al., 2008), a second application was made after 30 days to maintain the inhibition of ammonia oxygenase.

4.5.2 Hypotheses

Adding DCD to the soil will halt the conversion of ammonium to nitrite, thus ammonium concentrations will increase over time while the inhibitor is viable and showing differences in ammonium productions without the reducing effect of nitrification.

It is hypothesised that with the addition of biochar, the rate of ammonium production will alter according to the type of nitrogen source applied. This hypothesis states therefore that the addition of biochar will increase ammonium production in the organically managed soil, but lower it in the conventional as a result of the inherent and added SOM levels. These will be attributed by the respective increase and decrease in the production of carbon dioxide.

4.5.3 Soil Measurements and Analysis

Ammonification was measured by detecting the accumulation of ammonium throughout the incubation. With the addition of DCD, the production of nitrate should be minimal, though this was measured also, to confirm this. As with nitrification, extraction of mineral nitrogen compounds was using a 2 mol L^{-1} potassium chloride (KCl) solution (MAFF, 1986). A segmented flow analyser (Burkard Scientific Series 2000, Uxbridge, UK) was used to detect levels of ammonium; total oxides of nitrogen (TON: nitrate + nitrite); and nitrite. The difference between TON and nitrite determined the nitrate levels.

The activity of the microbial population with the addition of DCD was measured through carbon dioxide release using RABIT as detailed in **Chapter 4.4.3: Soil Measurements and Analysis**.

Periodically throughout the incubation, 10 g ± 0.05 wet soil was removed for extractable nitrogen analysis (MAFF, 1986). Of the 12 sampling events, sampling was more frequent during the first 30 days, when most of the nitrogen changes were anticipated. Less frequently

(7 sampling events), between 1 and 2 g fresh-soil was removed for measuring basal respiration via carbon dioxide release.

4.5.4 Data and Statistical Analysis

Changes in ammonium, nitrate and carbon dioxide release were analysed individually using a repeated measures ANOVA using STATISTICA V.12 (Statsoft Ltd, 2013). Statistical significance level was determined with $\alpha = 0.05$. For multiple comparisons, a Fisher's least significant difference (LSD) analysis was used to compare individual means (Sokal & Rohlf, 1995). The results of the ANOVAs are provided in Table S4-3.

Probability plots of residuals were used to determine the normality of the population distributions and anomalous data were occasionally removed prior to analysis, though data were left intact where possible.

4.5.5 Results

Ammonification appears to be occurring throughout the incubation, this is highlighted by an increase in ammonium levels over time (Figure 4-12) and no overall change in nitrate from start to finish. Although the ANOVA indicates that there is a difference in nitrate concentrations over time ($P < 0.001$; Table S4-3), this is due to the fluctuations throughout the incubation between 2 and 7.5 µg kg^{-1} dry soil (Figure 4-13).

Ammonium release (Figure 4-12) is higher in the conventionally managed soil compared to the organic ($P < 0.001$) and greater at the higher SMC of 50% compared to 25% for both soil management systems ($P < 0.001$; Table S4-3).

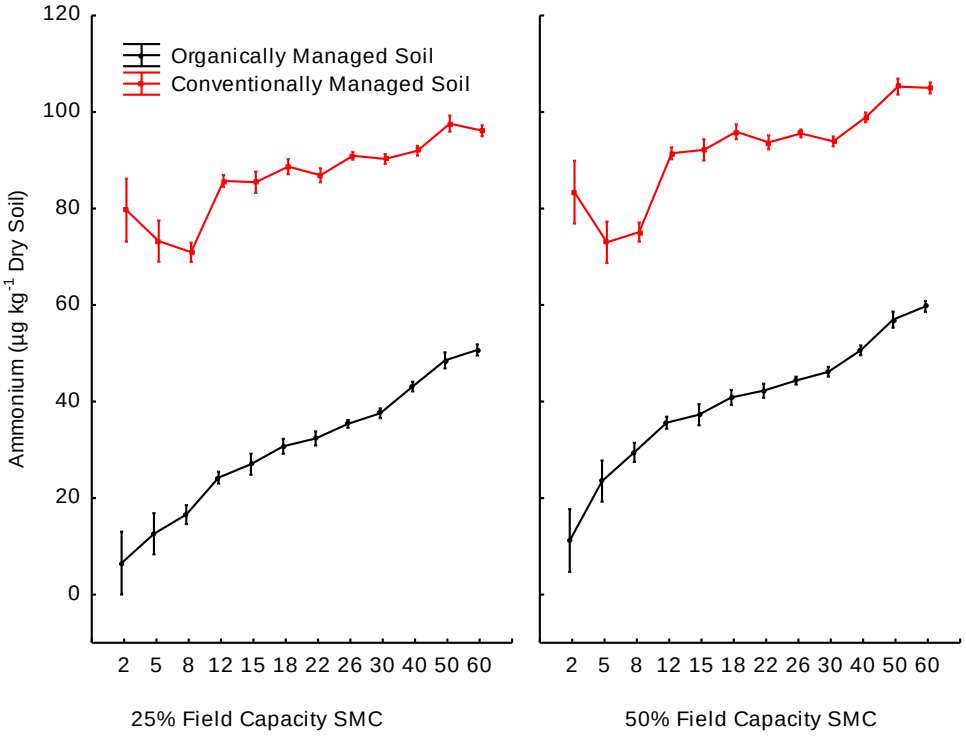


Figure 4-12: Ammonium concentration at 25 and 50% field capacity under organic and conventional soil management. Bars are standard errors ($N = 3$).

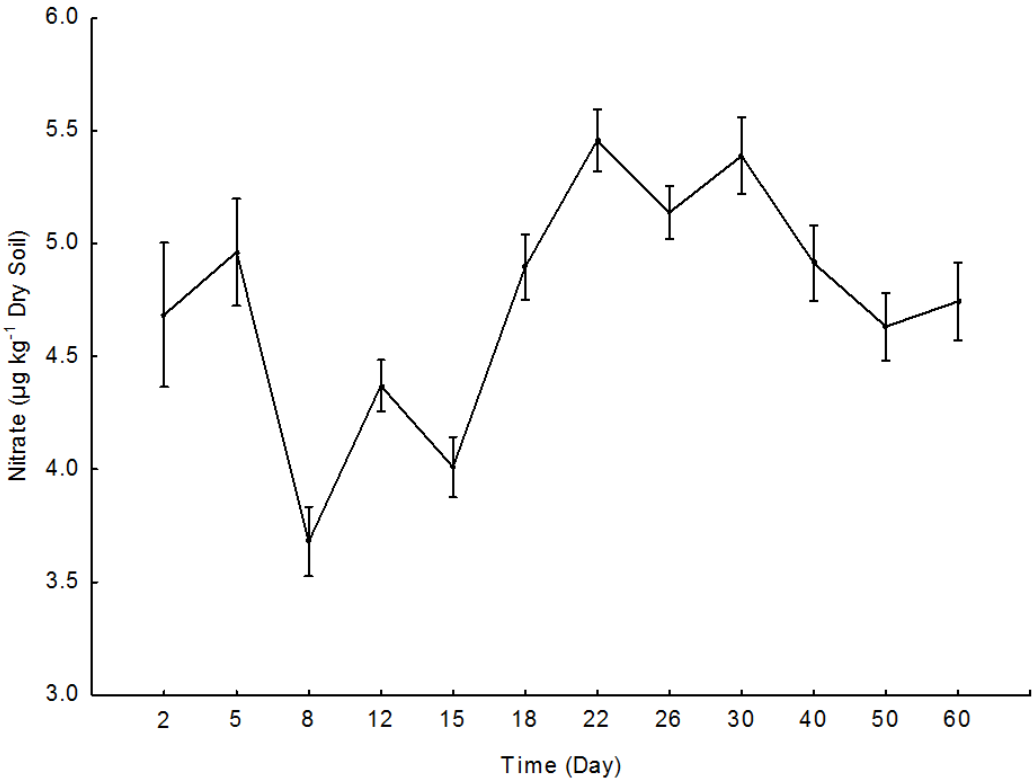


Figure 4-13: Change in nitrate concentrations over the 60 day DCD incubation. Bars are standard errors (N = 3).

Ammonium concentrations decreased with an increase in biochar application rate, this decrease became more prominent as the incubation progressed and was also more observable at the higher SMC of 50% field capacity where the addition of 30 and 60 t ha^{-1} biochar progressed from having no effect on ammonium (11.66 and 10.96 µg kg^{-1} dry soil respectively compared to control of 10.96 µg kg^{-1} dry soil) to showing decreases from 66.9 to 59.7 and 52.4 µg kg^{-1} dry soil (Figure 4-14).

A reduction in nitrate levels with the addition of biochar was also shown ($P < 0.001$; Table S4-3). These decreases were less consistent and showed more variability (Figure 4-14), there was no significant difference between nitrate concentrations with the biochar application rates.

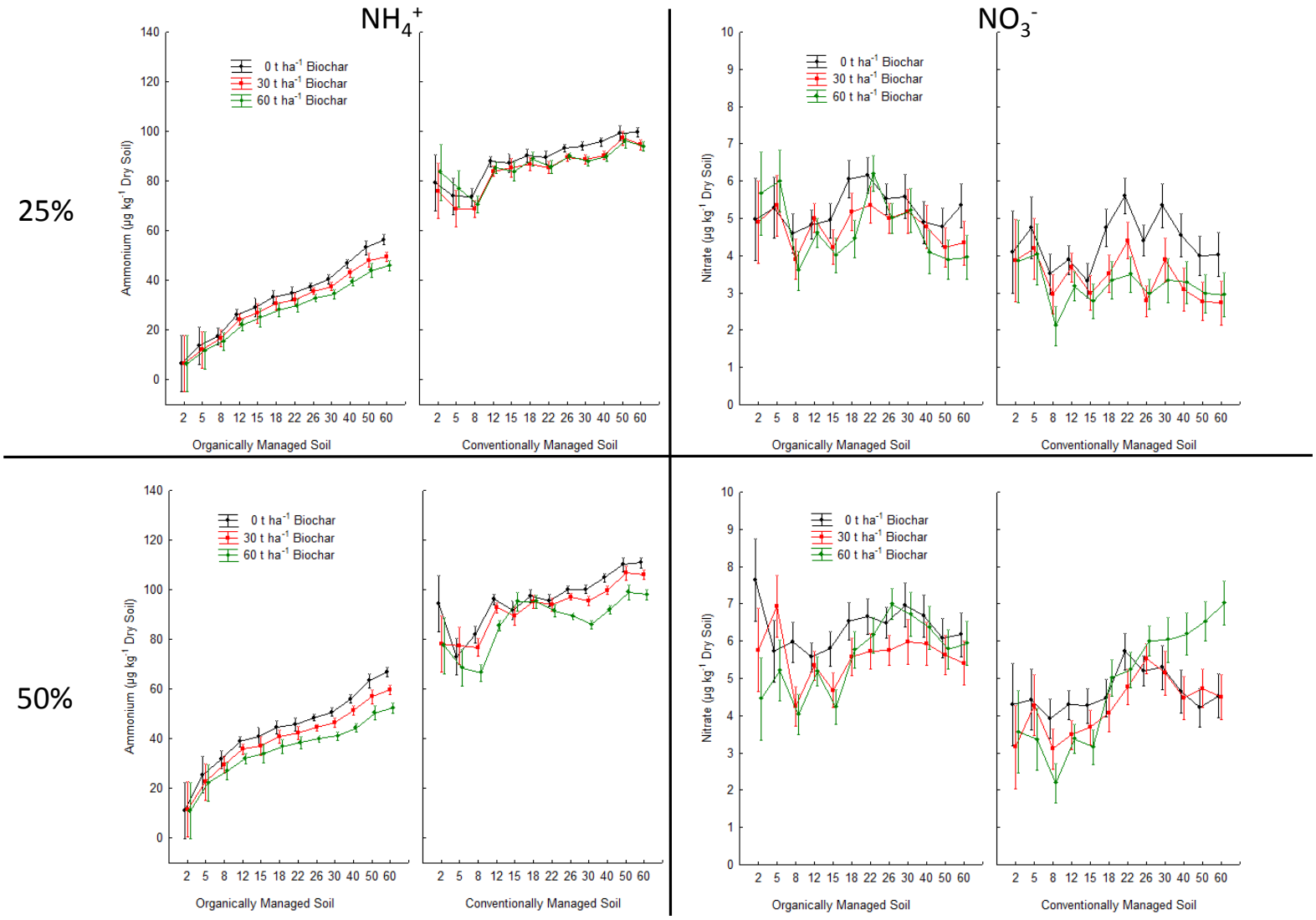


Figure 4-14: Soil ammonium and nitrate levels over 60 incubation with the addition of Dicyandiamide (DCD) nitrification inhibitor. Bars are standard errors (N = 3).

There were inconsistent increases in carbon dioxide release with the addition of biochar. These changes did not appear to be related to time or application rate of the biochar (Figure 4-15). There was a general trend for a lowering of carbon dioxide over the incubation ($P < 0.001$; Table S4-3).

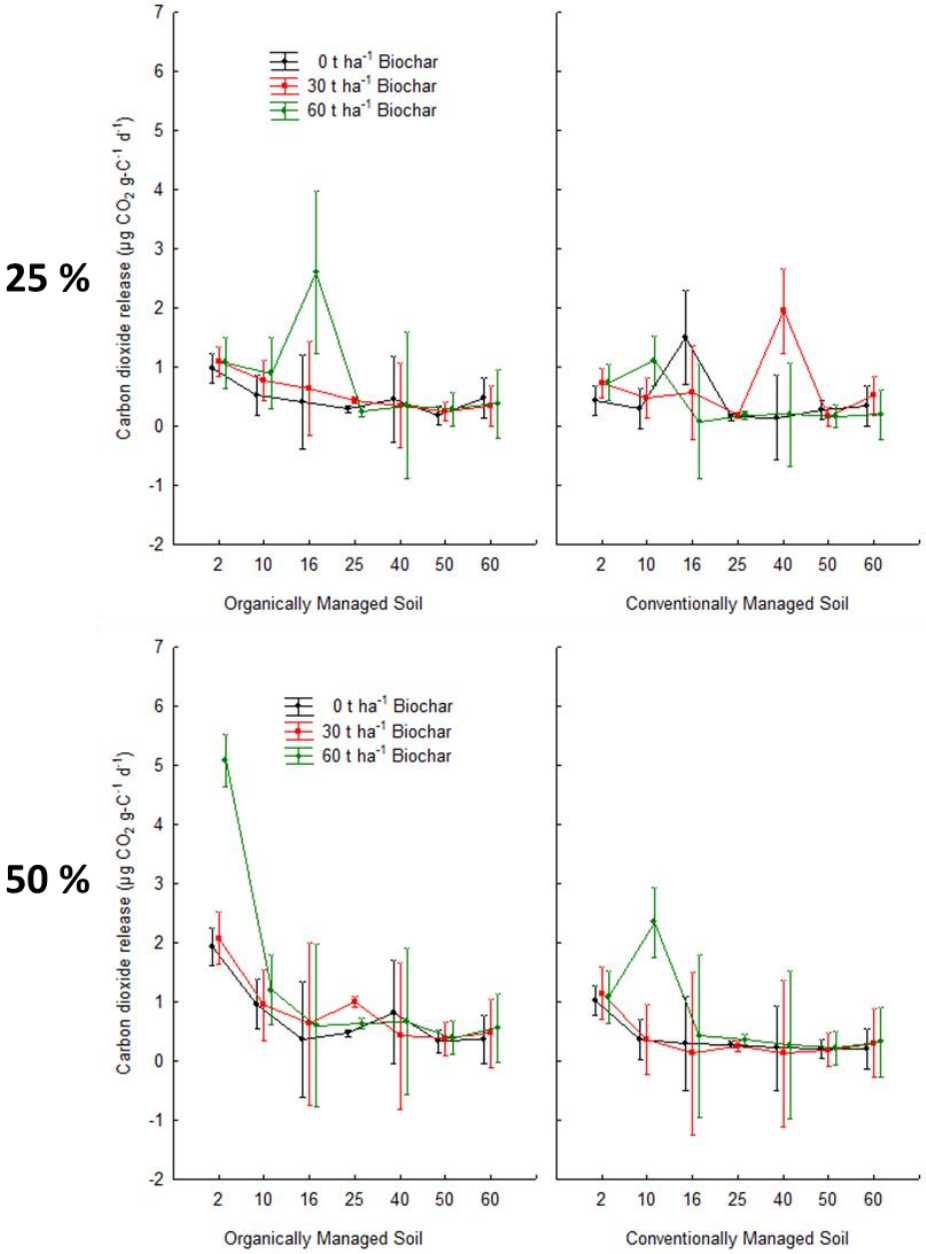


Figure 4-15: Carbon dioxide release over the 60 day DCD incubation. Bars are standard errors ($N = 3$).

4.5.6 Section Discussion

Previous research has suggested that DCD can be used to inhibit nitrification in agricultural soils (Camberato, 2001a). DCD was selected for this study for its high solubility in water and lower volatility than alternatives like nitrapyrin (Di & Cameron, 2002). DCD is bacteriostatic; the compound inhibits the bacterial conversion of ammonium but maintains cellular viability (Kelliher et al., 2008), which could otherwise release cell contents and influence available nutrient pools in the soil.

Disadvantages associated with DCD include a low residence time; degradation can occur within 30 days of application (Kelliher et al., 2008; Camberato, 2001a). This was mitigated by a second application of DCD after 30 days. It has also been reported that DCD has high mobility in the soil and can be easily leached; this was not an issue in the incubation as water was not leached through the soil.

DCD appeared to be an effective inhibitor throughout the incubation due to the progressive increase in ammonium levels and no temporal change in nitrate levels. This is suggested to be due to the DCD blocking the enzyme's active site. Fluctuations in the nitrate levels were noted, though there was no overall increase over time (Figure 4-13) (contrary to the repeated measures ANOVA – $P < 0.001$; Table S4-3) to suggest any degradation of DCD to the extent that the *Nitrosomonas* bacteria regained functionality.

The agricultural management, particularly the organic matter inputs, affects the pools of available nutrients. Burger & Jackson (2003) found that when comparing organic and conventional systems, the organically managed soil shower higher gross ammonification rates. A review by Booth et al. (2005) showed that the ammonification rate was positively correlated with both the soil's nitrogen and carbon content, implying the importance of substrate quantity on ammonification.

Given the positive relationship between ammonification rate with the soil's total carbon and nitrogen, it could be expected that the organically managed soil would show a greater release of ammonium over the incubation. The results however, show higher ammonification in the conventionally managed soil. This is likely a result of the immediate application of the ammoniacal nitrogen to the conventionally managed soil compared with the much slower release of the GWC.

Results show that ammonium concentration was higher at 50% field capacity compared to 25%. This was true for both soil management types (Figure 4-12). As ammonification is the mineralisation of organic matter by microbes to produce ammonium, the rate of ammonification is affected by the SMC. Rates of gross ammonification have been shown to be greater as the water potential increases towards field capacity (Chen et al., 2011). It is suggested that the lower SMC at 25% field capacity caused greater desiccation of and inhibition of microbial functioning more than the soils at 50% field capacity.

It was hypothesised that the addition of biochar to the DCD amended soil would affect the ammonium concentrations of the two soils differently. This was shown to be false, as under both management systems, the application of biochar reduced the concentration of ammonium (Figure 4-13). Due to the presence of DCD, this change is not attributed to the conversion to nitrate.

Although this lower concentration could be attributed to a decrease in ammonification rate, as shown in Figure 4-11, there is an alternative mechanism that could also have affected the ammonium levels: surface adsorption. It is not possible, from this experiment alone, to differentiate between mineralisation rate and a potential increase in adsorption to the biochar surface. Indeed, during a 14 day incubation, Gundale & DeLuca (2006) postulated that their observed decrease in ammonification with the addition of a wood biochar (2% by mass) was attributed to the increased adsorption.

To indicate whether it was mineralisation or surface adsorption that influenced the reduction in ammonium levels, the microbial activity was measured through carbon dioxide production (Ritz et al., 2006), which was expected to correspond with mineralisation.

It has been reported that the mineralisation of organic matter by the soil microbial biomass can be slow and is not comprehensively controlled by their activity or abundance (Kemmitt et al., 2008) and that a significant proportion of mineralisation can be influenced by abiotic processes. Paterson et al. (2009) discussed that the type of carbon and nitrogen is also influential. It was suggested that in the presence of labile high C:N sources (such as those found in freshly-applied compost and biochar - (Smith et al., 2010)), mineralisation of SOM by microbes is dominant compared to soils limited in labile substrates.

However, there was limited increase in carbon dioxide release with the addition of biochar. The high variability in the data measured by the RABIT leads to the necessity of caution

when interpreting the results; it cannot be concluded whether biochar is having an effect on microbial respiration though the inconsistency indicates that this is not the case. There is a need to reduce variability to ensure that the correct inferences are made from the data and that a type II error is not being made.

Indications are that microbial-induced ammonification is an unlikely mechanism for the decrease in ammonium levels, as hypothesised, and a more likely mechanism is the adsorption of ammonium to the biochar's surface. This will be tested in **Chapter 4.6: Ammonium Adsorption**.

There was also a reduction in nitrate levels with the addition of biochar. Although there have been suggestions that biochar can have an adsorptive capacity for anions such as phosphate (Collison et al., 2009; Verheijen et al., 2010) and thus could hold on to and reduce the nitrate concentrations in the soil, it is proposed that in this case the more likely cause of the reduction in nitrate with biochar addition is a result of the lower ammonium levels and thus reduction in substrate for the limited viable nitrifying bacteria population.

The accumulation of ammonium in the soil could be affected by losses through ammonia volatilisation. The volatilisation of ammonia is dependent upon substrate availability and soil pH (Chen et al., 2012). Indeed, a positive relationship has been described between ammonia volatilisation with soil ammonium concentration and soil pH (Rochette et al., 2013). Additionally in a field experiment that examined ammonia volatilisation following fertiliser application, it was stated that a low pH and high CEC might be key features that discourage losses in ammonia from soils (Hayashi et al., 2011). The increase in pH and potential CEC with the biochar could account for at least part of the decrease in ammonium though how much of this effect is influencing the ammonium concentration in the soil cannot be quantified by the experiment.

The decrease in ammonium concentrations in the DCD incubation highlight that the addition of biochar could impact subsequent nitrification rate and thus nitrogen availability to plants. It is suggested therefore that the changes observed in the nitrification incubation could be influenced by in this way by the biochar. It is indicated that the reduction in ammonium for the conventionally managed soil is primarily driven by nitrification due to the increase in nitrate, but some of this decrease could be due to the changes caused by the adsorption observed in the DCD experiment and that this difference is not substantial enough to counteract the nitrification process.

4.5.7 Section Conclusions

DCD was an effective inhibitor to nitrification and the two applications appeared to remain functional throughout the 60 day incubation.

As the incubation progressed, increasing biochar application rate reduced concentrations of ammonium for both soil management systems, thus falsifying the hypothesis suggested. It cannot be ascertained whether this is due to the adsorption of the ammonium to the biochar surface or a decrease in ammonium production from organic matter. Due to the limited effect of biochar application and soil respiration, it is indicated that mineralisation was not a predominant factor in the changes of ammonium production, which would be complemented by lower microbial activity with the addition of biochar. The respiration data must be treated with some caution, the high variability of the data may be masking any effect of treatment. Therefore efforts to reduce this variability, such as increasing sample size and repetitions could be considered to improve confidence that a type II error is not taking place.

With the potential for ammonium volatilisation at higher concentrations and increased pH, future work could include this, as this may be a contributing factor in the decrease of ammonium.

4.6 Ammonium Adsorption

4.6.1 Background

We have observed that the concentration of ammonium is lower with the application of biochar, and that the indication was that this was not a product of the microbial activity. As such, it is important to test whether the adsorption capacity of the biochar was affecting ammonium availability that may be causal in affecting nitrification rate.

From Figure 4-1, we can see that the holding of ammonium to the soil's surface is one potential pathway of the mineralised nitrogen. Soils with higher adsorption capacities measured through the cation exchange capacity (CEC), show reduced nitrogen mobility through leaching, but could also reduce uptake.

The cation exchange capacity of a soil is influential on the uptake of cationic nitrogen and the rate of movement in the soil; as the ability of a soil to hold onto an ion increases, mobility decreases. Fresh biochars can have a varied CEC (Lehmann et al., 2011), though with a higher CEC of the biochar used in the current study (66.33 ± 1.72 cmol+ kg^{-1}), compared to the initial CEC of the organically and conventionally managed soils (33.45 ± 0.58 and 23.60 ± 0.65 cmol+ kg^{-1} respectively), it could be proposed that the addition of biochar could increase the CEC of the soils reducing the potential for nitrifying bacteria utilisation and may have impacted on the reduction of ammonium observed in the nitrification incubation.

Increased holding capacity of ammonium can provide a useful mechanism for reducing nitrogen losses in field systems, however can be unfavourable if ions are held too strongly and lack the necessary mobility for uptake. This fixation and immobilisation of ammonium in the soil, in a manner that results in that they are un-exchangeable by cation exchange is known as non-exchangeable ammonium (NEA) (Nieder et al., 2010).

4.6.2 Hypotheses

1. It is hypothesised that, due to the high CEC of the biochar (66.33 ± 1.72 cmol+ kg^{-1}), increasing the application rate into the soils will increase the overall CEC also. It is predicted that the CEC will increase over the 90 day incubation due to oxidation of the biochar's surface.

2. The increase in CEC with biochar application and over time will result in a reduced ability of ammonium extraction and thus higher non-exchangeable ammonium levels(NEA).

4.6.3 Soil Measurements and Analysis

Sampling procedures followed that of the previous incubations: periodic removal of adequate soil for analysis over the experimental phase. The incubation lasted 90 days; the longer duration was used as changes were predicted to be slower than the biologically mediated changes associated with nitrification. Further preparation prior to the analysis is mentioned as required.

The CEC was measured on approximately 5 g soil (air-dried at 40˚ C) by ammonium acetate displacement method (MAFF RB427, 1986; Yuan et al., 2011; Gaskin et al., 2008) as discussed in **Chapter 2.1.2: Biochar Analysis**.

Non-exchangeable ammonium (NEA) was measured by the Potassium hypobromite-Dry Soil Combustion method, amended from the Silva-Bremner method, replacing the need for hydrofluoric acid with dry combustion (Nieder et al., 2010).

Soil was treated (0.5 g air-dried and finely ground) with 10 ml Potassium hypobromite (KOBr) and boiled for 10 minutes to oxidise organic compounds present (Nieder et al., 2010). Successive shaking with 30 ml KCl (0.5 mol L^{-1}) and centrifugation removed exchangeable ammonium ions and the supernatant was discarded, leaving the NEA in the residue.

Dry combustion of the residue using catalytic tube combustion (Vario EL III, CHNOS elemental analyser, Hanau, Germany, British Standards Institute, 1995) directly measures the remaining nitrogen in the soil.

Calculation of the NEA was using the formula:

$$NEA\ (mg\ kg^{-1}\ Original\ Soil) = \frac{Z \times 10 \times Y}{m}$$

Z = Total nitrogen in residue (%)

Y = Mass dry residue (g)

m = Mass original soil (g)

4.6.4 Data and Statistical Analysis

Changes in the CEC and NEA levels over the incubation period were analysed with a repeated measures ANOVA (General Linear Model) using STATISTICA V.12 (Statsoft Ltd, 2013).

Statistical significance level was determined with $\alpha = 0.05$. For multiple comparisons, a Fisher's least significant difference (LSD) analysis was used to compare individual means (Sokal & Rohlf, 1995). The results of the ANOVAs are provided in Table S4-4.

Probability plots of residuals were used to determine the normality of the population distributions and anomalous data were occasionally removed prior to analysis, though data were left intact where possible.

To determine whether CEC changed over the incubation period, a Pearson's product moment correlation coefficient was conducted using R (R Core Team, 2013).

4.6.5 Results

There was a significant ($P < 0.001$; Table S4-4) effect of CEC over the 90 days. However, Figure 4-16 shows large fluctuations in CEC throughout the incubation but no overall correlation between CEC and time which was confirmed with a Pearson's product moment correlation coefficient of 0.05 and $P = 0.4$, thus indicating that there was no significant relationship of CEC within 90 days of application.

CEC was higher in the organically managed soil throughout the incubation study ($P < 0.001$). This was supported by the repeated measures ANOVA. The addition of the biochar during the incubation however showed no impact on the total CEC (Table S4-4).

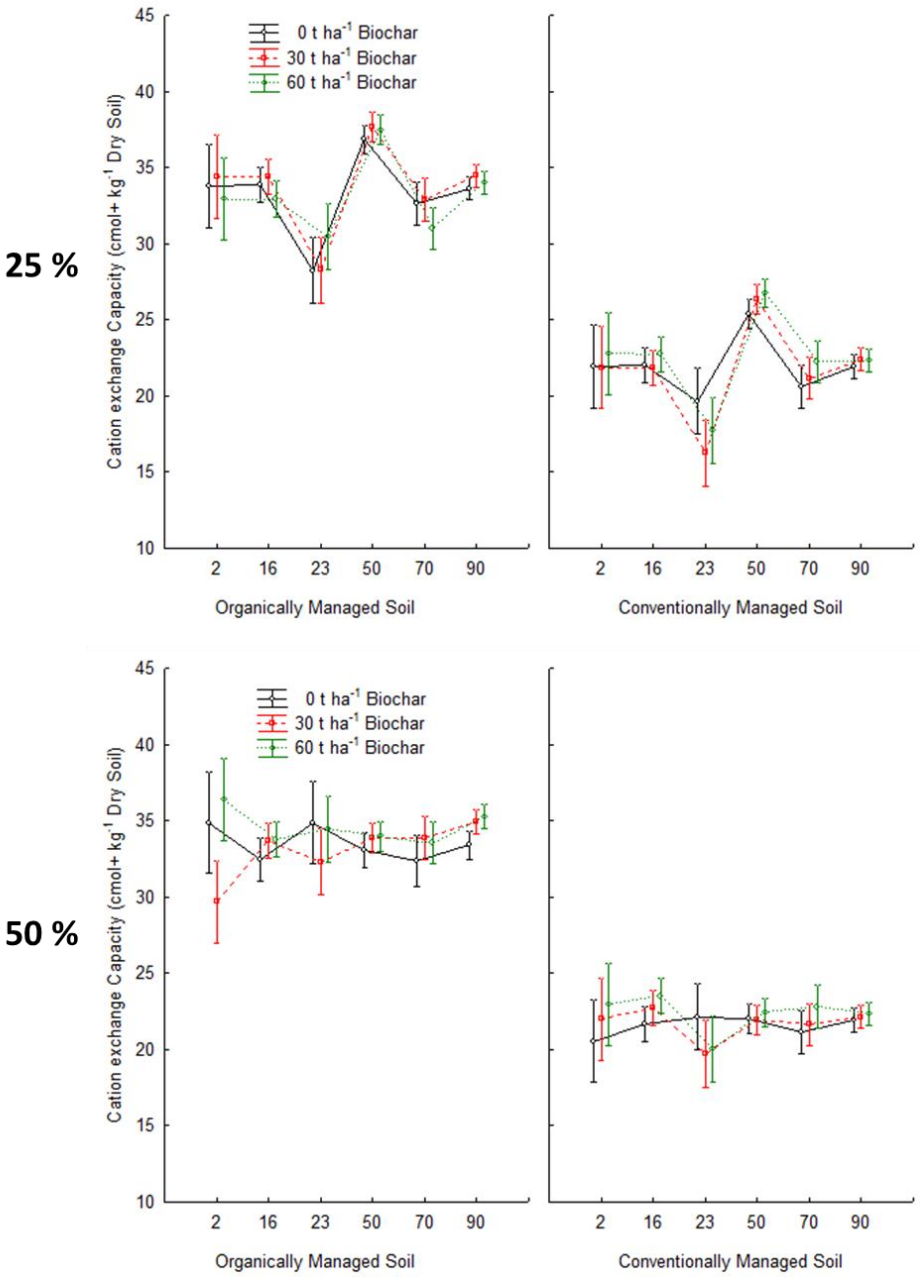


Figure 4-16: Cation exchange capacity over the 90 day incubation at 25 and 50% field capacity SMC. Bars are standard errors (N = 3).

The levels of NEA were low, ranging between 0.2 and 0.4 mg kg^{-1} dry soil. As a result of this, up to 85% of the data had absolute ammonium levels below the minimum working range of 0.3 mg kg^{-1} for the elemental analyser.

Despite this, NEA levels were higher in the biochar amended soils compared to the controls in both the organically and conventionally managed soils. This was as much as a 20 and a 40% increase with 30 and 60 t ha^{-1} biochar respectively compared to the control in the organically managed soil and a 35 and 60% increase with biochar application rates for the

conventionally managed soil (Figure 4-17), though there was no overall increase or decrease over time (Figure 4-18).

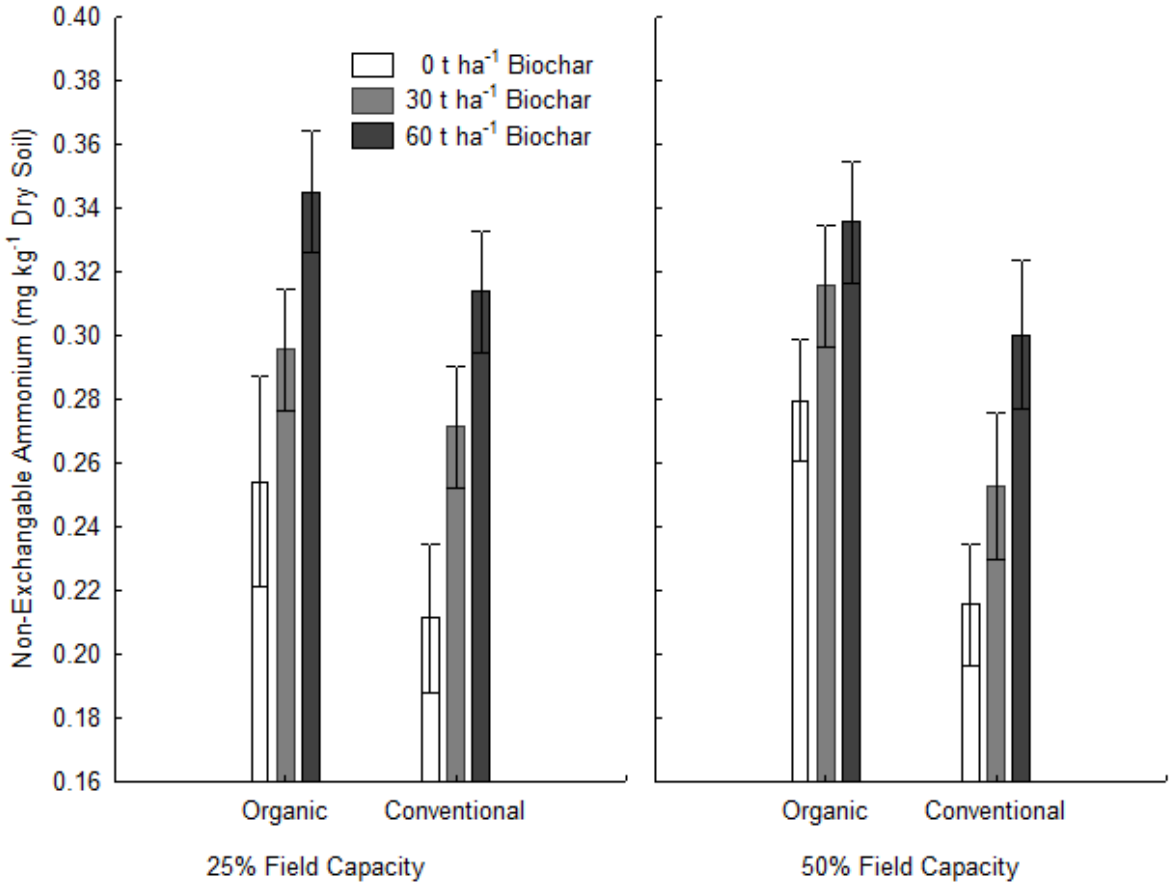


Figure 4-17: Non-exchangeable ammonium with the addition of biochar under organically and conventionally managed soils at 25% and 50% field capacity.

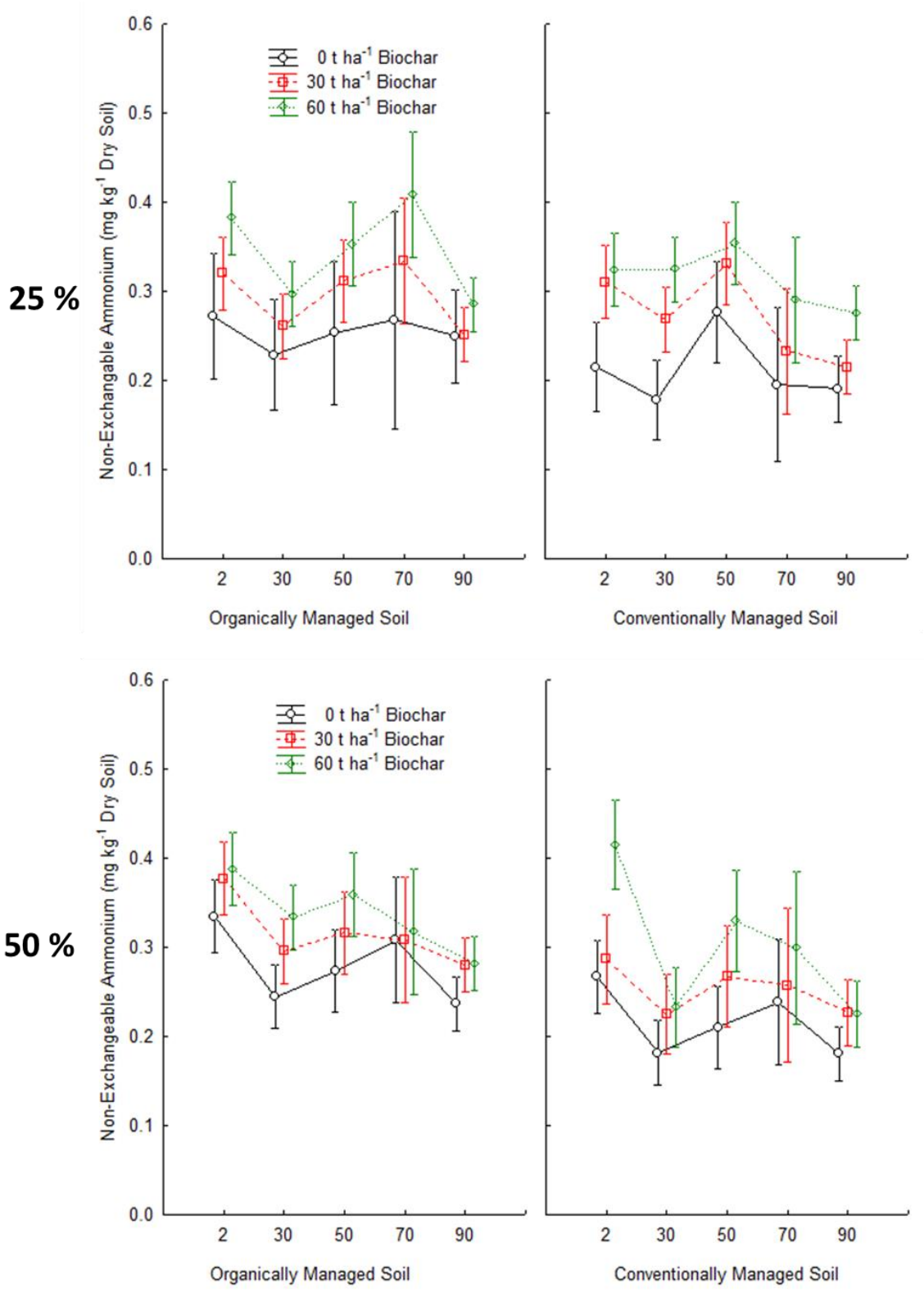


Figure 4-18: Non-exchangeable ammonium levels over the 90 day incubation at 25 and 50% field capacity. Bars are standard errors (N = 3).

4.6.6 Section Discussion

Although the repeated measures ANOVA suggested that the soil's CEC was significantly affected over the 90 days, it can be seen in Figure 4-16 that this is primarily due to fluctuations throughout the incubation, particularly at 25% field capacity, and there was no overall relationship between CEC and time during the incubation experiment, producing a Pearson's product moment correlation coefficient of 0.05 and $P = 0.4$, thus indicating that there was no trend between time and CEC.

CEC of the soil is affected by charge types and density in the soil. There are various sources of negatively charged surfaces such as SOM and clay particles and can be affected by environmental factors such as pH.

These factors are affected by long processes such as soil formation form parent materials and long term soil management practices and tend not to show quick responses over time and thus could explain the lack of a correlation over the relatively short time period of 90 days.

CEC was higher in the organically managed soil, shown by the repeated measures ANOVA ($P < 0.001$; Table S4-4). The CEC is affected by the soil management, particularly the addition of organic matter. The addition of compost, with nutrient ions such as Ca^{2+} and Mg^{2+} can increase the number of cation exchange sites and thus the CEC (Ge et al., 2010).

In a field trial (Bulluck et al., 2002), it was found that the CECs of various sandy loam soils after 2 years of applying organic waste were higher (7.97 cmol kg^{-1}) compared to an inorganic fertiliser (6.05 cmol kg^{-1}). Influencing CEC requires regular high loading rates of organic matter (Shiralipour et al., 1992) indicating that this is a result of long-term management changes.

There is much contemplation regarding the effects of biochar addition to the soil's CEC. A review by Ameloot et al. (2013) found increases in CEC with biochar addition varied from 10 to 100% depending on the feedstock and the production temperatures. Despite this high variability, there is a potential for biochar to positively impact on the soil's CEC and cation retention (Verheijen et al., 2010).

The addition of the biochar during the incubation however showed no impact on the total CEC, despite the higher CEC (66.33 cmol+ kg^{-1} dry soil) than the soil's baseline CEC (33.45 and 23.60 cmol+ kg^{-1} dry soil for the organically and conventionally managed soils respectively). It was suggested by Silber et al. (2010) that due to high CECs found within

native organic compounds (up to 2800 mmol+ kg^{-1} [C]), the addition of biochar may only show favourable increases in soils that exhibit low clay and SOM contents. Despite choosing soils with high sand contents, these were productive agricultural soils and are unlikely to be classified as degraded in organic matter.

It was proposed by Silber et al. (2010), that the effects of biochar on CEC would only be favourable on degraded soil, unless the surface oxidation of the biochar proves to be significant, which was suggested by Liang et al. (2006). During the incubation however, the CEC did not increase with biochar addition over time. As the biochar used in the incubation was fresh, it could be that more time or aging was required for a notable increase in CEC.

A review by Clough et al. (2013) highlighted several short-term studies in which the retention of ammonium was increased through the addition of biochar. These were often implied to be due to the higher CEC of the biochar itself. However the biochars used in short term studies are often with fresh biochar with lower CECs than compared to aged biochars.

Adsorbed ammonium through mechanisms such as cation exchange, which although has been shown to be resistant to leaching should be exchangeable as the name suggests and therefore available for uptake or extraction with KCl (Taghizadeh-Toosi et al., 2011b; Clough et al., 2013).

The levels of NEA were low. Although this resulted in up to 85% of the samples having concentrations below the minimum working range for the elemental analyser, this also indicates that the majority of ammonium added to the soil was bioavailable (Taghizadeh-Toosi et al., 2011b, 2011a).

Despite this, the higher NEA with biochar could account for some of the decreases in ammonium observed with the application of DCD. This complements the idea that the primary mechanism for a reduction in ammonium availability (Figure 4-14), is due to retention to the biochar rather than a reduction in microbial metabolism.

Similarly it was also found that the addition of a peanut-hull biochar could hold on to added ammonium concentrations without release. It was suggested that this was through physical entrapment within the biochar's pore structures (Saleh et al., 2012; Clough et al., 2013).

4.6.7 Section Conclusions

The addition of biochar to the soils did not significantly impact on the CEC. Despite this lack of effect, increasing the application rate of the biochar did result in higher levels of NEA found in the soil. However due to the high percentage of NEA samples that had absolute ammonium levels below the dynamic working limit of the elemental analyser, we can only indicate that the biochar additions could be holding onto higher levels of ammonium that cannot be removed through extraction and by extension by plants and microbes.

Despite a lack of significant difference between the application of biochar and CEC, there is still an increase in NEA in the soil. It is purported here that as the majority of ions held in cation exchange sites should be exchangeable and thus available for extraction by plants and microbes, the rationale behind an increase in NEA is by a separate mechanism, potentially physical entrapment.

4.7 Chapter Conclusions

The soil organic matter is a key source of carbon and nitrogen for plants and microbes. In agricultural systems, SOM is provided as a source of carbon and nitrogen. The type of nitrogen source over the long and short-term can have substantial impacts on the soil's functioning and ability to effectively deliver nitrogen to the plants and microbes.

SOM however cannot be effectively utilised in many situations and by many species of plants. Before uptake, the majority of nitrogen must be in a mineral (inorganic) form, a result of the process of mineralisation. Ammonification is one aspect of this process and results in the release of ammonium ions. As such, the rate and extent at which SOM is mineralised influences the levels of available nitrogen in the soil. How the addition of biochar affects nitrification and nitrogen availability cannot be answered without considering the effects biochar has on mineralisation rate.

Ammonium production changes according to the type of nitrogen provided to the soil. The addition of the ammoniacal nitrogen fertiliser to the conventionally managed soil resulted in higher increases in ammonium concentrations as hypothesised, due to the slower mineralisation rates and lower inherent ammonium levels of the GWC compared to the NPK fertiliser.

It was also hypothesised however that the addition of biochar would affect the mineralisation rate differently for each system due to these difference in SOM. It was predicted that the higher SOM content of the organically managed soil would show a higher mineralisation rate than the conventional despite the higher levels of ammonium and that this would be reflected in the microbial activity as the major influencer of mineralisation rate in systems with labile carbon.

It was shown however that the ammonium levels were lower with increasing application rate of biochar for both soil management systems. Examination of the microbial activity through respiration rate showed no significantly different changes in the rate of carbon dioxide release with the addition of biochar and it was concluded that the changes in ammonification were not primarily caused by microbial activity. It was postulated that this could be due to the adsorption of ammonium to the biochar's surface due to the higher CEC levels of the biochar compared to the inherent CEC of either the organically or conventionally managed soils.

For this experiment, it was hypothesised that as the major influence on the lower ammonium levels with addition of biochar in the mineralisation experiment was due to CEC, a higher CEC would be seen with biochar application. It was also hypothesised that this would reduce availability and extractability of ammonium, and therefore increase the non-exchangeable ammonium (NEA) levels.

Results showed that the addition of biochar did indeed hold more ammonium that was un-exchangeable. Although a lack of change with CEC led to the postulation that cation exchange was not a primary factor influencing this adsorption. It is suggested that this could be due to entrapment within the biochar's pores.

The low level of NEA suggests that the majority of ammonium added to a biochar amended soil is extractable and thus biologically available, although, these effects are the result of a short-term experimental approach. Whether these effects are meaningful given the large heterogeneity of a field system is debatable however these give an insight into the potential mechanisms behind the short term changes in nitrogen cycling. Nitrification is an important process that affects the availability of nitrogen in the soil. It was hypothesised that the addition of biochar to both soils would increase the nitrification rate and result in a larger available nitrate pool in the soil. The interactions between biochar and the differences in the soil's properties were more complex and thus the hypothesis was shown to be false. It was hypothesised that these changes were as a result of changes in microbial activity and thus would increase with nitrification rate.

The addition of biochar decreased the net ammonium levels in the conventionally managed soil and correspondingly increased net nitrate levels indicating nitrification. This was attributed to the more amenable conditions for higher microbial activity provided in the form of labile carbon directly from the biochar, and the depletion of this was suggested to cause the reduction in biochar improved nitrification after 30 days.

In the organically managed soil however, it was shown that at least part of the reductions in net ammonium levels with biochar during the nitrification incubation could be due to lowering substrate (ammonium) availability. But that this reduction in ammonium levels as a substrate for nitrification was not sufficient to cause a reduction in nitrification in the conventionally managed soil, thus the larger supply of ammoniacal nitrogen in the form of inorganic fertiliser was largely unaffected.

Biochar increased respiration in the organically managed soil, but did not affect the MBC. Conversely, in the conventionally managed soil, biochar addition increased the MBC but not respiration. With a higher inherent C:N ratio in the organically managed soil compared to the conventional the addition of further labile carbon from the biochar (but little nitrogen) resulted in the excess being released as carbon dioxide. The biochar addition to the more carbon limited conventionally managed soil resulted in less excess carbon and no increased release of carbon dioxide but greater uptake by the microbes resulting in the increased MBC.

This series of incubation experiments aimed to consider how the application of biochar could affect the nitrification in the soil and to explore the possible mechanisms behind this. It was concluded that a combination of different factors attribute to a change in nitrification. It appears however that the strength of retention and subsequent release is a strong contributing factor to the availability of ammonium as a substrate for nitrifying bacteria.

5 GLASSHOUSE STUDY

5.1 Introduction

Managing soil nitrogen dynamics in agricultural systems is essential for supporting crop productivity. This chapter presents the Glasshouse Study (Perennial Rye-grass Pot Trial). Previous chapters showed how the addition of biochar can affect the nitrogen transformation and availability in agricultural soils. This chapter was conducted to determine how the changes with biochar application rate can impact on the nitrogen uptake and resultant growth of plants.

A pot trial allows the measurement of yield while still having a partly controlled environment by providing the complete separation of different treatments and the flexibility to control factors such as nitrogen application.

5.2 Chapter Objectives

This chapter used a semi-controlled environment to determine the impact of the biochar application on plant growth and soil nitrogen availability. This used a glasshouse study over a three season period and aimed to utilise the information on how biochar affects the water and nutrient dynamics, as studied in **Chapter 3 and 4** respectively, and explore how these changes transfer to crop production and the utilisation of nitrogen by the crop. As such this pertains to Objectives 2 and 3 as supplied in **Chapter 1**.

5.3 Hypotheses

Crop yield will increase with the application rate of biochar to the soil due to higher nitrification rate and supply of available nitrogen.

5.4 Materials and Methods

5.4.1 Glasshouse Set-Up

The pot experiment was conducted in the glasshouse facility at Cranfield University (Figure 5-1), commencing in July 2010 and continuing for three season's growth. The soils used were the same as used in the incubation and soil water dynamics experiments as detailed in **Chapter 2: Material Characterisation**.

Figure 5-1: Pots of *Lolium perenne* in glasshouse study before dry matter sampling. Blocks 1 and 2 shown.

The pots were set-up with samples in triplicate as a randomised block design with soils from the two management practices: organic and conventional; four rates of biochar application equivalent to: 0, 20, 40 and 60 t ha^{-1} equivalents and two nitrogen application rates: 70 and 140 kg [N] ha^{-1} (totalling 48 pots, organised as shown in Figure 5-2).

BLOCK 1	O; 20; 140	O; 0; 140	O; 20; 70	O; 60; 140	O; 40; 140	
	O; 40; 70	C; 20; 70	O; 0; 70	C; 40; 140	C; 60; 70	C; 0; 140
	C; 60; 140	O; 60; 70	C; 20; 140	C; 40; 70	C; 0; 70	

BLOCK 2	C; 40; 140	C; 60; 70	O; 40; 70	C; 20; 70	C; 0; 140	
	O; 20; 70	O; 20; 140	O; 0; 70	O; 0; 140	C; 20; 140	O; 60; 70
	C; 0; 70	O; 60; 140	C; 40; 70	C; 60; 140	O; 40; 140	

BLOCK 3	C; 40; 70	O; 60; 70	C; 20; 140	O; 20; 140	O; 20; 70	
	C; 40; 140	O; 60; 140	C; 60; 70	O; 40; 70	O; 40; 140	O; 0; 70
	C; 20; 70	C; 60; 140	O; 0; 140	C; 0; 140	C; 0; 70	

Figure 5-2: Randomised block design of the pots within the glasshouse study. Each location is characterised by the key [Soil Management; Biochar Application Rate; Nitrogen Application Rate]. O: Organically Managed Soil, C: Conventionally Managed Soil.

Soil was prepared as discussed for **Chapter 3: Soil Water Dynamics**; the soil was air-dried at 40˚ C and ground to pass through a 2 mm mesh-diameter sieve (Cordovil et al., 2005). The pots had an approximate volume of 6L, to each of which was added a layer of course gravel to allow excess water drainage and prevent soil losses throughout the experiment. A bulk density of 1.2 g cm^{-3} was achieved by adding 5.3 kg of the prepared soil and pressing to the lower lip of the pot. Any excess water that leached through the soil was collected in the base and re-applied to the surface of the pot.

Prior to the glasshouse study commencing, the soils from the organically and conventionally managed farms were analysed to determine the chemical and nutritional properties before experimentation. The methods and results of this can be found in Chapter 2.2.1: Soil Analysis. A nitrogen source was applied to the organically and conventionally managed soils as GWC and inorganic fertiliser respectively at the start of each season. The application rates of 70 and 140 kg ha^{-1} equivalents were derived from RB209 DEFRA fertiliser guidelines (DEFRA, 2010).

The quantity of compost applied per pot was calculated on a by mass basis assuming a soil depth of 0.15 m and a bulk density of 1.2 g cm^{-3}. The nitrogen source and the biochar was surface applied and hand-incorporated to a depth of 0.15 m to emulate field conditions

The pots were sown with *Lolium perenne* (Perennial Ryegrass) at a seeding density of 4 g m^{-2} (Antille, 2011), equating to 0.15 g per pot. The seeds were covered with a thin layer of soil to

reduce the desiccation of the seeds and promote germination, to reduce the movement of seeds before the establishment of the grass watering was by water spray. *Lolium perenne* was selected as this allowed several harvests during each growing season. Each sampling event simulated an optimum grazing pattern. *Lolium perenne* is a monocotyledon; as the plant grows, a new leaf is produced periodically. During the growth of the fourth leaf, the first leaf dies as such the optimum time for grazing (and harvesting) is therefore after the growth of the third new leaf (EBLEX, 2012). Grasses have the advantage of having a large number of individual plants able to be cultivated in a small area allowing larger representation of plant growth.

Sacrificial pots were packed, saturated, and drained for 24 hours to estimate field capacity of the pots by mass of water. Using these calculations, water was added to the pots to achieve field capacity and then maintained for the duration of the experiment.

Maintaining the moisture content of the soil at field capacity was estimated using evapotranspiration readings by a ceramic plate atmometer (Figure 5-3).

Figure 5-3: Atmometer (evapotranspiration gauge) to estimate loss of water from pots. Photo courtesy of Grivin Chipula.

5.4.2 Measurements and Analysis

Lolium perenne Yield

Yield was measured through crop dry matter in a regular series of sampling events (Table 5-1). Plant material was collected by cutting the growth down to the top of the pots (Figure 5-4), approximately 3 cm from the base of the plant as suggested by Gunnarsson et al. (2010). The plant growth material was dried at 60˚C for a minimum of 72 hours before weighing.

Figure 5-4: *Lolium perenne* during yield collection. Photo courtesy of Arianne Hanson (Taken July 2012)

The total nitrogen (TN) of the plant material was performed on finely ground samples by catalytic tube combustion (Vario EL III, CHNOS Elemental Analyser, Hanau, Germany, British Standards Institute, 1995). Nitrogen uptake (NU) by *L. perenne* was calculated by the product of dry matter (DM) and total nitrogen (TN) content (Brink et al., 2001; Douglas et al., 2003).

Table 5-1 provides the frequency and timings of each sampling event and the corresponding season this took place in. The growing season was determined to be from spring through to autumn.

Table 5-1: Time table of sampling event for crop yield and soil analysis. Regarding 'Analysis Performed' Soil: Extractable N (with KCl); DM: Plant Dry Matter; TN: Plant Total Nitrogen; N/A: Not Applicable.

Season	Sampling Event Number	Date Undertaken	Cumulative Sampling Time (Weeks)	Analysis Performed
1	Setting-Up	01 July 2010	0	N/A
	1	20 September 2010	12	Soil; DM; TN
2	2	10 February 2011	32	Soil; DM; TN
	3	31 May 2011	48	Soil; DM; TN
	4	20 July 2011	57	Soil; DM; TN
	5	05 October 2011	66	Soil; DM; TN
3	6	10 May 2012	87	Soil; DM; TN
	7	11 July 2012	106	DM; TN
	8	20 October 2012	120	Soil; DM; TN

Soil Analysis

Soil samples were also initially taken at each sampling event then reduced when little significant difference was observed. Collection of the soil was with a 15 mm diameter auger, which was used to remove three cores and homogenised by hand prior to soil analysis. The resultant holes after sampling were re-filled with the corresponding prepared soil, without the addition of biochar.

Ammonium and total oxidisable nitrogen (TON) was analysed by 2 mol L^{-1} Potassium chloride solution (KCl) extraction and analysed by segmented flow analyser (Burkard Scientific Series 2000, Uxbridge, UK). Soil moisture content (% by mass) was calculated by oven-drying soil samples at 105˚ C.

5.4.3 Data and Statistical Analysis

Changes in *L. perenne* dry matter, nitrogen uptake and the soil's extractable nitrogen over the incubation were analysed using a repeated measures ANOVA (General Linear Models) using STATISTICA V.12 (Statsoft Ltd, 2013). Statistical significance level was determined with $\alpha = 0.05$. For multiple comparisons, a Fisher's least significant difference (LSD) analysis was used to compare individual means (Sokal & Rohlf, 1995).

Probability plots of residuals were used to determine the normality of the population distributions and anomalous data were occasionally removed prior to analysis, though data were left intact where possible.

5.5 Results

5.5.1 Dry Matter Yield of L. perenne

The dry matter yield of *Lolium perenne* was usually found to be higher under conventional soil management, although towards the end of a season's growth the difference in yield between organically and conventionally managed systems became non-significant or switched (Figure 5-5).

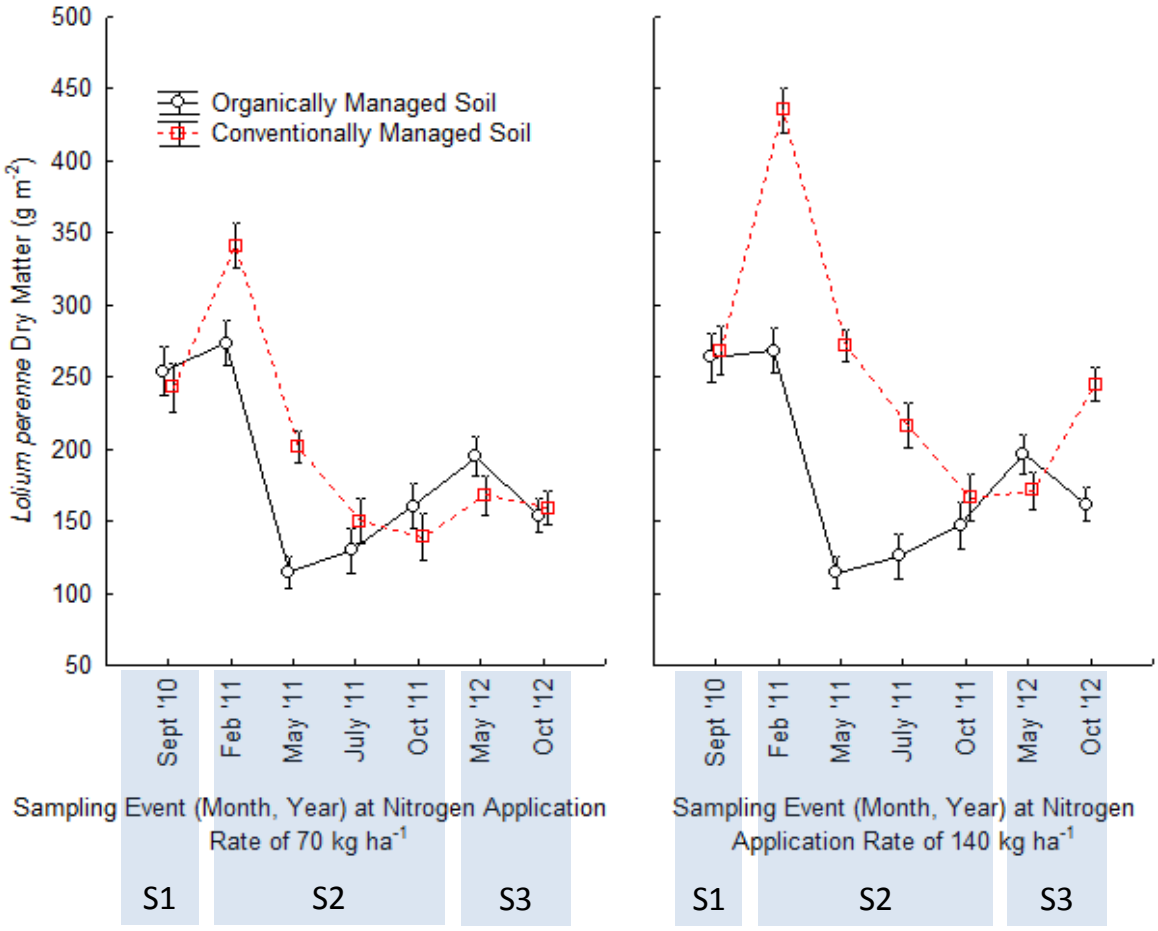


Figure 5-5: *Lolium perenne* yield throughout the 120 week glasshouse study showing effects of soil management and nitrogen application. S1: Season 1, S2: Season 2, S3: Seasons 3.

Shown in figure 5-6, there was a significant interaction between soil management and biochar application ($P < 0.001$; Table S5-1) which showed that dry matter production increased with fertiliser application rate, but only in the conventionally managed soil. Mean dry matter production after the addition of biochar decreased each season.

No significant response to dry matter was observed with the application rate of biochar (Figure 5-6) over the glasshouse study.

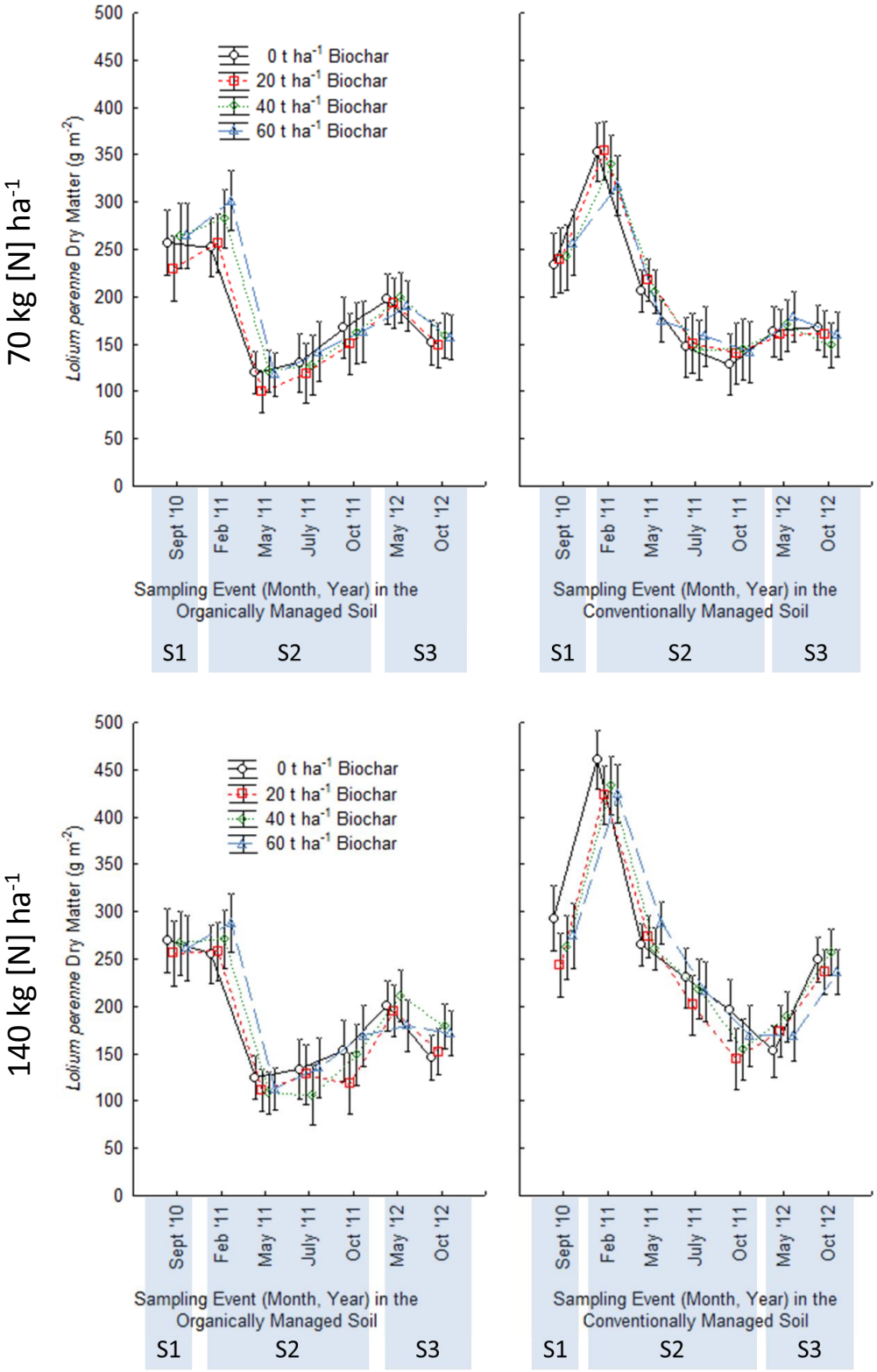


Figure 5-6: Effect of biochar addition on Lolium perenne yield (dry matter). S1: Season 1, S2: Season 2, S3: Seasons 3.

5.5.2 L. perenne Nitrogen Content and Uptake

Higher plant nitrogen concentrations and nitrogen uptake was observed in the cuts taken after the application of the fertilisers (Sampling events at 48 and 106 weeks). This was particularly noticeable within the conventionally managed soils (Figure 5-7; Figure 5-8).

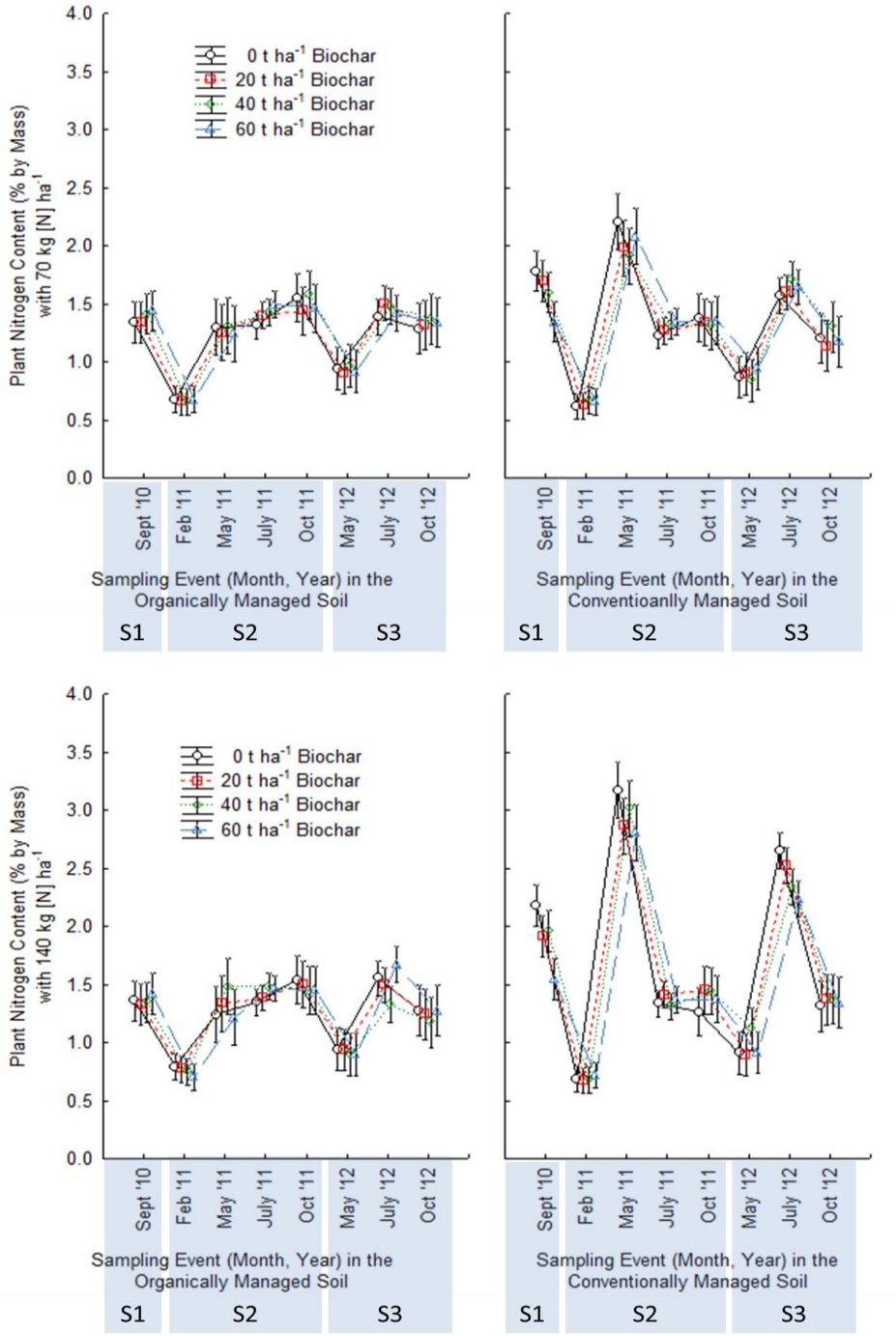


Figure 5-7: Total nitrogen within the plant over ground biomass with the addition of biochar (% by mass) S1: Season 1, S2: Season 2, S3: Seasons 3.

This effect reduced over time, as with dry matter yield, the increase in plant nitrogen content at 106 weeks (due to the addition of nitrogen after the post-winter cut at week 87) was less than that observed at week 48.

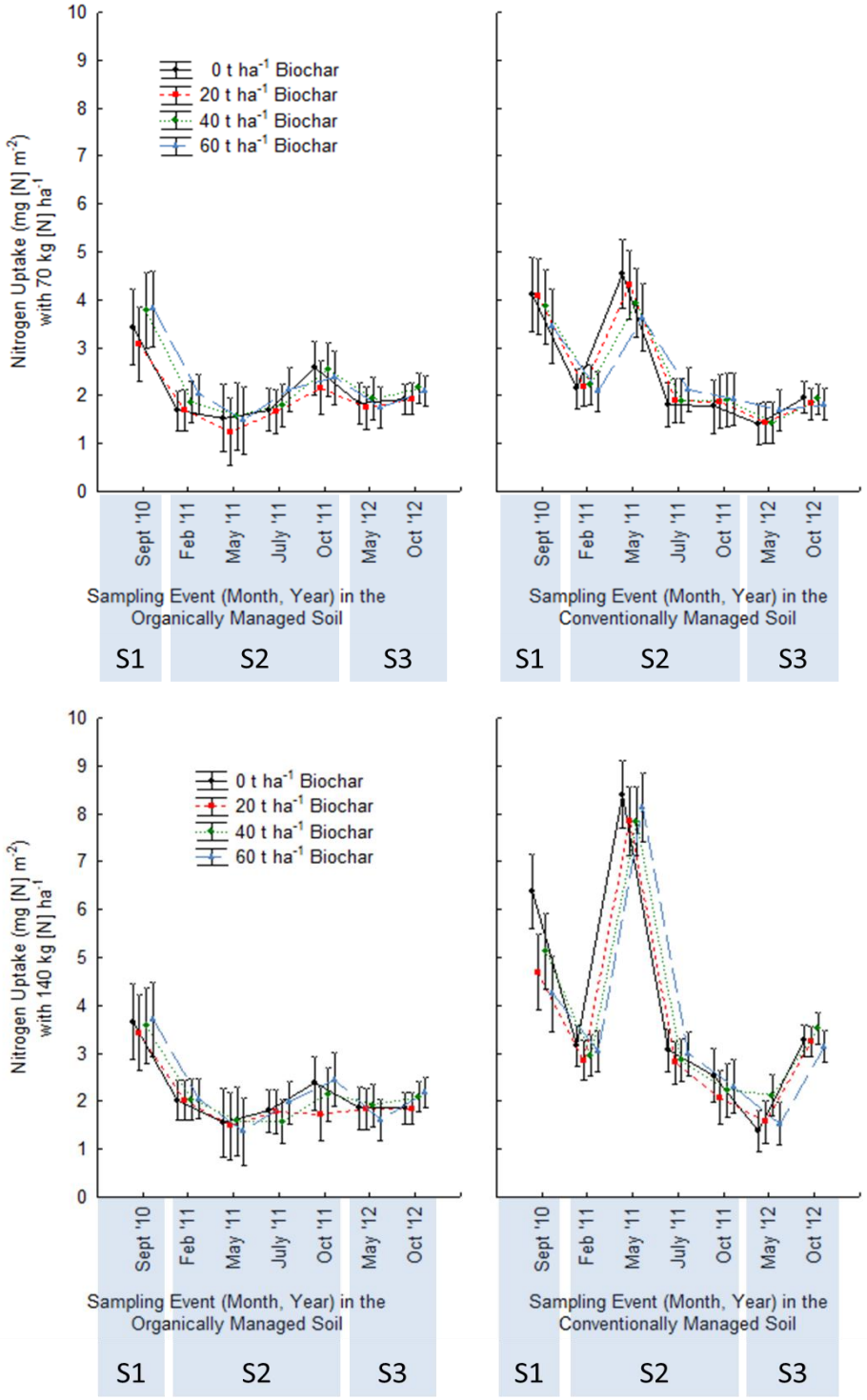


Figure 5-8: Effects of biochar application on nitrogen uptake into *Lolium perenne*. Nitrogen uptake is a product of the yield (g m^{-2}) and plant nitrogen content (%) S1: Season 1, S2: Season 2, S3: Seasons 3.

The repeated measures ANOVA suggested that the addition of biochar did not impact on the plant nitrogen content (P = 0.33; Table S5-1) however a Fisher's test of least significant difference showed that a reduction in plant nitrogen content (Figure 5-7) was shown with increasing biochar application rate at weeks 48 and 106 (cuts after the application of nitrogen) but only in the conventionally managed soils with a high application of nitrogen (140 kg ha^{-1}).

5.5.3 Soil Extractable Nitrogen

No significant difference in soil extractable nitrogen (both ammonium and nitrate) can be detected between nitrogen application rates (Figure 5-9).

There was higher ammonium levels found in the organically managed soil (P = 0.01) but no difference with nitrate (P = 0.1; Figure 5-9; Table S5-2).

The high variability and low values (between 0 and 4 mg kg^{-1} dry soil) resulted in no difference in ammonium or nitrate levels with the addition of biochar (Figure 5-10).

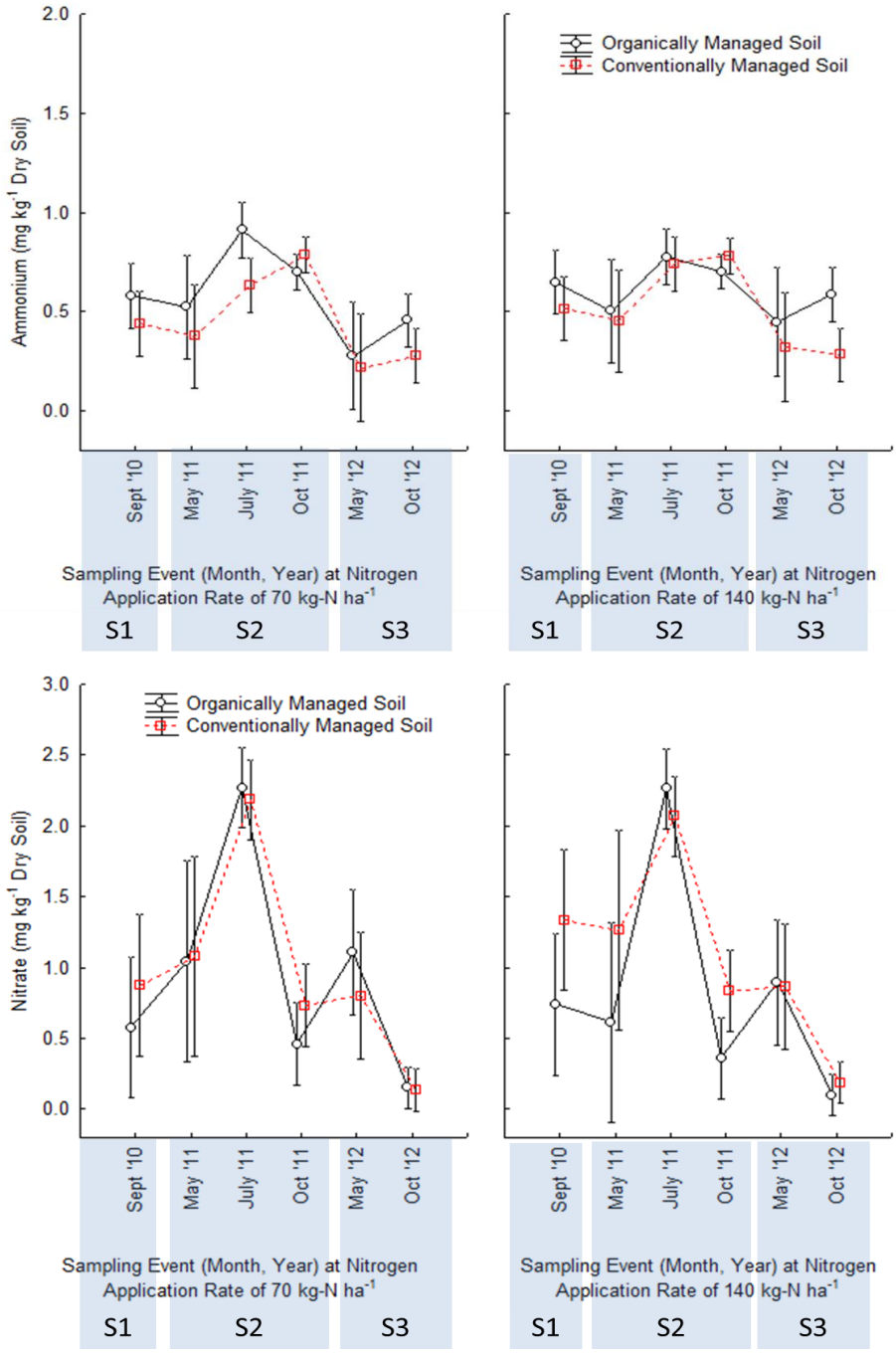


Figure 5-9: Soil ammonium and nitrate levels over the glasshouse study in response to soil management and nitrogen application rate.

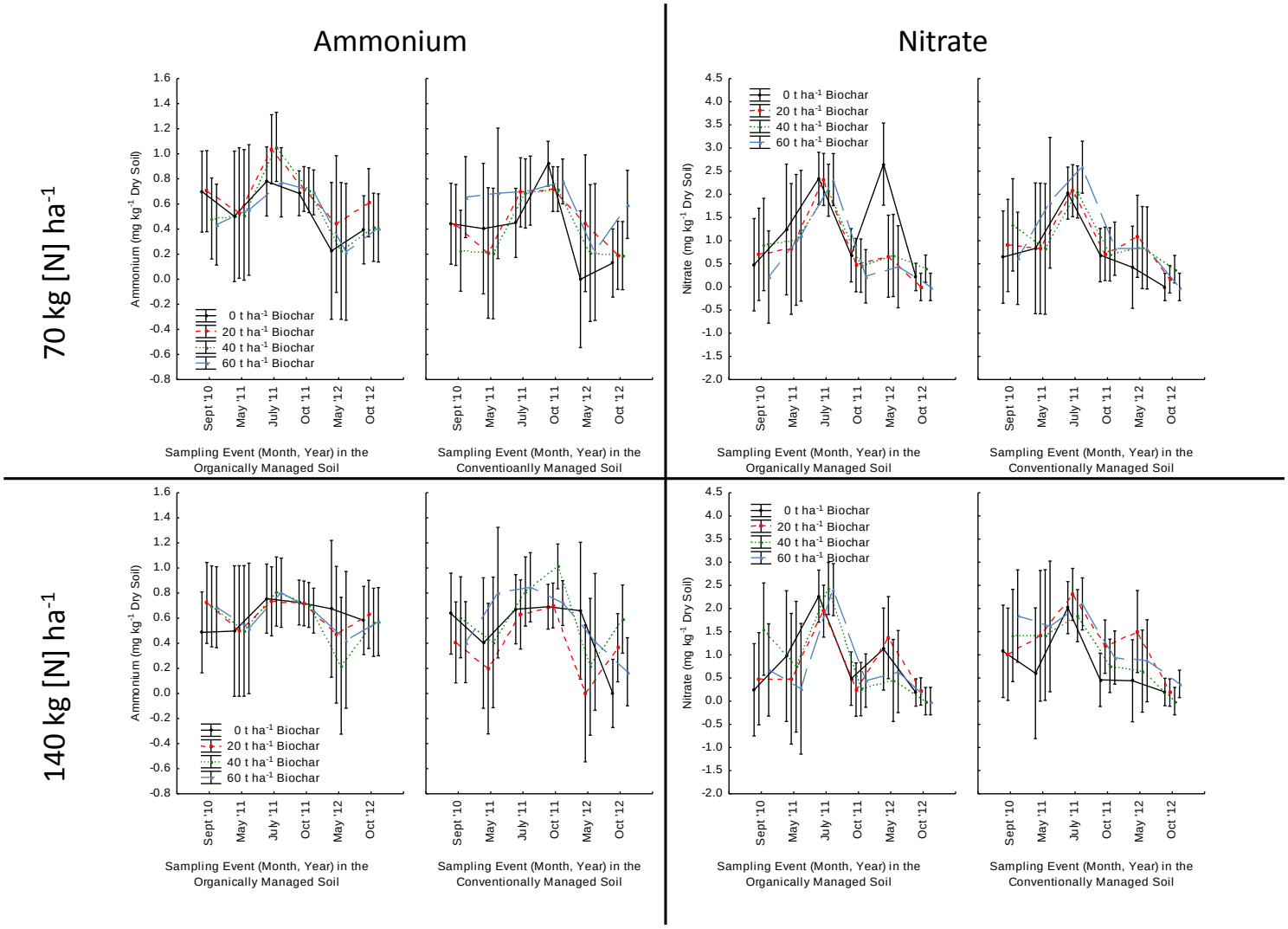


Figure 5-10: Soil ammonium and nitrate levels over the glasshouse study in response to soil management, nitrogen and biochar application rate.

5.6 Chapter Discussion

The objective of the chapter was to determine the effect of biochar application on soil nitrogen availability over several growth seasons of a semi-controlled environment and how the impacts of biochar as observed in the incubation experiments translate to the uptake of nitrogen and thus growth of *Lolium perenne*. This chapter also considers how time and the potential oxidation during the aging process of the biochar impacts on the soil and plant environment.

Each season within the glasshouse study commenced in spring (March) and cuts were taken frequently until the following March. The time intervals between sampling events was affected by the external conditions as influenced by the weather. Colder weather reduced growth and thus lengthened the time between harvests. Cutting at regular time-intervals as used by Frame & Morrison (1991) risked having too little material over the winter months and did not emulate the seasonal use of rye-grass as a grazing crop.

At each sampling event, the removal of soil left a core within the pot that was filled with the corresponding prepared soil without biochar. Filling the cores removed the large channels that would have otherwise affected the infiltration and retention of water in the pots. This led to the eventual dilution of the biochar application rates in the pots after each sampling event. However this allowed the study to determine the effects of biochar aging without the continuous addition of fresh biochar. Markers were placed in the location of the freshly filled soil cores to allow identification and avoidance of previous sampling sites.

The biochar application rates covered a wide range, potentially higher than those that would be reasonably found applied to a field site. This was to provide theoretical implications to how biochar might be impacting on the yield of a rye-grass and was not therefore intended to accurately reflect practices within a field study or practical agricultural practices. A review (Jeffery et al., 2011) showed that the most positive increase in yield was shown with up to 100 t ha^{-1} biochar.

Dry matter production of *L. perenne* was typically higher under conventional soil management, though this was not always the case as shown in Figure 5-5. It appears that the addition of a comparatively quick release nitrogen source with inorganic fertiliser compared to the GWC caused higher yields of dry matter early in each season. This was observed also

in the nitrification incubation (**Chapter 4**) which showed a faster release of nitrate in the conventionally managed soil compared to the organic. The nitrogen in the pot trial is quickly taken up by the plants however as shown by the nitrogen uptake (Figure 5-8). As the seasons progress however it is suggested that the depletion of nitrogen from the inorganic fertiliser causes a reduction in rate of growth, the slower release of nitrogen from the GWC continues to release nitrogen resulting in the higher yields of dry matter in the sampling events towards the end of each respective season (Seufert et al., 2012). The slower release of nitrogen from the GWC however limited the overall yield of dry matter and did not provide the crop with the optimum level for growth (Seufert et al., 2012).

As the duration of the glasshouse study goes beyond that of the nitrification incubation's 60 days, it could be indicated that higher ammonium levels in the soil within the organic systems towards the end of each season shows continuing release of nitrogen from GWC for longer than the inorganic fertiliser. Although due to the presence of plants in the system taking up excess available nitrogen, the effect of this is difficult to ascertain and is inconsistent.

The immediate growth response of *L. perenne* with the addition of fertiliser showed a pronounced reduction in subsequent seasons. Frame & Morrison (1991) also observed reductions in the mean levels of dry matter yields over subsequent season's growth of various grasses including *L. perenne*.

Dry matter production, along with the uptake of nitrogen into the plants increased with fertiliser application rate, but only in the conventionally managed soil. The increase in nitrogen application rate from 70 to 140 kg ha^{-1} appeared to increase the level of nitrogen availability of nitrogen for plant uptake. No significant difference in soil extractable nitrogen (both ammonium and nitrate) can be detected between nitrogen application rates (Figure 5-9), though this is likely due to the rapid uptake into the plants, masking any potential differences. The increase of nitrogen application, within the GWC, from 70 to 140 kg ha^{-1} however did not increase dry matter production or plant uptake of nitrogen as observed in the conventionally managed soil, the rate of compost mineralisation and the release of nitrogen is slower than that of the inorganic fertiliser (Flavel & Murphy, 2006).

Studies have indicated the potential for using biochar to increase yields (Atkinson et al., 2010). A review of biochar application on soil nitrogen dynamics (Clough et al., 2013) highlighted that the effect of biochar application to crop yields is inconsistent and is dependent on many factors including soil nutrient status, soil type and biochar type although

higher yield is associated with biochars produced at higher temperatures and from hard-wood feedstocks. A pot trial also utilising *L. perenne* with biochar application showed no effect on yield (O'Toole et al., 2013).

The findings suggested that increasing biochar application rate did not change yields of dry matter. Jeffery et al. (2011) in a meta-analysis of crop yields in response to biochar application also highlighted that the results of biochar application can be variable. Overall this showed a slight positive increase in yields with the application of biochar, however also noted that dry-matter yields of *L. perenne* decreased. Within Jeffery et al. (2011), the reviewed biochars were all derived from biosolids and as such it was not ascertained whether the decrease in yield was due to the crop type (Rye-grass) or due to an interaction with the type of biochar.

Despite the reduction in ammonium concentration observed with increasing biochar as shown in the DCD incubation, this was not translated to a reduction in crop yield with biochar either. This could be due to the high bioavailability of nitrogen added (Taghizadeh-Toosi et al., 2011b, 2011a) shown by the low levels of NEA and the lack of increase in CEC with the biochar (**Chapter 4**) although there is a limitation when comparing the short term incubation experiment (90 days) with the 120 week glasshouse study and it should be noted that CEC may have changed over this period.

The uptake of nitrogen by crops and thus the concentration of nitrogen within the crop's biomass are dependent upon the availability and mobility of nitrogen in the soil (Masclaux-Daubresse et al., 2010). If higher levels in the soil are available for the plant, then greater levels will be taken up.

Higher plant nitrogen concentrations and uptake of nitrogen into plants were observed in the dry-matter yields taken after the application of the fertilisers (Sampling events May 2011 (Season 2; 48 weeks) and June 2012 (Season 3; 106 weeks). This was particularly noticeable within the conventionally managed soils with the application of the inorganic nitrogen source contained more ammoniacal nitrogen than the GWC. This effect reduced over time, as with dry matter yield, the increase in plant nitrogen content at 106 weeks - due to the addition of nitrogen after the post-winter cut in Season 3 at May 2012 (week 87) was less than that observed at week 48 (May 2011, Season 2) (Frame & Morrison, 1991). This effect cannot be observed in the dry matter however due to a missing sampling event.

The repeated measures ANOVA suggested that the addition of biochar did not impact on the plant nitrogen content ($P = 0.33$; Table S5-1) however a Fisher's test of least significant difference showed that a reduction in plant nitrogen content (% by mass) was shown with increasing biochar application rate at weeks 48 and 106 (cuts after the application of nitrogen) but only in the conventionally managed soils with a high application of nitrogen (140 kg ha^{-1}).

It is possible though that the reduction shown in the plant TN within the conventional system after the addition of an ammonical nitrogen source is a product of the higher NEA levels observed and thus a greater retention of ammonium, reducing the availability in the soil for uptake. The lack of significance in the organically managed soil however, may be a factor of the slower release nitrogen reducing the concentration available in the soil. The release of inorganic nitrogen from SOM is through biotic processes (Flavel & Murphy, 2006), as it was shown that it is unlikely that biochar had a significant effect on this (**Chapter 4.5: Dicyandiamide Incubation**).

As the effect within the incubation could be described as short term, it is possible that the reduction in TN within *L. perenne* is only evident for a short time after the application of the nitrogen source as shown.

5.7 Chapter Conclusions

The addition of biochar showed limited effect when applied to a glasshouse study with *Lolium perenne*. A repeated measures ANOVA suggested that the application of biochar did not impact on the growth and productivity of *L. perenne* over the three growth seasons.

It was observed however that the nitrogen content within the leaves of *L. perenne* was affected by the application rate of biochar on two occasions during the study. A decrease in the nitrogen (% by mass) occurred on the harvests after the application of the NPK nitrogen source (weeks 48 and 106) and thus were only observed under conventional management and at the higher nitrogen application rate of 140 kg ha^{-1}.

The nitrogen content of the plant is affected by the availability of the nitrogen within the soil. Thus it is suggested that the higher nitrification rate that was observed in the conventionally managed soil during the incubation, provided more nitrogen for uptake. It is also suggested that the slower release of nitrogen within the organically managed system is mediated by microbial activity, and it was indicated during the incubation experiments that the microbial activity responsible for the mineralisation of SOM was unaffected by the addition of biochar (**Chapter 4.5: Dicyandiamide Incubation**).

It could be concluded that the effect of biochar on nitrogen transformations and availability cannot be translated effectively to changes in yield of *L. perenne* although crop selection may be a contributing factor in this. Correlations between the crop yield and nitrogen content cannot be made with the nitrogen extractable from the soil as plant uptake maintained a consistent low level of nitrogen in the soil.

6 FIELD TRIALS

6.1 Introduction

Laboratory based experiments are ideal for isolating the controlling mechanisms of an effect. A more useful method for decision making from a farming perspective however comes from a more realistic setting such as field trials.

This chapter aims to use field trials to observe whether any changes in nitrogen and water dynamics, as caused by the addition of biochar, impacts on crop yield. Where feasible, factors were not controlled, but left to normal farming practice.

It has been described that there is a lack of studies that consider the effects of biochar addition to soil using field-scale experiments within temperate regions (Hammond et al., 2013; Jones et al., 2012). Field-scale experiments can also vary in size; a report by the International Biochar Initiative (Tomlinson et al., 2012) summarised published global field-studies and showed that only 3 of 24 studies were categorised as large (> than 30 x 30 m) scale.

Within the UK alone, 7 field-scale experiments studied by Hammond et al.(2013) showed that there was a mean positive effect of biochar on crop yield (+ 0.4 t ha^{-1}), though of these 3 showed no significant effect of biochar, 3 positive and 1 negative effect.

A meta-analysis of the effect of biochar addition on crop yield in field and pot trials also indicated a mixed response (Jeffery et al., 2011). This also showed a small (~ 10%) but significant increase in crop productivity with biochar addition compared to controls. Also positive impacts of biochar tended to be associated with biochars derived from feedstocks such as wood and paper.

The effect of biochar application rate is also varied, Hammond et al. (2013) show that the highest benefits to plant growth are with application rates under 20 t ha^{-1} whereas Jeffery et al. (2011) showed there is a tendency for plant growth to increase with biochar rate with the highest effect (increase of 39%) with 100 t ha^{-1}biochar.

As such previous research suggests that biochar can have a range of effects ranging from positive to negative and this can depend on the type of biochar, the soil type and the type of crop.

6.2 Chapter Objectives

Like the glasshouse study, this chapter aims to bring together aspects of previous incubation studies to determine how biochar addition affects soil properties in a field-scale environment and how this impacts on plant growth. As such this chapter relates to Objectives 1, 2 and 3 as portrayed in Figure 1-3.

6.3 Hypotheses

It is predicted that increasing the biochar application rate within each field trial will increase crop yield in comparison to the control through an increase in soil moisture contents (SMC) and higher the rates of nitrification.

6.4 Materials and Methods

6.4.1 Establishment

Four sites were selected for the field trial which included an organic farm and a conventional farm in England (East Anglia) and Scotland (Dumfries) (Table 6-1).

In England the organic farm, (Rushbrooke Farm: 52°13'05.0"N, 0°46'01.1"E) was located in Bury St. Edmunds, Suffolk and the conventional farm (Silsoe Farm: 52°00'37.0"N, 0°26'03.2"W) was at the experimental farm of Cranfield University, Bedfordshire. In Scotland, the organic farm (Barfil Farm: 55°02'21.7"N, 3°48'38.0"W) was located in Crocketford and the conventional (Barrasgate Farm: 54°59'31.1"N, 3°19'50.6"W) in Cummertrees (Figure 6-1).

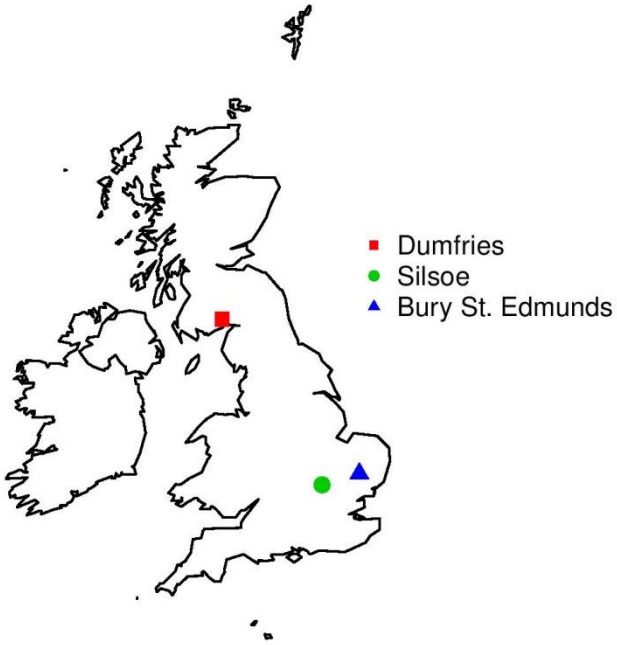


Figure 6-1: Locations of field sites in UK. Map created using R Project for Statistical Computing (R Core Team, 2013).

Table 6-1: Initial soil properties for each of the field sites. Means and (Standard Errors) shown. Sample size (*N* = 3). TN: Total Nitrogen; TC: Total Carbon; TOC: Total Organic Carbon; TON: Total Oxidisable Nitrogen (nitrite + nitrate). Org: Organically Managed Soil, Con: Conventionally Managed Soil.

	TN	TC	TOC	C:N Ratio
		%		
Scotland Org	0.422 (0.014)	4.276 (0.109)	4.1 (0.03)	10.143 (0.085)
Scotland Con	0.303 (0.017)	3.124 (0.193)	3.1 (0.10)	10.310 (0.081)
England Org	0.149 (0.004)	1.714 (0.061)	1.3 (0.03)	11.535 (0.667)
England Con	0.157 (0.002)	1.732 (0.115)	1.5 (0.03)	11.083 (0.882)

Through interviews with farm managers, the farms and location of the plots were selected due to their predicted similarity in soil type. An analysis of the particle size distribution (British Standards Institute, 1998a) showed that, according to the UK classification system three of the soils were Sandy Loams but the organically managed soil in Scotland (Barfil Farm) was a Clay Loam (Figure 6-2; Table 6-2).

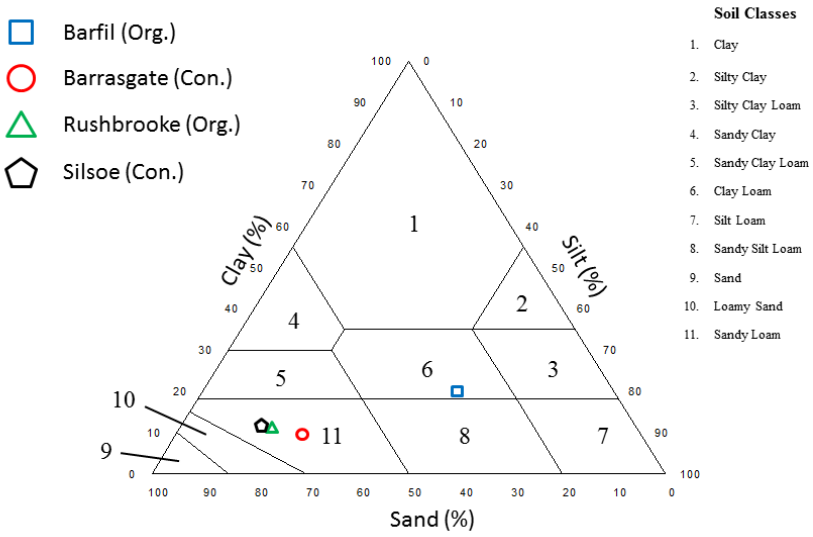


Figure 6-2: Soil textures of the field locations. Texture triangle created using Texture Auto-Lookup (TAL) (Teh, 2002).

Table 6-2: Particle size distribution for the four locations used in the field trials.

	Dumfries, Scotland		**East Anglia, England**	
	Organic	**Conventional**	**Organic**	**Conventional**
% Sand	28.09	66.17	71.13	72.60
% Silt	20.58	9.24	10.85	11.75
% Clay	51.33	24.59	18.02	15.65
UK Texture Class	**Clay Loam**	**Sandy Loam**	**Sandy Loam**	**Sandy Loam**

At each of the farms, in both locations, biochar was applied at three rates (0, 10 and 40 t ha^{-1}). Each trial consisted of 2 x 5 m plots, set up in triplicate as a randomised block design to account for slope and associated moisture content changes (Figure 6-3*a*).

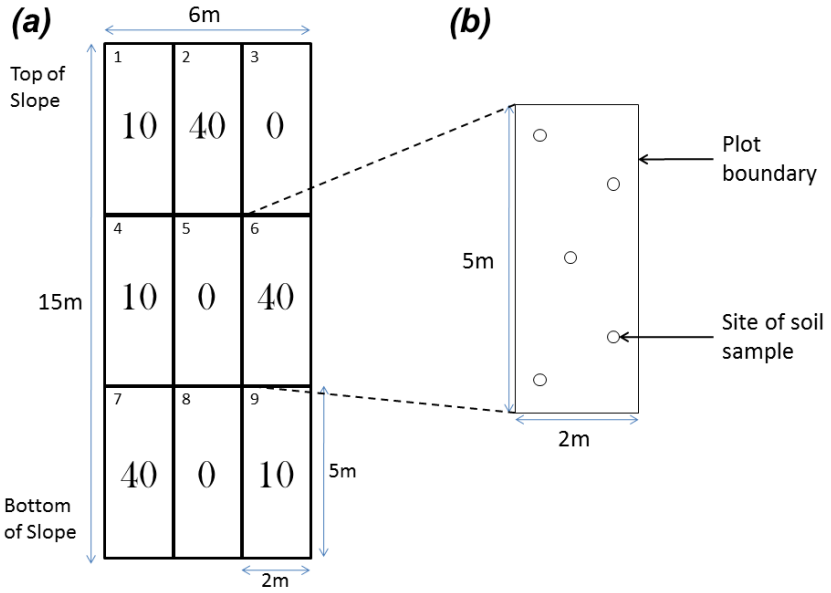


Figure 6-3: Schematic showing establishment of field trials including the layout of plots (*a*) and soil sampling locations (*b*).

Figure 6-4: Establishment of field trial at Barrasgate Farm (conventionally managed in Scotland) trial showing surface application of biochar, before incorporation

Locations were marked using triangulation from permanent fixtures, for repeated sampling over seasons. Each plot had the required equivalent mass of biochar surface applied (Figure 6-4), and machine incorporated to a depth of 0.15 m using a tined harrow and crumbler roller.

6.4.2 Sampling Events

Initial soil properties were ascertained by sampling before biochar application, and bi-annually subsequent to this; once approximately 5 weeks after sowing ('Mid-Season Sampling') and once at the time of harvest ('Harvest Sampling'). At harvest, samples of the crop were also taken for yield measurements.

Due to the potential for biochar to move under the soil with tillage and cultivation, the edge of each plot was avoided when sampling (Figure 6-3*b*). For each tri-replicated plot, 5 soil cores were taken in 'W-shaped' pattern to account for heterogeneity of the soil. These sub-samples were taken between 0 and 150 mm depth (depth of biochar incorporation) and combined before analysis.

Fresh samples were analysed for soil moisture content (SMC) by drying at 105˚ C and plant available nitrogen through extraction with 2 mol L^{-1} Potassium chloride solution (MAFF, 1986) and analysed using a segmented flow analyser (Burkard Scientific Series 2000, Uxbridge, UK). Plant available nitrogen compounds included ammonium (NH_4^+) and total oxidisable nitrogen (TON).

Yield measurements were taken using quadrats, by the removal of the entire above ground biomass, leaving less than 5 cm stalk. Yields for the first season of the Scottish trials were estimated using a 0.25 m^2 for each plot. After analysis of the first year's crop yield data in Scotland, 0.25 m^2 was thought to be under-representative. Yields after this, therefore, were measured by taking two replicates of 1 m^2 from the centre of each plot. Samples were dried at 40˚ C and weighed to 1 d.p.

In the England trials, winter crops were grown, only a single season was covered after the organic farm subsequently changed management system. The three seasons of Scotland trials were all spring crops. Details of the cropping can be found in Table 6-3.

Table 6-3: Cropping details of the field trials. Winter Wheat: *Tritium aestivum*. Winter Oats: *Avena sativa*. Forage crops are a mixture of crops including Oats (*Avena sativa*) and Alfalfa (*Medicago sativa*).

	Season	Organically Grown Crop	Conventionally Grown Crop
England	1	Winter Wheat	Winter Oats
Scotland	1	Oats	Forage
	2	Rye-grass	Forage
	3	Rye-grass	Spring Wheat

6.4.3 Statistical Analysis

Differences between means of crop dry matter and extractable nitrogen compounds were analysed using STATISTICA V.12 (Statsoft Ltd, 2013). Statistical significance level was determined with $\alpha = 0.05$. For post-hoc multiple comparisons, a Fisher's least significant difference (LSD) analysis was used to compare individual means (Sokal & Rohlf, 1995). A statistical comparison of means was not performed between locations and soil management due to confounding factors such as changes in crop selection and soil textures. Differences in crop yields within the same trial also could not be compared over different seasons due to changes in crop selection. As such, a One-way ANOVA was used to compare differences in mean crop yield between biochar as opposed to a repeated measures ANOVA and trials were analysed independently of one another.

Probability plots of residuals were used to determine the normality of the population distributions and anomalous data were occasionally removed prior to analysis, though data were left intact where possible. Plots were created with groups of trials for presentation only, not for statistical comparison.

6.5 Results

6.5.1 England Trials: Soil Moisture Content

Within the England field trials, the initial (before biochar application) SMC (% by mass) was higher in the organically managed soil than the conventional The SMC did not differ between plots for either of the soil management systems. (Figure 6-5*i*).

After the application of biochar however, at the mid-season sampling event, applying 40 t ha^{-1} biochar increased the SMC compared to the control from 8.8% by mass to 11.5% (P = 0.0009; Figure 6-5*ii*). Adding 10 t ha^{-1} biochar though did not change SMC (P = 0.20). This was shown in the conventionally managed soil.

As shown in Figure 6-5*iii* , at the harvest sampling event, under conventional management, the addition of biochar did not affect SMC. Addition of 40 t ha^{-1} biochar did increase (P = 0.01) the SMC for the organically managed soil to 9.32% by mass from the control (7.58%). No difference was found with the addition of 10 t ha^{-1} biochar (P = 0.69).

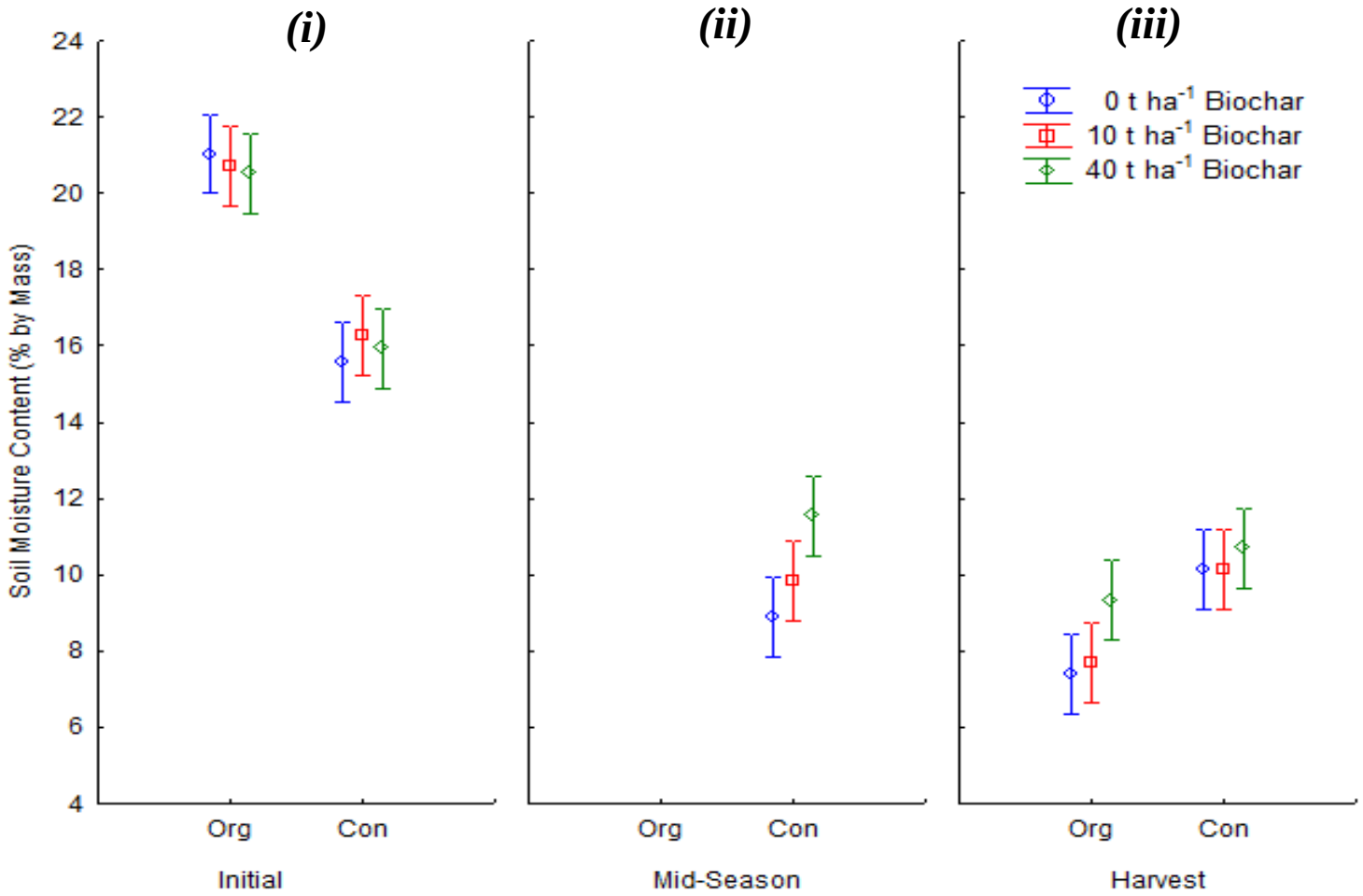


Figure 6-5: Soil moisture content (SMC) over the England field trial, bars are standard errors (N = 3). 'Initial' refers to plots before the application of biochar rather than the amount of biochar applied. No data is available for the organically managed soil mid-season.

6.5.2 England Trials: Plant Available Nitrogen

There were no significant differences in the means of ammonium and TON between plots before the application of biochar (Figure 6-6 and Figure 6-7 respectively) for either the organically managed soil or the conventional.

At the harvest sampling event, both ammonium and TON levels had reduced to comparable levels with initial and showed no significant differences with biochar application rate for either soil management system.

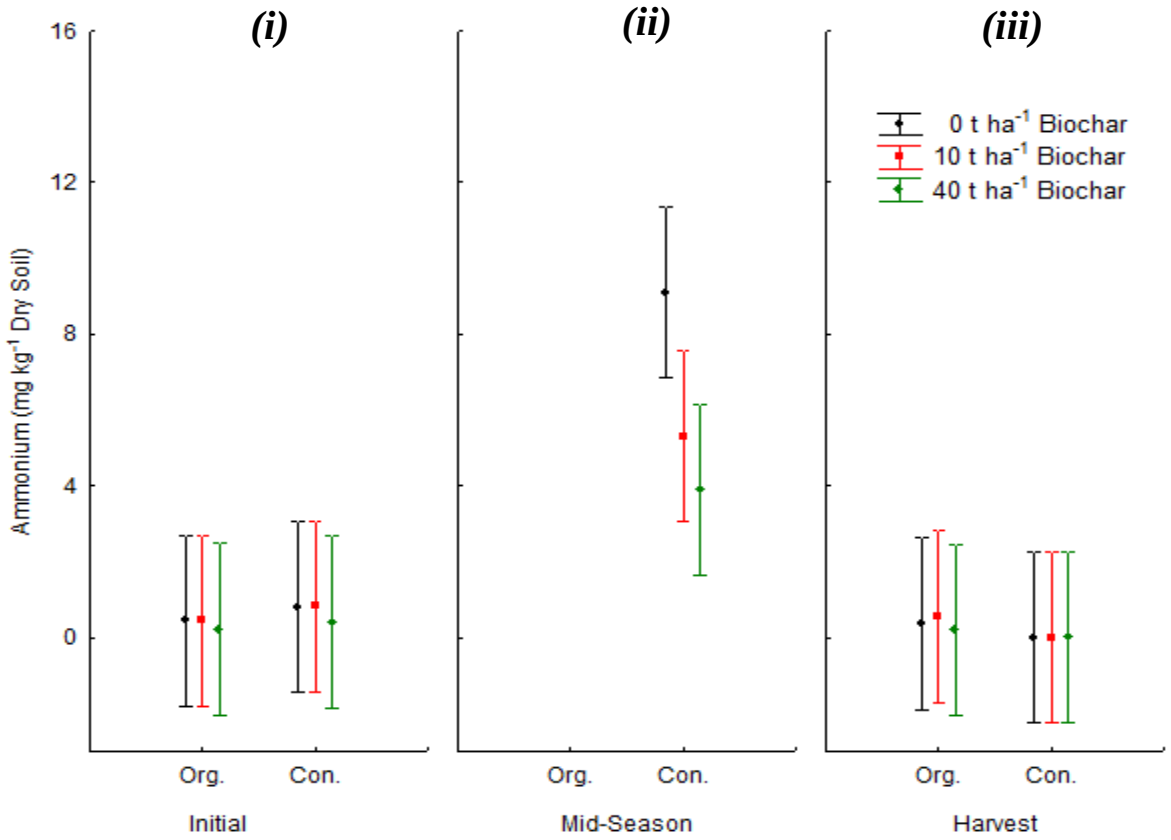


Figure 6-6: Ammonium levels over the England field trial. Bars are standard errors ($N = 3$). 'Initial' refers to plots before the application of biochar rather than the amount of biochar applied. No data is available for the organically managed soil at mid-season sampling.

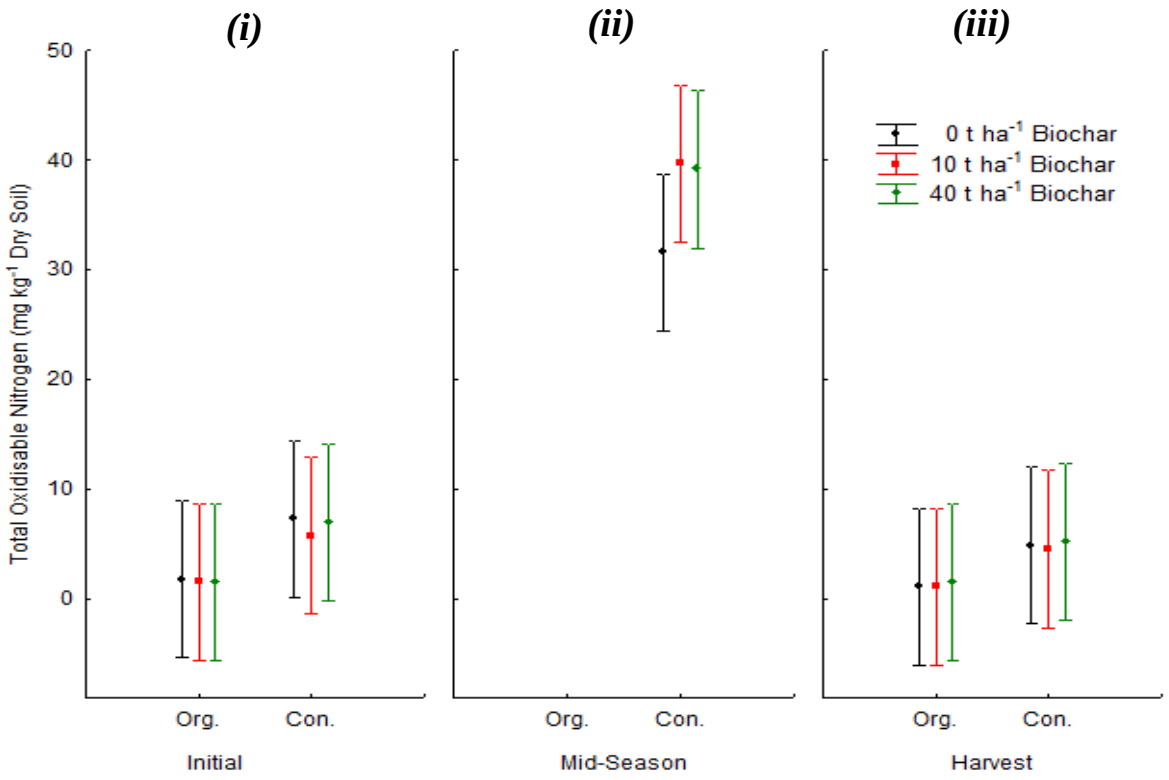


Figure 6-7: Total oxidisable nitrogen (TON) levels over the England field trial. Bars are standard errors (*N = 3*). 'Initial' refers to plots before the application of biochar rather than the amount of biochar applied. No data is available for the organically managed soil mid-season.

6.5.3 England Trials: Crop Yields

A comparison between the two systems cannot be made due to difference in crop types. Under organically managed soil, increasing biochar application rate did not affect crop growth (yields of 283, 239 and 284 g m^{-2} with 0 to 10 and 40 t ha^{-1} respectively; Figure 6-8). Under conventional management, increasing the biochar rate to 40 t ha^{-1} lowered the crop yield ($P = 0.03$) from 1014 g m^{-2} to 872.

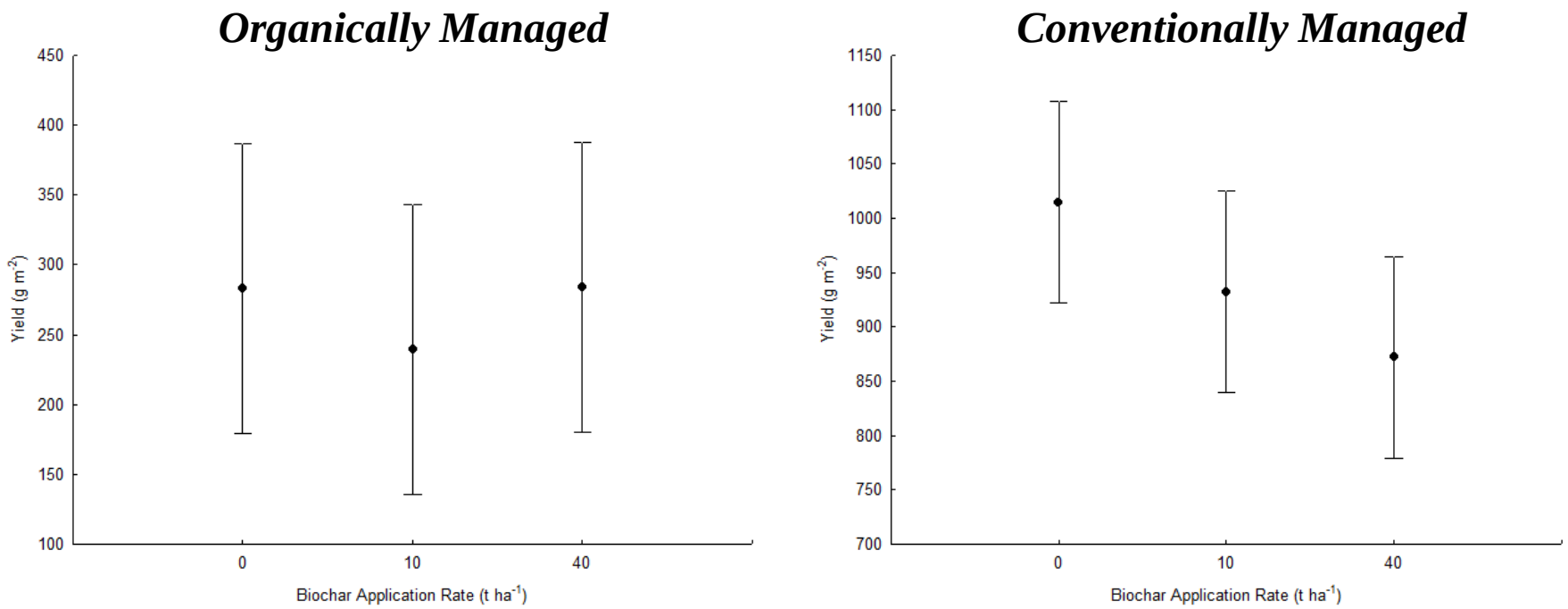


Figure 6-8: Crop yields for the England field trial, for both organically conventionally managed farms. Bars are standard errors (*N = 3*).

6.5.4 Scotland Trials: Soil Moisture Content

Initially SMC did not differ between plots for either the organically or conventionally managed system, though SMC was typically higher in the organically managed soil (47.8% by mass) than the conventional (28.4% by mass) as shown in Figure 6-9.

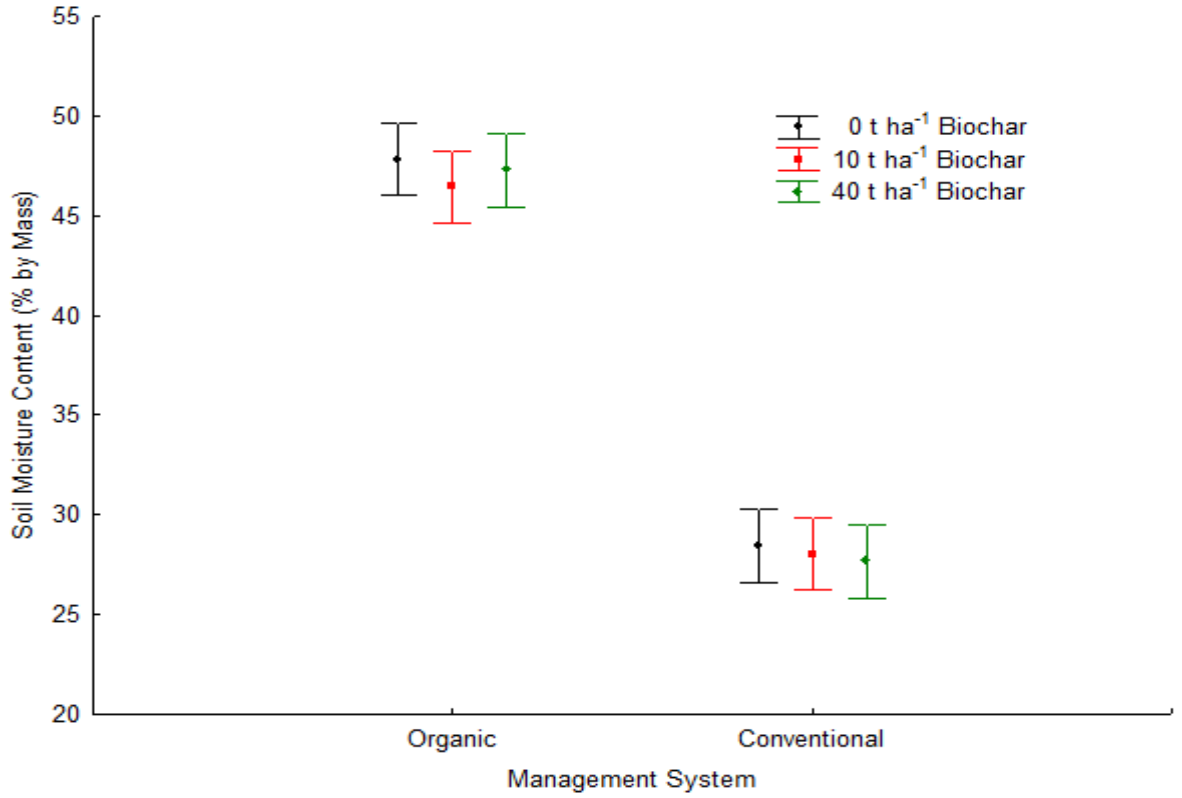


Figure 6-9: Initial soil moisture contents (SMC) of the Scotland field trial before the application of biochar. Bars are standard errors (N = 3).

Over season 1, increasing the biochar application rate in the organically managed soil increased the mean SMC at both the mid-season (from the control of 38.1% by mass to 40.4 and 41.9 respectively) and harvest sampling times (from 37.4% by mass to 39.2 and 40.8 respectively) as shown in Figure 6-10.

In the conventionally managed soil, the biochar addition of 40 t ha^{-1} increased SMC at the harvest sampling from 15.6% (control) to 18.1%, but not at mid-season.

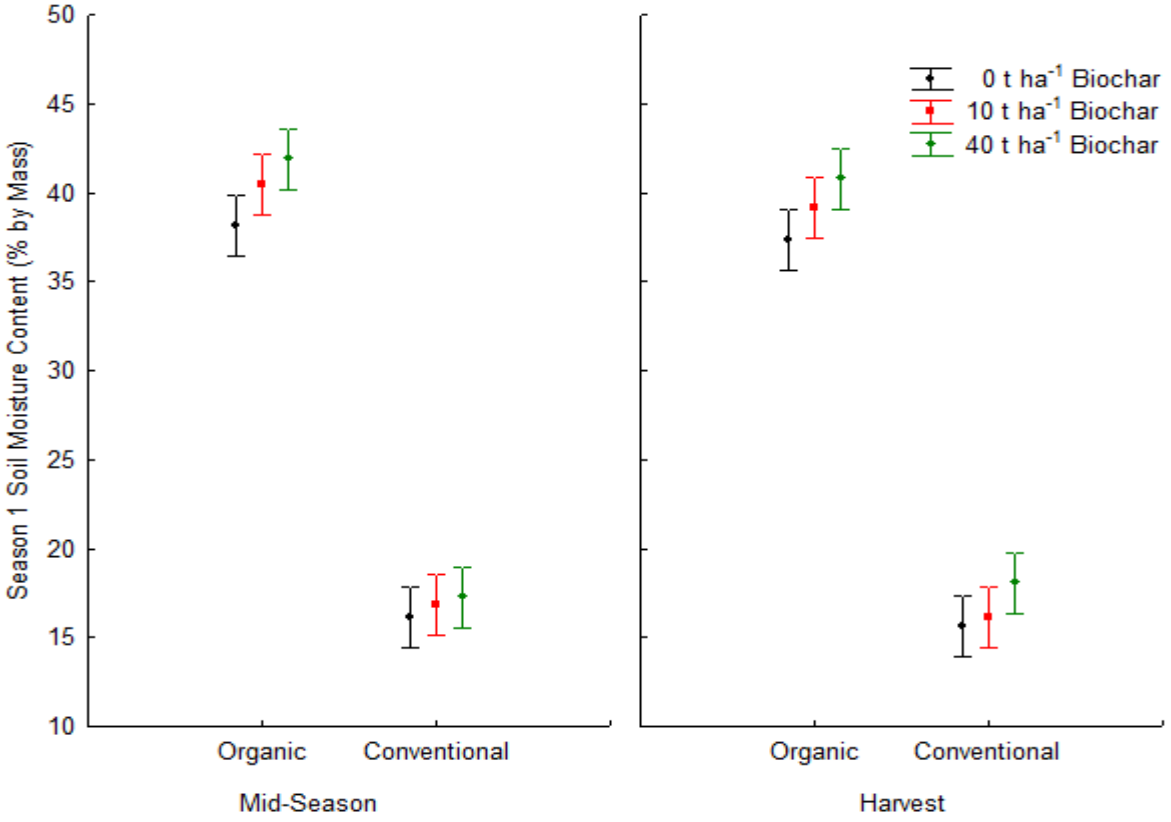


Figure 6-10: Soil moisture contents (SMC) over Season 1 (after the application of biochar) of the Scotland trials. Bars are standard errors (N = 3).

Subsequently to season 1, (seasons 2 & 3), no effect of biochar was observed (Figure 6-11 & Figure 6-12 respectively). It was indicated however, that adding could have reduced the SMC in the organically managed soil at the harvest of Season 3 (Figure 6-12) as significance was approached according to the Fisher's test of LSD (P = 0.056 and 0.06 with the application of 10 and 40 t ha^{-1} biochar respectively).

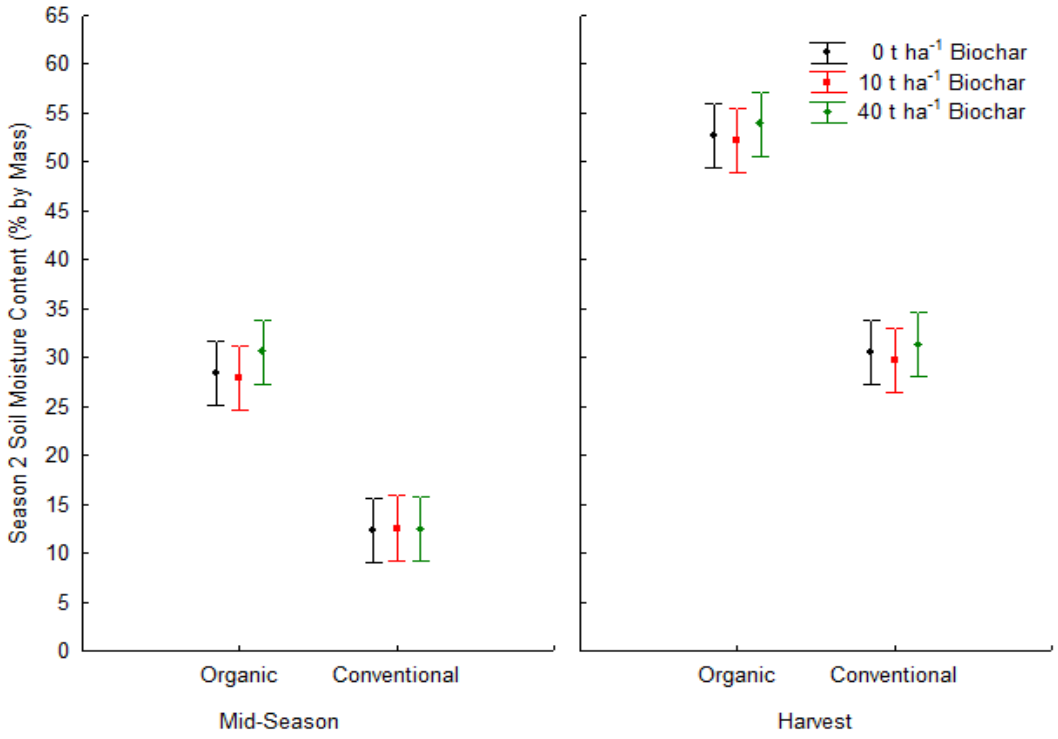


Figure 6-11: Soil moisture content (SMC) over Season 2 of the Scotland field trial. Bars are standard errors (N = 3).

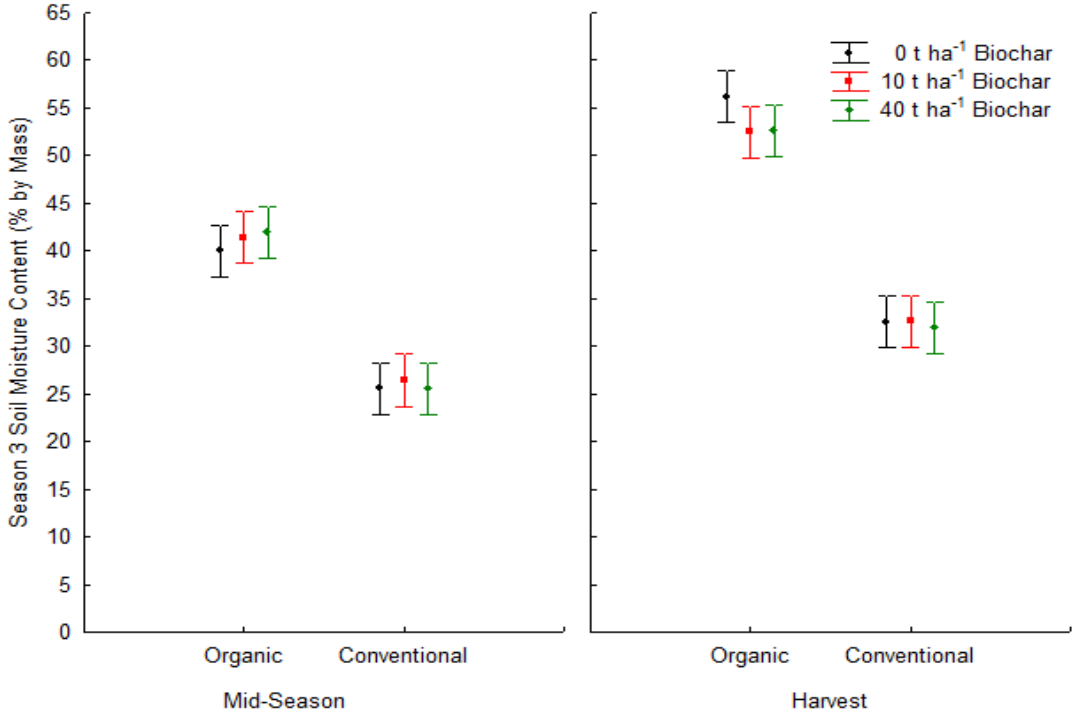


Figure 6-12: Soil moisture content (SMC) over Season 3 of the Scotland field trial. Bars are standard errors (N = 3).

6.5.5 Scotland Trials: Plant Available Nitrogen

Under the organically managed system, ammonium levels did not change from the initial sampling through the three seasons. Levels were low and remained below 2.5 mg kg^{-1}. The addition of biochar also did not affect the levels of ammonium in the soil.

Under the conventionally managed system, ammonium levels increased at the mid-season sampling for the first two seasons, although ammonium levels did change with application of biochar, these differences were not consistent with application rates (Figure 6-13).

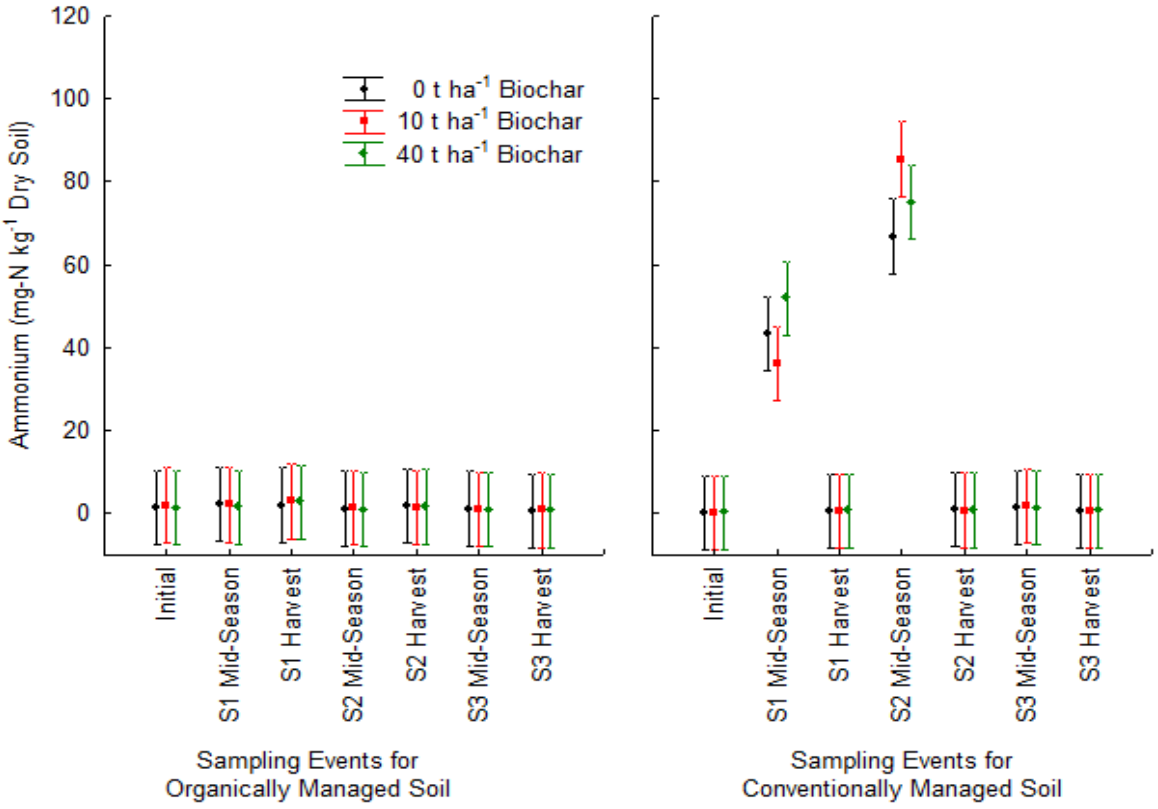


Figure 6-13: Ammonium levels over the Scotland field trial. 'Initial' refers to plots before the application of biochar rather than the amount of biochar applied. Bars are standard errors (N = 3).

Under organic management, TON levels were lower with both biochar application rates at the mid-season soil sampling in the first season. Under the conventionally managed system, the addition of 40 t ha^{-1} biochar reduced TON levels were lower at mid-season season 2 (Figure 6-14).

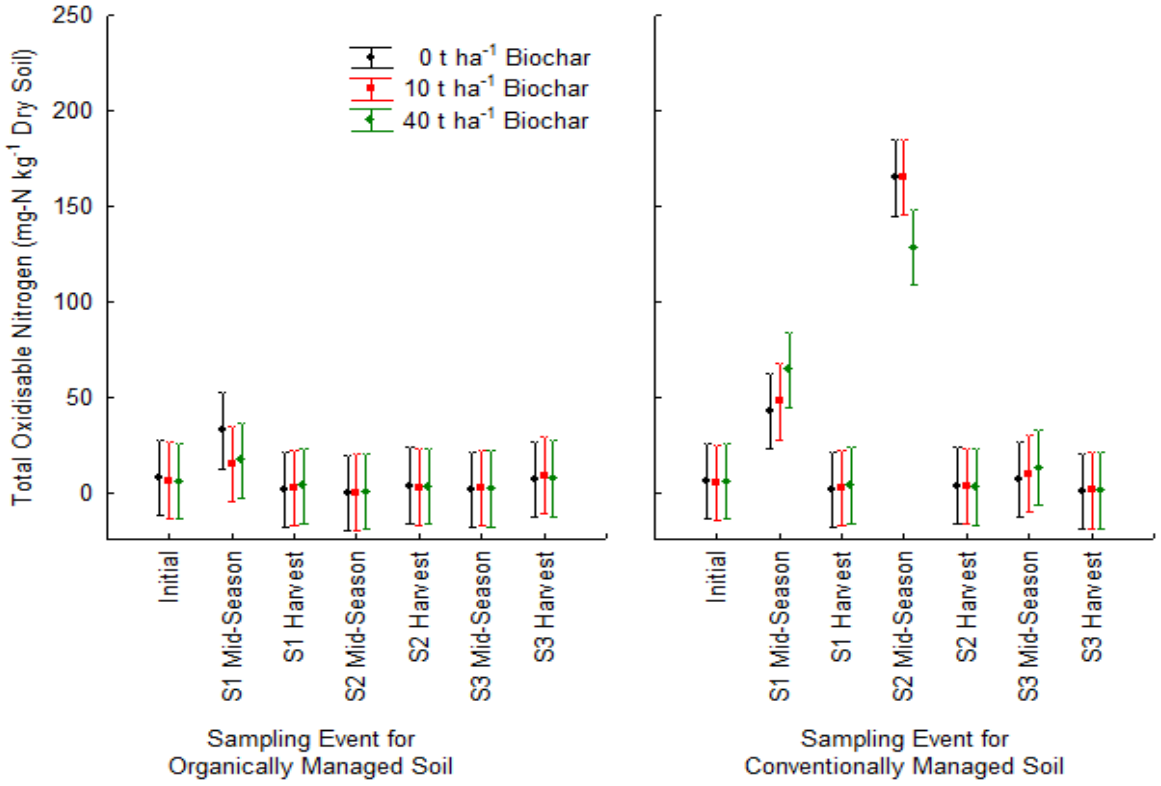


Figure 6-14: TON levels over the Scotland field trial. 'Initial' refers to plots before the application of biochar rather than the amount of biochar applied. Bars are standard errors (N = 3).

6.5.6 Scotland Trials: Crop Yields

In the conventionally managed soil, increasing the application rate of biochar produced higher crop yields from 616 g m^{-2} (control) to 738 and 837 g m^{-2} (for 10 and 40 t ha^{-1} biochar application rates respectively). In the organically managed soil, crop yield reduced with 10 t ha^{-1} biochar in season 1 (from (773 g m^{-2} to 555), but 40 t ha^{-1} biochar did not affect the yield (793 g m^{-2}) compared to the control (Figure 6-15).

Subsequently to season 1, biochar did not elicit an effect on crop yield. Yield from the conventional plots in season 3 was not taken due to an error in sampling times.

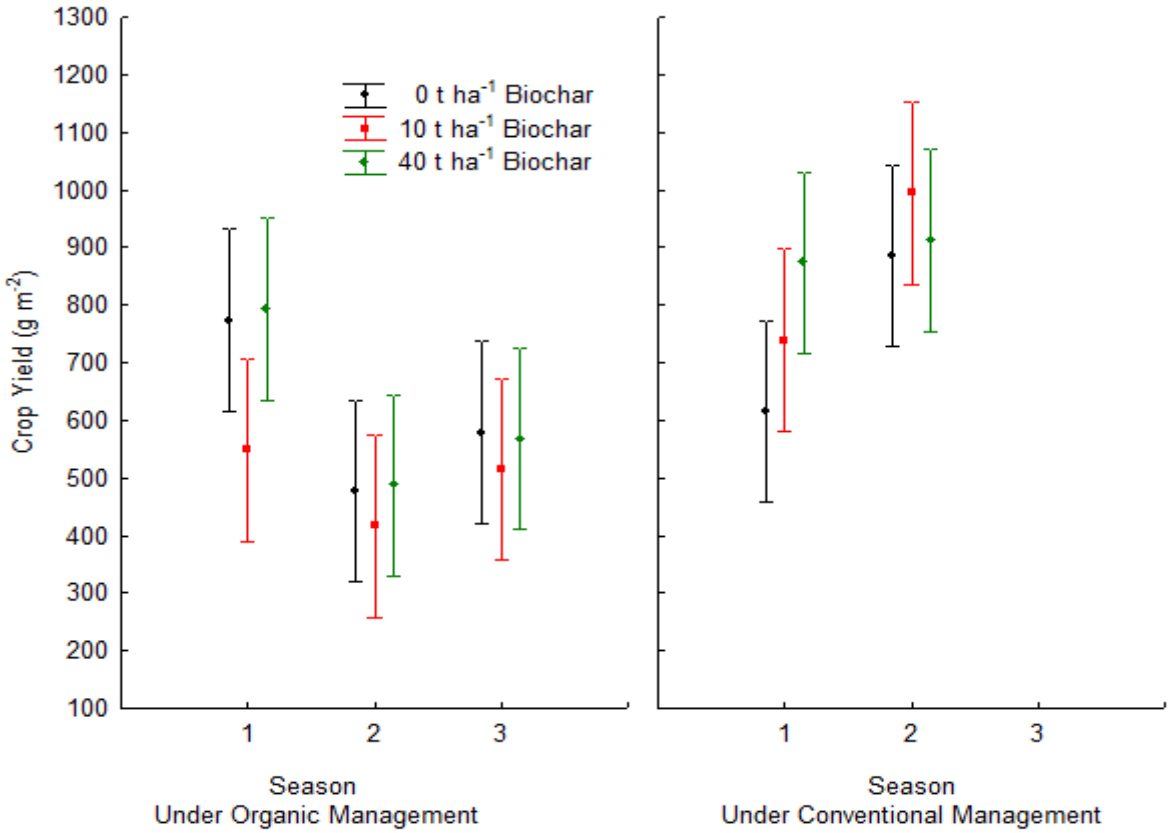


Figure 6-15: Crop yields over the Scotland field trial. Bars are standard errors (N = 3).

6.6 Chapter Discussion

6.6.1 Soil Water Retention

Initial measurements (prior to the application of biochar) showed there were no significant differences in SMC between plots within the organically and conventionally managed soils. This indicates that there was no spatial variability in SMC that might have affected results after biochar application. This was true for both the England and Scotland trials.

The addition of biochar to the soil did show some increases in SMC, though these were inconsistent. Within the England trial, and the first season of the Scotland trial, some SMCs were observed to be higher with the addition of biochar. Many studies have also shown higher water retention in soils with addition of biochar, though the investigation of how biochar can ameliorate soil physical properties is not comprehensive and could be improved (Abel et al., 2013). Many studies focus on overall effects such as water holding capacity (WHC) and available water. Beck et al. (2011), showed that the addition of 7% biochar to a sandy soil increased water retention and thus reduced leaching in a turf-grass roof. Asai et al. 2009) also showed improvements in WHC in addition to greater permeability to water. A short-term (4 months) field trial (Liu et al., 2012) showed that after 2 months the addition of a compost/ biochar mixture (20 t ha^{-1} biochar) increased the volumetric soil water content by a factor of 2 when compared to just a compost addition.

Mulcahy et al. (2013) suggested that biochar addition to soils could provide higher resistance to drought particularly in sandy soils due to their rapid drainage. Given this suggestion that biochar might be effective for resisting water restriction, it could be expected that the biochar amended plots would show greater improvements to SMC in soils with lower water potentials. Results however, showed that improvements in water retention were observed over a range of water contents.

The effects biochar has on the soil's properties is dependent on factors such as feedstock, production temperatures and soil type (Mukherjee & Lal, 2013), hydrological properties in particular are linked to changes in surface area, porosity and bulk density, primarily due to changes in the distribution and connectivity of pores (Manyà, 2012). Given that results and effects of biochar can vary so much according to feedstock and that the properties of the biochar can also vary there is a need to link the specific properties of this biochar to the effects observed.

Given that, according to the mercury porosimetry results, the highest frequency of pores were found in the larger pore sizes, this could explain the improvement in water retention at higher water potentials. This is supported by the WRC which also showed that increases in water retention were found as soil water potential increased towards field capacity.

There could also be an effect of biochar movement with each year's tillage. This could spread out the biochar over time and dilute the biochar's effect over time. A larger plot size would help negate these effects. Although it has been suggested by Wang et al. (2013) that this effect is more pronounced with smaller biochar particles with an average size of 100 nm, when compared to larger particles of less than 2 µm.

Although increases in SOM have been linked with greater soil water retention (Toth et al., 2007) the differences in SMC between organically and conventionally managed soils, cannot be compared due to the differences in location and potential precipitation. A larger number of both organic and conventionally managed farms in various locations would allow a more accurate comparison of systems.

6.6.2 Plant Available Nitrogen

Before the application of biochar the little available nitrogen (ammonium and TON) present, and could be due to uptake by the previous year's plant growth and none replaced through fertilisation or through leaching.

Regular increases in available nitrogen at the mid-season's sampling events are due to the application of fertilisers; in the England trials, at the mid-season sampling event, ammonium and TON levels were higher for this reason. Here irrespective of the rate, the application of biochar lowered the ammonium levels and increased nitrate levels. This could imply greater nitrification as found in the nitrification incubation. Greater nitrate release from nitrification is important for increased crop growth

It is not possible to form strong conclusions about the effect of biochar on nitrogen in the field setting as for the majority of the time, both ammonium and nitrate levels are low, probably due to the lability of nitrogen and fast uptake by the plants. The occasional decrease in TON with biochar in the Scotland trials is opposite to that found in the England trial, though there is no complementary change in ammonium.

Larger scale trials would provide more reliable results. Larger plots would eliminate potential edge effects and a more representative sampling procedure.

6.6.3 Crop yields

Some changes in crop yield were noted with the application of biochar, over the first season's growth for both the Scotland and England trials. These changes however, were not consistent with the rate of biochar application. Within the Scotland's first season, due to a low sample size taken (0.25 m^2 per plot) it could be that the differences are an incorrect rejection of the null hypothesis (type I Error) and that either greater number of samples was required or larger samples.

To counteract this, future crop sample sizes were increased to 2 x $1m^2$ samples. Following this alteration, no significant differences in crop yield were seen with the application of biochar. It cannot be shown, whether the lack of significance in seasons 2 and 3 are due to a more representative sampling procedure or that biochar only offered a short term improvement in crop yields as water content.

With a realistic setting such as a field trial, many variables cannot be controlled. This includes controlling the crop type. As such it is not possible to compare over the three years for the Scotland trial. The effect of biochar on crop yield, particularly in a field environment can show varying effects (Jeffery et al., 2011) dependent on various parameters including crop type.

6.7 Chapter Conclusions

Biochar has shown some beneficial effects in the field scale environment, such as increases in water storage capacity of the soil. These effects did not persist in the longer 3 year trial in Scotland. Larger scale plots would help prevent any cross contamination and measurement inaccuracies the may have contributed to this effect. Any changes in SMC at this stage appears to be short-lived. Changes in nitrogen are few and far between but can offer glimpses into potential effects of biochar. These changes however are soon eradicated by uptake into plants.

Any effect of the biochar on crop yield is varied and inconsistent. Comparison between years and systems are hindered by changes in crop type and location. It is unlikely that differences in crop yield are a product of biochar but of sampling misrepresentation.

A greater number of trials across the area would allow a comparison between management systems, though maintaining comparable parameters such as crop type, fertiliser type and timings would remain a difficulty.

7 INTEGRATED DISCUSSION

7.1 Introduction

This chapter discusses the various experiments contained within the study and aims to assimilate the results of the water dynamics' experiments and the nitrogen incubations, comparing these to the characteristics of the biochar and ultimately how the biochar might affect consequent crop growth with the glasshouse study and field trials. A synthesis of how the experiments relate to the objectives and to each other, as first mentioned in **Chapter 1**, can be found in Figure 7-1.

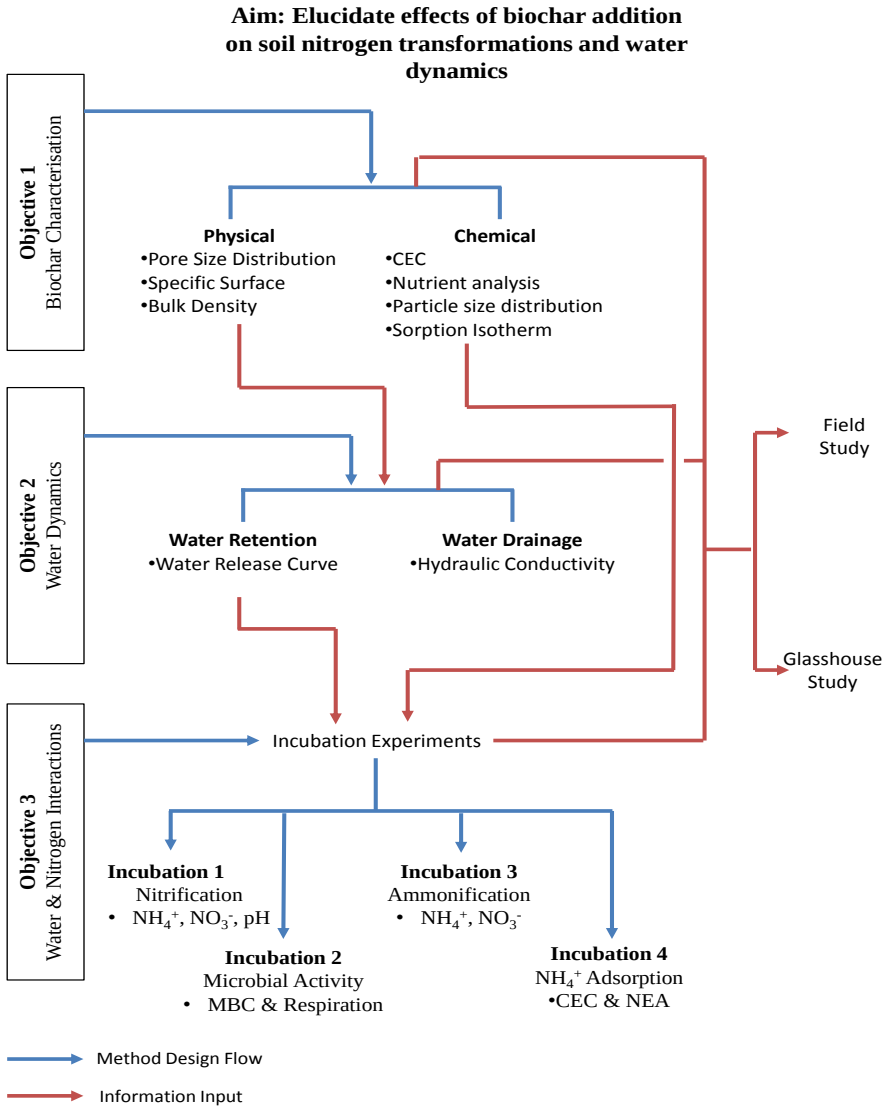


Figure 7-1: Schematic showing the experimental aspects of the projects and how they relate to the objectives.

7.2 Impacts of Biochar on Water Dynamics

It was hypothesised that the addition of a porous material such as biochar (Atkinson et al., 2010), would increase water retention in both the organically and conventionally managed sandy loam soils through a lowering in bulk density increasing water availability. It was also hypothesised that the increase in porosity and reduction in bulk density would increase the hydraulic conductivity of the soil.

The dry bulk density measurements of the WRC rings showed lower densities with increasing biochar application rate (Figure 3-4) but only in soils that did not receive aging by incubation; this was attributed to a difference in packing quality. The change in bulk density can be directly linked to the change in porosity caused by the biochar. The lowering of the bulk density and the consequent increase in water retention is therefore attributed to the high porosity of the biochar.

The low bulk density and thus high porosity of biochars is well documented (Verheijen et al., 2010). Physical characteristics of the biochar are controlled by the parameters during production and the feedstocks (Mukherjee & Lal, 2013), with increased porosity associated with higher production temperatures (Mukome et al., 2013). The total pore volume of the biochar used in the study was 1.31 $cm^3\ g^{-1}$ (N = 2; **Chapter 2**). Biochar from a Pine and Oak feedstock produced at similar temperatures were found to have total pore volumes of 0.61 and 0.45 $cm^3\ g^{-1}$ (Manyà, 2012), showing that the biochar used in the current study had a high porosity.

The porosity of a biochar determines the surface area also and results in a correlation between the two (Atkinson et al., 2010). This was not reflected by the biochar used in the study. The high porosity of the biochar was shown to have a low surface area of 13.99 $m^2\ g^{-1}$ as measured by BET (Brunauer-Emmett-Teller) nitrogen adsorption. This is thought to be due to the presence of volatiles on the surface that prevented full adsorption of the nitrogen. These volatiles, although lower in biochars produced from high-temperature pyrolysis (Deenik et al., 2010), are still present in similar biochars (Figure S1-1).

An increase in water retention was not observed over all the soil water potentials (SWPs) (Figure 3-5). There was no difference in water retention at the lower SWPs (-0.5 kPa and less) or at saturation (0 kPa). The strength of water retention is due to the distribution of pore sizes. Biochars do not have a consistent pore size distribution and is dependant again on

feedstock and production temperature (Mukherjee & Lal, 2013). Mercury porosimetry showed that the largest frequency of pore sizes were in a bi-modal distribution between 1 and 50 µm, small contribution to the overall pore volume was through pore sizes over 50 µm and under 1 µm (**Chapter 2:** Figure 2-7). These pores would contribute to the retention of water at low and high SWPs respectively. It is the large frequency of pores between 1 and 50 µm that could be contributing to the retention of water in the specific ranges of SWPs.

Laird et al. (2010) found during a WRC from saturation (0 kPa) to the permanent wilting point (-1500 kPa) that the addition of a hardwood biochar to a loamy soil, increased the retention of water up to 15%, but only between -5 and -500 kPa SWP. This is similar to the results found in the current study; however a direct comparison between biochar studies can be problematic due to the wide variety of pore size distributions possible that change with biochar type. Thus biochar does not have a typical response in the soil and future research could include modelling to predict likely response to the application of certain soil types.

The potential for biochar to increase plant available water content is highlighted as a potential advantage of biochar use (Brockhoff, 2010). It was noted from the field trials that sporadic increases in water retention with increasing biochar application rate occurred under both soil management systems, in both field locations and at varying SMCs.

Changes in soil water retention are due to changes in the physical characteristics of the soil. The inconsistent nature of the increases in the field study could reveal the effects of the irregular distribution of pores within the biochar (Chapter 2: Figure 2-7). This was revealed in the restricted effect of the biochar in the WRC experiment. The field experiment however could be exhibiting more than the direct effects of the biochar's porosity, over time indirect effect of the biochar can affect the soil's physical properties. Research showing the direct link of biochar to increased aggregation is limited, however it has been shown that biochar can increase mycorrhizal fungi through alteration of physio-chemical properties such as elevated bio-available nutrients and pH (Warnock et al., 2007) which can stimulate aggregation (Koide et al., 2011). Busscher et al. (2010) however showed positive direct effects of the biochar on water retention but did not observe any effects on aggregation, and attributed their lack of effect a potential interaction of the specific biochar used (pecan shells) and the temperature during production (700˚ C).

Compression and higher bulk densities lowers the available macro-pore space and thus the proportion of air to water ratio in a soil (Beylich et al., 2010). Gregory et al. (2010) showed

that the pores most susceptible to compression were those larger than 30 µm, equivalent of a SWP of -10 kPa. Results indicate therefore that the application of the biochar to the sandy loam soil on a larger scale may show some beneficial increase in water retention at specifically higher water potentials, particularly in sandy soils that show poor water retention and soils that exhibit low organic matter.

The effect of the biochar additions on the soil bulk density cannot be translated to the field trials. It was not possible to measure bulk density in the field trials due to the presence of large stones. Also, the objective of the work was to determine the effects of biochar on water retention and conductivity and thus identical methodological formats were used to compare the two values. This could not be done if using two different methodological approaches such as laboratory and field trials. However previous research indicates that the addition of biochar reduces the bulk density of the soil (Case et al., 2012; Dugan et al., 2010).

The saturated hydraulic conductivity experiment is laboratory based and measured the direct effect of the biochar addition, namely the porosity. Draining water from saturation is attributed to the movement of water through macropores that do not hold water by gravity (Lipiec et al., 2006). It was shown that the addition of biochar did not affect the saturated hydraulic conductivity of the soil and as such could be attributed to the low proportion of macro-pores (between 50 and 200 µm) within the biochar itself as described by the mercury porosimetry analysis or due to a difference in packing consistency at two separate events.

Previous research however has shown that, the addition of biochar can increase hydraulic conductivity (Clough et al., 2013; Asai et al., 2009). This can depend on the specific pore size distribution of the type of biochar used, and on the indirect effects of the biochar on the soil properties that develop over time such as increased aggregation and improved structure as mentioned previously (Koide et al., 2011; Busscher et al., 2010).

Influences such as aggregation would not occur in the destructive soil environment used in the experiment and highlights the restrictions of using such controlled samples, but the experiment helps to ascertain the direct effect of pores size. An interesting comparison would determine the relative effects of direct biochar addition, in terms of porosity, and the indirect aggregation effects by comparing ‘disturbed’ and ‘undisturbed’ field sample cores.

7.3 Impacts of Biochar on Nitrogen Dynamics

7.3.1 Nitrogen Mineralisation

Although different plants can utilise several forms of nitrogen including organic nitrogen (Jones et al., 2005), the release of inorganic nitrogen, via mineralisation, is still important in these systems as the main supporter of plant growth (Nordin et al., 2001; Burgos, 2006).

The mineralisation of organic matter is controlled by several environmental factors including soil moisture content (SMC), pH and SOM composition (Thangarajan et al., 2013; Guntiñas et al., 2012). As the C:N ratio of a substrate increases, nitrogen mineralisation decreases, an optimum ratio for nitrogen mineralisation was estimated between 15 and 40 (Burgos, 2006). The slower rate of nitrogen mineralisation in the organically managed soil was attributed to a higher overall C:N ratio; a product of adding GWC, which had a C:N ratio of 13 to the organically managed soil of 9.6. This is in comparison to the conventionally managed soil which had a C:N ratio of 9.3 with an ammonically based fertiliser (C:N ratio of 0). Results of the soil and fertiliser analysis can be found in **Chapter 2**. The compost, with a C:N ratio of 13, still exhibits the potential for net mineralisation but is below the optimum ratio range from 15 to 40.

Nitrogen mineralisation is characterised by the production of ammonium ions. Increasing the application rate of biochar reduced the concentration of ammonium in the soil for both the organically and conventionally managed soils. The addition of biochar could have reduced the soil ammonium levels via several pathways; these data alone cannot ascertain the mechanism of this reduction, though the addition of Dicyandiamide (DCD) nitrification inhibitor eliminates conversion to nitrate as a cause. Indeed, it was confirmed over the 60 day incubation (with a Spearman's rank correlation co-efficient of 0.05) that nitrate levels, although fluctuated, did not change.

It is argued here that the primary cause of the reduction in the levels of extractable ammonium is due to increased adsorption to the biochar as opposed to a reduction in mineralisation rate or volatilisation. This conclusion was following a series of further incubation experiments, detailed below.

Ammonium is considered less mobile and less liable to leach out of a soil than nitrate due to the cation exchange capacity of a soil being typically higher than the anion exchange capacity. Ammonium ions held onto cation exchange sites, have the potential to be replaced

by other cations and as such are largely available for uptake by plants and microbes for synthesis into organic nitrogen (Taghizadeh-Toosi et al., 2011b; Clough et al., 2013). These are also said to be extractable through chemical means (notably Potassium chloride) as an indication for availability.

The binding of ammonium to the extent that the ions cannot be readily exchanged i.e. non-exchangeable ammonium (also known as fixed ammonium) was measured over an incubation period and it was shown that the addition of 30 t ha^{-1} and 60 t ha^{-1} biochar increased these levels as much as 20 and 40% respectively compared to the control. Despite the higher cation exchange capacity (CEC) of the biochar itself (66.33 cmol+ kg^{-1} ± 1.72), the addition of biochar did not increase the CEC of the soil. As such, this retention could be through an alternate mechanism. It is noted that ammonium ions can enter between layers of a clay mineral and become trapped without cation exchange (Nieder et al., 2010). It has been suggested that ammonium can be retained by biochar through physical entrapment in the wide range of pore structures available (Saleh et al., 2012; Clough et al., 2013).

It was concluded that a lowering of mineralisation rate is an unlikely mechanism for the reduction in ammonium concentration. Mineralisation can occur through a variety of microbial species, though there is suggestion that mineralisation is also controlled by abiotic processes (Kemmitt et al., 2008). The relative impact of biotic to abiotic mineralisation in the soil has been linked to the composition of the carbon and nitrogen sources in the organic matter; Paterson et al. (2009), noted that the presence of labile high C:N sources, mineralisation of SOM by microbes is more likely than in soils where there is limited labile substrates. It is put forward therefore, that the addition of compost and inorganic fertiliser would indicate a prevalence of microbial mineralisation over abiotic. Considering this, with the lack of a significant difference in carbon dioxide production from microbial respiration with the addition of biochar (Figure 4-15) indicates that a reduction in microbial activity is not contributing to the lower ammonium levels.

The C:N ratio of the soil can influence the mineralisation rate. With a high C:N ratio of 117.46, adding biochar to the soil could lower the mineralisation rate, however, between 50 and 90% of the biochar's carbon content is recalcitrant (Verheijen et al., 2010) and thus unavailable for microbial degradation. It is suggested that this did not cause the reduction in ammonium concentration shown.

7.3.2 Nitrification

The conversion of ammonium to nitrate (nitrification) is an important process in the soil as the rate of nitrification can regulate the relative forms of nitrogen on the soil (Barnard & Leadley, 2005). The surface chemistry of a soil can fall into two categories; permanent charge and variable charge, variable charged soils have both positive and negative charges which change according to mineral composition and pH. Permanently charged soils are more common in temperate regions, and attain their charge from clay particles (Sollins et al., 1988) thus making CEC more influential in these regions than AEC. Due to this, permanent charged soils can retain ammonium thus reducing the mobility (Xiong et al., 2010).

Adding biochar to the organically managed soil did not observably alter the ammonium levels in the soil. This was thought to be due to the low levels of ammonium present where they soon approached zero. Thus any effect of the biochar on the ammonium was not evident. By the addition of DCD, we know that that ammonium is being produced in the organically managed soil, but appears that any produced was immediately immobilised or otherwise transformed. This effect was observed under both SMCs.

The corresponding nitrate levels of the organically managed soil showed a decrease with the addition of biochar. It is surmised that the increased adsorption of ammonium to the biochar subsequently reduced the nitrification rate due to a reduction in ammonium concentration as a substrate. Although Taghizadeh-Toosi et al. (2011) noted that the majority of ammonium adsorbed to biochar is bioavailable, with a lack of effect of biochar on CEC, it is suggested that the ammonium retained by the biochar is not available for microbial transformation.

Despite no apparent effect of the biochar on the ammonium in the organically managed soil, a lower ammonium concentration was observed with the addition of biochar in the conventionally managed soil. The addition of biochar increased the adsorption of NEA for both soil management types, some of the decrease in ammonium concentration therefore could be attributed to this effect.

After considering the increase in nitrate levels with biochar application, it is evident that the fixation of ammonium is not the only factor affecting the ammonium concentration; the increase is also attributed to higher conversion to nitrate. This response occurred at both levels of SMC but with a faster rate at the higher SMC of 50% field capacity. This supports the idea that nitrification is taking place and not just adsorption to the biochar.

Organically and conventionally managed systems differ in regards to their method of crop fertilisation. This translates to a difference in nitrogen transformation and availability (Burger & Jackson, 2003). Identical biochar was added to the soils and according to Stockdale et al. (2002) the microbial communities between the two systems are similar, and yet they produced different responses to this addition of the biochar.

Table 7-1 shows a summary of the effects of biochar addition on the microbial activity in the soil. This shows that the addition of biochar to the organically managed soil did not result in a detectable change in microbial biomass carbon.

Table 7-1: Summary of microbial biomass and activity effects with the addition of biochar for each soil. N.S: Not Significant

Soil Management	Effect of biochar on Microbial Biomass	Effect of biochar on CO_2 Evolution
Organic	N.S	Increased
Conventional	Increased	N.S

As nitrification is mediated by bacteria, the microbial activity was measured by biomass carbon and carbon dioxide release. The addition of biochar could affect the microbial population through several factors. The pH increase caused by the biochar is more favourable to the nitrifying bacteria which show maximal growth in alkaline conditions (Kuenen & Robertson, 1988).

It was shown that increasing the SMC can provide more amenable environment for bacterial growth with nitrifying activity peaking at ~60% water holding capacity. However as the SMC was maintained at equal water potentials, the availability of the water for microbial uptake should also be equal (Case et al., 2012).

It was shown that in the organic soil, biochar increased microbial respiration but not the biomass. This higher microbial activity with biochar was not enough to counteract the non-exchangeable adsorption of ammonium to the biochar.

In the conventionally managed soil, the microbial biomass carbon increased but the respiration was not affected. Biochar is known to be a high carbon material (Vaccari et al., 2011). The stability of the carbon fractions within can vary according to the production

parameters, with labile carbon decreasing as pyrolysis temperature and residence time increases (Cross & Sohi, 2011). Although a small fraction, the labile carbon in biochar is a significant factor influencing the microbial activity of a soil, particularly short-term (Farrell et al., 2013) and even biochar produced at high temperatures (> 550˚ C), have small levels (up to 0.5%) of labile carbon contents (Cross & Sohi, 2011).

In the conventionally managed soil, the level of carbon substrate as TOC is lower than that found in the organically managed soil. The labile carbon in the biochar therefore, could exhibit a greater effect on the microbial biomass. The higher level of TOC in the organically managed soil could be providing the required necessary substrate therefore any extra provided by the biochar is not effective.

Overall, it is suggested that in a slow release system, the adsorption of ammonium has the potential to be greater than the release through nitrification. But in a fast nitrogen-release system this is overcome by the level of substrate available for nitrification and the higher pH could be causing the higher rate of nitrification. This could be more beneficial for soils with a lower pH, such as the conventionally managed soil shown here.

8 CONCLUSIONS

This chapter summarises the major conclusions of the study, with each conclusion presented in **bold**. Following this, details the results and research that led to this conclusion.

It was concluded that the biochar had chemical and physical attributes that were judged to be suitable for use as an amendment to the sandy loam soil.

A wide range of effects on soil properties have been noted by previous studies as a result of biochar application. In many ways the biochar used in the current study was typical of other biochars produced within similar feedstocks and peak temperatures.

Regarding physical characteristics, mercury porosimetry showed that the mean porosity of the biochar was 1.31 $cm^3\ g^{-1} \pm 0.03$. The biochar displayed a bimodal pore distribution and the majority of pores were detected between 1 and 50 µm. This indicated that the addition of the biochar might impact primarily on the soil's macro-porosity and thus could potentially alter the water retention capabilities at higher soil water potentials and also the saturated hydraulic conductivity.

Brunauer, Emmett and Teller (BET) nitrogen adsorption detected that the biochar's surface areas was between 14 to 30 $m^2\ g^{-1}$. This was lower than surface areas as suggested by previous. The difference may be due to the adsorption of volatiles. High surface areas are correlated with prevalence of micro-pores, with high surface-area : volume ratios, and the lower result may also be indicative of the dominance of larger pores.

Chemical analysis of the biochar showed that nitrogen levels (TN, ammonium and TON) were low. The low levels of inherent nutrients were indicative of the high peak temperature during pyrolysis, which increases volatilisation. The biochar showed a high degree of carbonisation with a H:C ratio of 0.04, this was comparable to similar biochars which typically show ranges between 0.01 and 0.05.

The pH (10.05) and CEC (66.33) were high in comparison to the soils. Indicating that the biochar could increase the soil's pH and CEC after application. The biochar also indicated an ability to effectively adsorb ammonium.

Within the controlled conditions, the addition of biochar increased water retention, but did not appear to influence hydraulic conductivity under both management practices.

It is known that hydrological properties are associated with physical properties such as porosity, bulk density and surface area. It was suggested that due to the experimental set-up and use of disturbed soils samples, the increase in retention was due to the direct alteration of the pore size distribution as caused by the biochar. With the addition of biochar, was also observed a reduction in bulk density which is also attributed to the increase in porosity.

Specifically, it was noted that the biochar increased water retention at higher water potentials (between 0 and -50 kPa) but not at lower, which suggested that biochar was affecting larger pore distributions compared to smaller, micro-pores. The mercury porosimetry supported this and showed that the largest frequency of pores were between 1 and 50 µm.

Some increase in water retention was observed in the field trials although it is not possible to attribute the increased retention to the direct effect of the biochar's porosity, as other factors may be contributing such as aggregation and improvements in structure.

The lack of effect of biochar on saturated hydraulic conductivity could be indicative of the limitations of using disturbed soil samples and the difficulty in achieving comparable and consistent packing densities across a column of soil.

Increasing the application rate of biochar to the organically managed soil resulted in lower nitrification rates and release of nitrate, but this did not impact on crop growth.

The lower rate of nitrification in the organically managed soil was characterised by the reduced release of nitrate and the decline in pH over the incubation period.

Enzymatic reactions are affected by the availability of the substrate; as such nitrification is affected by the ammonium levels. It was observed that ammonium did not change in response to biochar addition in the organically managed soil, though this was attributed to the levels approaching zero and thus too low to exhibit differences.

Measuring ammonium production (gross ammonium levels) through the addition of a nitrification inhibitor DCD resulted in lower gross ammonium levels with increasing biochar application rates. It was suggested that there were two potential mechanisms for this reduction: a lowering of ammonification rate or adsorption to the biochar's surface.

It is proposed that the lower gross ammonium levels are due to adsorption to the biochar. It is known that mineralisation is controlled by both microbial and abiotic processes, however in systems with presence of labile carbon such as in working agricultural soils or after the addition of fresh compost, it is expected that the microbial mineralisation is dominant. A measure of microbial activity was determined by carbon dioxide releases but this did not show any change with the addition of biochar with DCD.

Higher levels of non-exchangeable ammonium, were found however adsorbed to the biochar, though this was found to not be related to the cation exchange capacity (CEC) of the biochar which did not impact on the soil's CEC as such it is hypothesised that an alternative mechanism may be causing this such as physical entrapment.

A driving factor of nitrification is the activity of the nitrifying bacteria that process the ammonium. It is suggested that the addition of labile carbon from the biochar to the already carbon rich organically managed soil, provides excess carbon which is released producing higher respiration rates, but not affecting the microbial biomass.

Increasing the application rate of biochar to the conventionally managed soil resulted in higher nitrification rates and greater release of nitrate, but this did not impact on crop growth.

This was characterised by a lowering of ammonium levels coupled with higher release of nitrate as biochar application rate also increased. Soil pH also reduced over time (indicating nitrification) but was higher with increasing application of biochar demonstrating that the biochar was acting as a buffer to the acidification caused by nitrification.

As in the organically managed soil, measuring gross ammonium levels (after the application of the nitrification inhibitor DCD) resulted in a reduction with biochar application rate. As discussed for the organically managed soil, this was also attributed to increased adsorption due to a lack of influence on CEC and higher NEA levels. It appears that biochar is exerting the same influence on ammonium regardless of the soil management technique.

Despite this reduction in ammonium availability, nitrification did increase in the conventionally managed soil. Due to the presence of higher levels of ammonium as a result of the addition of ammoniacal fertiliser, it is suggested that the reduction in ammonium availability as caused by the biochar is outweighed by the amount of ammonium. The

increased rate of nitrification however is attributed to the increased amenable conditions provided by the biochar such as higher pH and increased water retention.

It is perhaps not surprising that the effects of and the mechanisms that govern biochar amendment to the soil on nitrogen transformations and plant growth are so variable, given that the attributes of any individual biochar can vary. A key part of future research will include highlighting underpinning characteristics of tailor-made biochars sourced from appropriate feedstocks and relate this to its effect on soil systems.

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SUPPLEMENTARY TABLES

Table S1-1: A summary of physical and chemical properties of various biochar's found in the literature. Ordered by Pyrolysis Temperature. TC: Total Carbon. TN: Total Nitrogen. CEC: Cation Exchange Capacity. VM: Volatile Matter. SA: Surface Area. Current biochar produced for this report is highlighted in blue.

Feedstock	Pyrolysis Temperature. (˚ C)	pH	TC (%)	TN (%)	C:N	CEC ($cmol+\ kg^{-1}$)	VM (%)	SA ($m^2\ g^{-1}$)	Reference
Ponderosa pine	100		50.6	0.05	1012		77.1	1.6	(Keiluweit et al., 2010)
Ponderosa pine	100		50.6	0.05	1012		77.1	2	(Spokas, 2010)
Hard Wood	200		47.2	1.29	37			0.58	(Harvey et al., 2011)
Pine	200		51.8	0.27	192			0.8	(Harvey et al., 2011)
Ponderosa pine	200		50.9	0.04	1273		77.1	2.3	(Keiluweit et al., 2010)
Ponderosa pine	200		50.9	0.04	1273		77.1	2	(Spokas, 2010)
Bubinga	250						66.4	5.4	(Zimmerman, 2010)
Laurel Oak	250						66	1.8	(Zimmerman, 2010)
Loblolly Pine	250						61.1	139.7	(Zimmerman, 2010)
Eastern Red Cedar	250						62.6	68.1	(Zimmerman, 2010)
Oak hardwood	250		55.2				66	2	(Spokas, 2010)
Corn stover	300	7.30	59.9	7.3	8	75.28	51.9		(Enders et al., 2012)
Oak	300	4.20	63.9	0.1	639	41.37	61.1		(Enders et al., 2012)
Pine	300	6.70	67.2	0.1	672	28.85	55.3		(Enders et al., 2012)
Quercus rotundifolia	300		58.8	0.3	196		65.1		(Cordero et al., 2001)
Pinus halepensis	300		57.8	0.2	289		68.1		(Cordero et al., 2001)
Hard Wood	300		62.1	1.6	39			1.28	(Harvey et al., 2011)
Pine	300		63.8	0.3	213			1.13	(Harvey et al., 2011)
Ponderosa pine	300		54.8	0.05	1096		70.3	3	(Keiluweit et al., 2010)
Ponderosa pine	300		54.8	0.05	1096		70.3	3	(Spokas, 2010)
Corn stover	350		65.2	1.2	54		48.9		(Enders et al., 2012)

Oak 350	350		74.9	0.2	375		60.8		(Enders et al., 2012)
Pine	350		70.7	0.1	707		56.3		(Enders et al., 2012)
Quercus rotundifolia	350		75.7	0.6	126		43.4		(Cordero et al., 2001)
Pinus halepensis	350		72.1	0.2	361		49.5		(Cordero et al., 2001)
Hard Wood	350		63.5	1.7	37			1.82	(Harvey et al., 2011)
Pine	350		68.3	0.4	171			2.03	(Harvey et al., 2011)
Pine wood chip	350	4.60	74.7	0.45	166		45.2	n/a	(Spokas et al., 2011)
Aspidosperma australe	350		74	1.2	62		66.8	2	(Spokas, 2010)
Aspidosperma quebracho	350		76	1.7	45		69.5	2	(Spokas, 2010)
Corn stover	400	9.20	65.2	1.1	59	79.62	44.7		(Enders et al., 2012)
Pine	400	4.60	76.3	0.1	763	30.36	45.5		(Enders et al., 2012)
Wood waste	400	6.90	76.9	0.8	96		25.8	3.5	(Spokas et al., 2011)
Quercus rotundifolia	400		76.9	0.4	192		34.5		(Cordero et al., 2001)
Pinus halepensis	400		74.7	0.2	374		36.5		(Cordero et al., 2001)
Oak	400	4.60	78.8	0.2	394	26.05	40.9		(Enders et al., 2012)
Pine Chips	400	7.55	73.9	0.255	290	7.27			(Gaskin et al., 2008)
Ponderosa pine	400		74.1	0.06	1235		36.4	28.7	(Keiluweit et al., 2010)
Eucalyptus saligna	400	7.70	69.4	0.21	330	47			(Singh et al., 2010)
Eucalyptus saligna	400	6.90	69.7	0.21	332	39			(Singh et al., 2010)
Beech sawdust	400		84.3				16.3		(Spokas, 2010)
Oak hardwood	400		69.6				52	2	(Spokas, 2010)
Ponderosa pine	400		74.1	0.06	1235		36.4	29	(Spokas, 2010)
Bubinga	400						41.1	6.1	(Zimmerman, 2010)
Laurel Oak	400						51.9	2.2	(Zimmerman, 2010)
Loblolly Pine	400						58.6	2.9	(Zimmerman, 2010)
Eastern Red Cedar	400						52	7.2	(Zimmerman, 2010)
Wood	410	7.10	65.7	0.21	313	10		2.82	(Mukome et al., 2013)
Corn stover	410		42.1	1	42			2.23	(Spokas & Reicosky, 2009)

Corn stover	410		42.1	1	42			2	(Spokas, 2010)
Corn stover	450		68.3	1.1	62		42.7		(Enders et al., 2012)
Oak	450		85.1	0.2	426		44.4		(Enders et al., 2012)
Pine	450		80.5	0.1	805		48.8		(Enders et al., 2012)
Wood waste	450	8.40	77.9	0.7	111		22.8	26.8	(Spokas et al., 2011)
Quercus rotundifolia	450		81.2	0.4	203		21.8		(Cordero et al., 2001)
Pinus halepensis	450		78.3	0.2	392		27.4		(Cordero et al., 2001)
Corn stover	450		33.2	1.4	24		12.7	12	(Spokas, 2010)
Maple	450		70.87	1.19	60			6.74	(Zheng et al., 2010)
Elm	450		70.66	1.21	58			7.29	(Zheng et al., 2010)
Oak woodchips	450		71.79	1.15	62			7.57	(Zheng et al., 2010)
Oak barks	450		71.18	1.15	62			7.56	(Zheng et al., 2010)
Pine wood chip	465	6.80	75	0.3	250		34.9	0.1	(Spokas et al., 2011)
Pine wood chip	465	6.80	71	0.2	355		72.3	0.2	(Spokas et al., 2011)
Pine woodchips	465		74.5	0.3	248			0.1	(Spokas & Reicosky, 2009)
Pine woodchips	465		71.2	0.2	356			0.19	(Spokas & Reicosky, 2009)
Pine wood chip	465		74.5	0.3	248			<1	(Spokas, 2010)
Pine wood chip	465		71.2	0.2	356			<1	(Spokas, 2010)
Corn stover	500	9.90	70.3	1.1	64	51.66	31.1		(Enders et al., 2012)
Oak	500	5.80	85.3	0.2	427	14.72	30.7		(Enders et al., 2012)
Mixed Hardwood	500						56.8		(Enders et al., 2012)
Wood Waste	500	5.00	68.7	0.1	687		33.6	66.3	(Spokas et al., 2011)
Pine wood chip	500	7.20	87.2	0.43	203		45.8		(Spokas et al., 2011)
Pine wood chip	500	7.30	73.3	0.2	367				(Spokas et al., 2011)
Oak	500	8.90	72.4	0.4	181		n/a	n/a	(Spokas et al., 2011)
Oak (sawdust)	500	8.00	61.8	0.21	294		5	46	(Spokas et al., 2011)
Corn stover	500		37.8	0.8	47		14.9	7	(Brewer et al., 2009)
Corn stover	500		62.8	1.3	48		11.1	20.9	(Brewer et al., 2009)

Quercus rotundifolia	500		83	0.6	138		17.5		(Cordero et al., 2001)
Pinus halepensis	500		81.8	0.2	409		20.2		(Cordero et al., 2001)
Mixed Softwood	500						45.5		(Enders et al., 2012)
Pine	500	5.60	83.4	0.1	834	23.97	37		(Enders et al., 2012)
Pine	500								(Enders et al., 2012)
Mixed Woodchips	500	7.90	85.9	0.4	215		26.9		(Enders et al., 2012)
Pine Chips	500	8.30	81.7	0.223	366	5.03			(Gaskin et al., 2008)
Ponderosa pine	500		81.9	0.08	1024		25.2	196	(Keiluweit et al., 2010)
Corn stover	500		24.6	0.6	41			4.2	(Spokas & Reicosky, 2009)
Corn stover	500		62.8	1.3	48		11.1	21	(Spokas, 2010)
Corn stover	500		37.8	0.8	47		14.9	7	(Spokas, 2010)
Corn stover	500		24.6	0.6	41			4	(Spokas, 2010)
Hardwood sawdust	500		67	0.3	223		29	10	(Spokas, 2010)
Ponderosa Pine	500		81.9	0.08	1024		25.2	196	(Spokas, 2010)
Corn stover	505		65.7	1.2	55			17.3	(Spokas & Reicosky, 2009)
Corn stover	505		65.7	1.2	55			17	(Spokas, 2010)
Wood feedstock	510	7.30	83.9	0.36	233	12		165.8	(Mukome et al., 2013)
Corn stover	515		84.6	0.5	169		28.3		(Enders et al., 2012)
Corn stover	515		50.7	1	51			9.85	(Spokas & Reicosky, 2009)
Corn stover	515		50.7	1	51		10		(Spokas, 2010)
Charcoal Green	520	9.20	87.3	0.59	148	9.13		164.1	(Mukome et al., 2013)
Oak hardwood	525		75.1				36	38	(Spokas, 2010)
Bubinga	525						35	500.9	(Zimmerman, 2010)
Laurel Oak	525						36.4	38.2	(Zimmerman, 2010)
Loblolly Pine	525						25.7	206.1	(Zimmerman, 2010)
Eastern Red Cedar	525						39.1	386.5	(Zimmerman, 2010)
Oak	538	9.80	53.4	0.4	134		32.5	33.7	(Spokas et al., 2011)
Hardwood char	538		53	0.4	133			7.2	(Spokas & Reicosky, 2009)

Hardwood char	538		93	0.7	133			7	(Spokas, 2010)
Oak	540	6.60	73.3	0.3	244		n/a	n/a	(Spokas et al., 2011)
Oak	550	10.20	52	0.2	260		4.3	134.8	(Spokas et al., 2011)
Oak	550	10.50	56	0.2	280		4.4	116.8	(Spokas et al., 2011)
Quercus rotundifolia	550		87.1	0.5	174		14.7		(Cordero et al., 2001)
Pinus halepensis	550		86.1	0.2	431		18.1		(Cordero et al., 2001)
Corn stover	550		72.2	1	72		37.3		(Enders et al., 2012)
Oak	550		87.9	0.2	440		38.5		(Enders et al., 2012)
Pine	550		86.8	0.1	868		40.2		(Enders et al., 2012)
Eucalyptus saligna	550	9.50	79.2	0.23	344	39			(Singh et al., 2010)
Eucalyptus saligna	550	8.80	83.6	0.26	322	35			(Singh et al., 2010)
Mixed woodchip	550		71.1	0.11	646		33.6	66	(Spokas, 2010)
Corn stover	600	9.90	41.6	0.4	104	278		178	(Hale et al., 2011)
Quercus rotundifolia	600		89.4	0.4	224		13.2		(Cordero et al., 2001)
Pinus halepensis	600		87.4	0.3	291		13.4		(Cordero et al., 2001)
Corn stover	600	10.00	70.7	1.1	64	38.54	23.5		(Enders et al., 2012)
Corn stover	600		29.1				25.7		(Enders et al., 2012)
Oak 600	600	6.40	87.6	0.2	438	12.58	27.5		(Enders et al., 2012)
Pine 600	600	6.00	91.1	0.1	911	15.38	27.7		(Enders et al., 2012)
Ponderosa pine	600		89	0.06	1483		11.1	392	(Keiluweit et al., 2010)
New Earth Pine	600	7.90	71.2	0.91	78	3.18		4.97	(Mukome et al., 2013)
Mixed waste wood	600	11.80	27.2	0.3	91		18.8	144	(Spokas et al., 2011)
Ponderosa pine	600		89	0.06	1483		11.1	392	(Spokas, 2010)
Wood pellets	600		69	0.1	690		12	24	(Spokas, 2010)
Mixed Deciduous	600	10.02	75.1	0.64	117	66.33		39.5	Current Biochar in Use
Hard Wood	650		72.6	1.47	49			107	(Harvey et al., 2011)
Pine	650		83.8	0.26	322			81.7	(Harvey et al., 2011)
Hard Wood	650	7.50	68.2	0.51	134	26.21		25.15	(Mukome et al., 2013)

Wood Chip	650	9.80	69.3	0.2	347		11.7	177.2	(Spokas et al., 2011)
Oak hardwood	650		78.8				21	219	(Spokas, 2010)
Bubinga	650						22.3	548.9	(Zimmerman, 2010)
Laurel Oak	650						20.7	218.7	(Zimmerman, 2010)
Loblolly Pine	650						25.2	393.9	(Zimmerman, 2010)
Eastern Red Cedar	650						30.9	490.1	(Zimmerman, 2010)
Eucalyptus saligna	700		92.7	0.4	232		6.6		(Cordero et al., 2001)
Ponderosa pine	700		92.3	0.08	1154		6.3	347	(Keiluweit et al., 2010)
Enhanced biochar	700	6.80	58.1	0.41	142	66.96		2.03	(Mukome et al., 2013)
Corn stover	700		33.5	1	34		7.6	29	(Spokas, 2010)
Ponderosa pine	700		92.3	0.08	1154		6.3	347	(Spokas, 2010)
Corn stover	730		38.5	0.7	55		5.5	23.9	(Brewer et al., 2009)
Carbonized Pine	750						18.7		(Enders et al., 2012)
Corn stover	760		38.5	0.7	55		5.5		(Spokas, 2010)
Corn stover	815		44.7	0.5	89			4.38	(Spokas & Reicosky, 2009)
Corn stover	815		44.7	0.5	89			4	(Spokas, 2010)
Aspidosperma austral	850		92.8	0.8	116		8.1	3	(Spokas, 2010)
Aspidosperma quebracho	850		97.4	1.2	81		14.6	2	(Spokas, 2010)
Beech sawdust	1000		94.8				1.3		(Spokas, 2010)

Table S3-1: Results from repeated measures ANOVA of Bulk density within the rings for the WRC. Soil: Soil Management; Biochar: Biochar Application Rate; Time: Incubation period of the soil before packing (Without incubation and after 3 months). SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/ Interaction	Bulk Density					
	SS	DF	MS	F - Ratio	*P* - Value	
Soil	0.2037	1	0.2037	54.33	0.000	***
Biochar	0.1075	2	0.0538	14.34	0.000	***
Soil x Biochar	0.0074	2	0.0037	0.98	0.387	
Time	0.8307	1	0.8307	519.41	0.000	***
Time x Soil	0.0624	1	0.0624	38.99	0.000	***
Time x Biochar	0.0140	2	0.007	4.36	0.022	*
Time x Soil x Biochar	0.0062	2	0.0031	1.93	0.163	
Residuals	0.0480	30	0.0016			

Table S3-2: Results from repeated measures ANOVAs from the water release curve (Without soil incubation and after a 3 month incubation prior to packing). Soil: Soil Management; Biochar: Biochar Application Rate; Pressure: Incremental soil water potential (KPa). SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/ Interaction	WRC: Without Incubation						WRC: After 3 Month Incubation					
	SS	DF	MS	F - Ratio	*P* - Value		SS	DF	MS	F - Ratio	*P* - Value	
Soil	253.83	1	253.83	235.81	0.000	***	383.08	1	383.08	180.02	0.000	***
Biochar	0.56	2	0.28	0.26	0.776		35.12	2	17.56	8.25	0.011	*
Soil x Biochar	2.83	2	1.42	1.32	0.315		2.65	2	1.33	0.62	0.560	
Pressure	3835.47	4	958.87	786.12	0.000	***	6218.62	5	1243.72	1227.98	0.000	***
Pressure	45.96	4	11.49	9.42	0.000	***	60.99	5	12.20	12.04	0.000	***
Pressure x Biochar	55.89	8	6.99	5.73	0.000	***	14.54	10	1.45	1.44	0.200	
Pressure x Soil x Biochar	9.10	8	1.14	0.93	0.502		4.80	10	0.48	0.47	0.897	
Residuals	43.91	36	1.22				40.51	40	1.01			

Table S3-3: Results from factorial ANOVA of the coefficient of permeability from saturated hydraulic conductivity. Soil: Soil Management; Biochar: Biochar Application Rate. SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/ Interaction	Coefficent of permeability					
	SS	DF	MS	F - Ratio	*P* - Value	
Soil	0.0067	1	0.0067	2.135	0.147	
Biochar	0.0078	2	0.0039	1.233	0.296	
Soil x Biochar	0.0230	2	0.0115	3.650	0.029	*
Residuals	0.3220	102	0.0032			

Table S4-1: Results from repeated measures ANOVA of ammonium and nitrate for incubation experiment. Soil: Soil Management; Biochar: Biochar Application Rate; SMC: Soil Moisture Content; Time: Sampling Event (Day). SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/Interaction	Ammonium						Nitrate						pH					
	SS	DF	MS	F-ratio	*P*-Value		SS	DF	MS	F-ratio	*P*-Value		SS	DF	MS	F-ratio	*P*-Value	
Soil	316178.4	1	316178.4	22108.0	0.000	***	11952.8	1	11952.8	1044.0	0.000	***	37.95	1	37.95	8922	0.000	***
Biochar	8494.7	2	4247.4	297.0	0.000	***	273.2	2	136.6	11.9	0.000	***	2.74	2	1.37	323	0.000	***
SMC	26959.7	1	26959.7	1885.1	0.000	***	69617.0	1	69617.0	6080.5	0.000	***	1.07	1	1.07	251	0.000	***
Soil × Biochar	6600.1	2	3300.0	230.7	0.000	***	2227.2	2	1113.6	97.3	0.000	***	0.07	2	0.04	9	0.002	**
Soil × SMC	24989.7	1	24989.7	1747.3	0.000	***	13328.2	1	13328.2	1164.1	0.000	***	2.49	1	2.49	585	0.000	***
Biochar × SMC	0.6	2	0.3	0.0	0.978		71.5	2	35.8	3.1	0.062		0.33	2	0.17	39	0.000	***
Soil × Biochar × SMC	35.3	2	17.6	1.2	0.309		371.6	2	185.8	16.2	0.000	***	0.04	2	0.02	4	0.028	*
Time	96153.1	13	7396.4	777.2	0.000	***	179231.3	13	13787.0	4086.7	0.000	***	10.78	7	1.54	315	0.000	***
Time × Soil	57765.0	13	4443.5	466.9	0.000	***	16846.8	13	1295.9	384.1	0.000	***	2.64	7	0.38	77	0.000	***
Time × Biochar	3763.5	26	144.7	15.2	0.000	***	2875.1	26	110.6	32.8	0.000	***	0.15	14	0.01	2	0.009	**
Time × SMC	17200.5	13	1323.1	139.0	0.000	***	21215.4	13	1632.0	483.7	0.000	***	0.22	7	0.03	6	0.000	***
Time × Soil × Biochar	3646.8	26	140.3	14.7	0.000	***	2333.0	26	89.7	26.6	0.000	***	0.19	14	0.01	3	0.001	***
Time × Soil × SMC	12661.5	13	974.0	102.3	0.000	***	12567.7	13	966.7	286.6	0.000	***	0.79	7	0.11	23	0.000	***
Time × Biochar × SMC	3844.0	26	147.8	15.5	0.000	***	2060.0	26	79.2	23.5	0.000	***	0.21	14	0.01	3	0.000	***
Time × Soil × Biochar × SMC	4367.3	26	168.0	17.6	0.000	***	2433.7	26	93.6	27.7	0.000	***	0.20	14	0.01	3	0.001	***
Residuals	2969.3	312	9.5				1052.6	312	3.4				0.82	168	0.00			

Table S4-2: Results from repeated measures ANOVA of microbial biomass carbon and carbon dioxide efflux for the incubation experiment. . Soil: Soil Management; Biochar: Biochar Application Rate; SMC: Soil Moisture Content; Time: Sampling Event (Day). SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/Interaction	Microbial Biomass Carbon						Carbon Dioxide Efflux					
	SS	DF	MS	F-ratio	*P*-Value		SS	DF	MS	F-ratio	*P*-Value	
Soil	14331.0	1	14331.0	138.4	0.000	***	43260.3	1	43260.3	60.59	0.000	***
Biochar	105.0	2	52.0	0.5	0.611		17128.7	2	8564.3	12.00	0.001	**
SMC	5.0	1	5.0	0.1	0.820		12743.3	1	12743.3	17.85	0.001	**
Soil × Biochar	91.0	2	45.0	0.4	0.651		4139	2	2069.5	2.90	0.084	
Soil × SMC	417.0	1	417.0	4.0	0.058		853.3	1	853.3	1.20	0.290	
Biochar × SMC	823.0	2	412.0	4.0	0.035		2191.6	2	1095.8	1.53	0.246	
Soil × Biochar × SMC	1244.0	2	622.0	6.0	0.009		1184.4	2	592.2	0.83	0.454	
Time	57089.0	7	8156.0	106.2	0.000	***	214188	7	30598.2	24.70	0.000	***
Time × Soil	4414.0	7	631.0	8.2	0.000	***	44328.6	7	6332.7	5.11	0.000	***
Time × Biochar	1938.0	14	138.0	1.8	0.044	*	11329.9	14	809.3	0.65	0.814	
Time × SMC	28126.0	7	4018.0	52.3	0.000	***	6542.4	7	934.6	0.75	0.626	
Time × Soil × Biochar	3108.0	14	222.0	2.9	0.001	**	15511.4	14	1108.0	0.89	0.567	
Time × Soil × SMC	1952.0	7	279.0	3.6	0.001	**	6033.9	7	862.0	0.70	0.675	
Time × Biochar × SMC	3405.0	14	243.0	3.2	0.000	***	22728.2	14	1623.4	1.31	0.212	
Time × Soil × Biochar × SMC	2947.0	14	210.0	2.7	0.001	**	18961.3	14	1354.4	1.09	0.371	
Residuals	10751.0	140	77.0				138720	112	1238.6			

Table S4-3: Results from repeated measures ANOVA of ammonium, nitrate and carbon dioxide release for the Dicyandiamide incubation experiment. . Soil: Soil Management; Biochar: Biochar Application Rate; SMC: Soil Moisture Content; Time: Sampling Event (Day). SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/Interaction	Ammonium						Nitrate						Carbon Dioxide Release					
	SS	DF	MS	F-ratio	*P*-Value		SS	DF	MS	F-ratio	*P*-Value		SS	DF	MS	F-ratio	*P*-Value	
Soil	315649	1	315649	13226.77	0.000	***	175.966	1	175.966	205.76	0.000	***	3.04	1	3.04	25.96	0.000	***
Biochar	2769	2	1385	58.02	0.000	***	35.083	2	17.542	20.51	0.000	***	2.00	2	1.00	8.51	0.005	**
SMC	5978	1	5978	250.51	0.000	***	89.246	1	89.246	104.36	0.000	***	0.92	1	0.92	7.87	0.016	*
Soil × Biochar	27	2	13	0.56	0.580		0.884	2	0.442	0.52	0.603		0.65	2	0.32	2.76	0.103	
Soil × SMC	410	1	410	17.2	0.000	***	0.005	1	0.005	0.01	0.938		1.06	1	1.06	9.02	0.011	*
Biochar × SMC	499	2	250	10.46	0.001	**	5.844	2	2.922	3.42	0.049	*	0.81	2	0.41	3.45	0.065	
Soil × Biochar × SMC	125	2	63	2.62	0.094		11.964	2	5.982	6.99	0.004	**	0.24	2	0.12	1.02	0.389	
Time	49429	11	4494	329.91	0.000	***	108.401	11	9.855	46.75	0.000	***	19.00	6	3.17	23.43	0.000	***
Time × Soil	4938	11	449	32.96	0.000	***	7.103	11	0.646	3.06	0.001	**	4.66	6	0.78	5.74	0.000	***
Time × Biochar	662	22	30	2.21	0.002	**	21.564	22	0.980	4.65	0.000	***	4.12	12	0.34	2.54	0.007	**
Time × SMC	231	11	21	1.54	0.116		40.855	11	3.714	17.62	0.000	***	8.51	6	1.42	10.50	0.000	***
Time × Soil × Biochar	337	22	15	1.12	0.321		15.364	22	0.698	3.31	0.000	***	6.59	12	0.55	4.06	0.000	***
Time × Soil × SMC	283	11	26	1.89	0.041	*	9.162	11	0.833	3.95	0.000	***	2.67	6	0.45	3.30	0.006	**
Time × Biochar × SMC	265	22	12	0.88	0.616		36.769	22	1.671	7.93	0.000	***	3.06	12	0.26	1.89	0.050	
Time × Soil × Biochar × SMC	337	22	15	1.12	0.321		8.379	22	0.381	1.81	0.017	**	5.74	12	0.48	3.54	0.000	***
Residuals	3596	264	14				55.649	264	0.211				9.73	72	0.14			

Table S4-4: ANOVA of Cation Exchange Capacity and Non-Exchangeable Ammonium for the 60 day incubation experiment. Soil: Soil Management; Biochar: Biochar Application Rate; SMC: Soil Moisture Content; Time: Sampling Event (Day). SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/Interaction	Cation Exchange Capacity						Non-Exchangeable Ammonium					
	SS	DF	MS	F-ratio	*P*-Value		SS	DF	MS	F-ratio	*P*-Value	
Soil	6953.2	1	6953.2	2463.14	0.000	***	0.06576	1	0.06576	53.061	0.000	***
Biochar	16.4	2	8.2	2.91	0.075		0.15320	2	0.07660	61.806	0.000	***
SMC	0.8	1	0.8	0.29	0.593		0.00006	1	0.00006	0.050	0.826	
Soil × Biochar	0.6	2	0.3	0.10	0.904		0.00220	2	0.00110	0.887	0.428	
Soil × SMC	3.4	1	3.4	1.22	0.282		0.00392	1	0.00392	3.159	0.092	
Biochar × SMC	8.8	2	4.4	1.56	0.232		0.00376	2	0.00188	1.517	0.245	
Soil × Biochar × SMC	10.8	2	5.4	1.92	0.170		0.00196	2	0.00098	0.791	0.468	
Time	369.0	5	73.8	41.02	0.000	***	0.12731	4	0.03183	20.295	0.000	***
Time × Soil	9.7	5	1.9	1.08	0.375		0.01058	4	0.00265	1.687	0.162	
Time × Biochar	46.1	10	4.6	2.56	0.008	**	0.00835	8	0.00104	0.666	0.720	
Time × SMC	261.0	5	52.2	29.01	0.000	***	0.01528	4	0.00382	2.436	0.054	
Time × Soil × Biochar	38.0	10	3.8	2.11	0.029	*	0.00209	8	0.00026	0.167	0.995	
Time × Soil × SMC	10.0	5	2.0	1.11	0.358		0.01725	4	0.00431	2.749	0.034	*
Time × Biochar × SMC	27.3	10	2.7	1.52	0.142		0.01133	8	0.00142	0.903	0.518	
Time × Soil × Biochar × SMC	28.2	10	2.8	1.57	0.125		0.01523	8	0.00190	1.214	0.302	
Residuals	206.9	115	1.8				0.11919	76	0.00157			

Table S5-1: Results from the repeated measures ANOVA of Plant Dry Matter, Total Nitrogen and Nitrogen Uptake. Soil: Soil Management; Biochar: Biochar Application Rate; Nitrogen: Nitrogen Application Rate; Time: Sampling Event. SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/Interaction	Dry Matter					Plant Total Nitrogen					Nitrogen Uptake				
	SS	DF	MS	F-ratio	*P*-Value	SS	DF	MS	F-ratio	*P*-Value	SS	DF	MS	F-ratio	*P*-Value
Soil	2.94	1	2.94	122.98	0.000 ***	5.22	1	5.22	213.79	0.000 ***	82.73	1	82.73	236.60	0.000 ***
Biochar	1.09	1	1.09	45.53	0.000 ***	2.28	1	2.28	93.28	0.000 ***	34.74	1	34.74	99.36	0.000 ***
Nitrogen	0.10	3	0.03	1.38	0.267	0.09	3	0.03	1.18	0.332	1.71	3	0.57	1.63	0.203
Soil × Biochar	1.08	1	1.08	45.26	0.000 ***	2.04	1	2.04	83.52	0.000 ***	35.02	1	35.02	100.16	0.000 ***
Soil × Nitrogen	0.09	3	0.03	1.23	0.314	0.15	3	0.05	2.02	0.131	1.56	3	0.52	1.49	0.237
Biochar × Nitrogen	0.05	3	0.02	0.67	0.579	0.06	3	0.02	0.84	0.480	0.50	3	0.17	0.48	0.699
Soil × Biochar × Nitrogen	0.04	3	0.01	0.52	0.672	0.07	3	0.02	0.96	0.423	0.74	3	0.25	0.71	0.554
Time	46.38	6	7.73	312.8	0.000 ***	54.86	7	7.84	332.77	0.000 ***	233.15	6	38.86	198.50	0.000 ***
Time × Soil	16.60	6	2.77	111.94	0.000 ***	18.02	7	2.57	109.31	0.000 ***	197.19	6	32.86	167.88	0.000 ***
Time × Biochar	2.02	6	0.34	13.62	0.000 ***	3.10	7	0.44	18.82	0.000 ***	30.08	6	5.01	25.61	0.000 ***
Time × Nitrogen	0.48	18	0.03	1.09	0.365	0.54	21	0.03	1.10	0.353	4.19	18	0.23	1.19	0.274
Time × Soil × Biochar	1.73	6	0.29	11.67	0.000 ***	2.43	7	0.35	14.73	0.000 ***	27.18	6	4.53	23.14	0.000 ***
Time × Soil × Nitrogen	0.84	18	0.05	1.88	0.019 *	0.89	21	0.04	1.80	0.020 *	4.10	18	0.23	1.16	0.296
Time × Biochar × Nitrogen	0.21	18	0.01	0.48	0.964	0.42	21	0.02	0.85	0.654	2.17	18	0.12	0.62	0.884
Time × Soil × Biochar × Nitrogen	0.17	18	0.01	0.39	0.988	0.31	21	0.01	0.63	0.892	1.97	18	0.11	0.56	0.925
Residuals	4.75	192	0.02			5.28	224	0.02			37.59	192	0.20		

Table S5-2: Results from the repeated measures ANOVA of ammonium and nitrate levels. Soil: Soil Management; Biochar: Biochar Application Rate; Nitrogen: Nitrogen Application Rate; Time: Sampling Event. SS: Sum of Squares, DF: Degrees of Freedom, MS: Mean squares. Significance is denoted by stars: $P \leq 0.001$ (***), $P \leq 0.01$ (**), $P \leq 0.05$ (*).

Term/Interaction	Ammonium						Nitrate					
	SS	DF	MS	F-ratio	*P*-Value		SS	DF	MS	F-ratio	*P*-Value	
Soil	0.84	1	0.84	6.6657	0.0146	**	1.57950	1	1.57950	2.568	0.1189	
Biochar	0.123	3	0.041	0.3258	0.8067		0.07000	3	0.02330	0.038	0.9899	
Nitrogen	0.168	1	0.168	1.3347	0.2565		0.00620	1	0.00620	0.010	0.9204	
Soil × Biochar	0.609	3	0.203	1.611	0.2062		5.84910	3	1.94970	3.170	0.0375	*
Soil × Nitrogen	0.011	1	0.011	0.0909	0.7649		0.96480	1	0.96480	1.569	0.2195	
Biochar × Nitrogen	0.349	3	0.116	0.9245	0.4401		1.06060	3	0.35350	0.575	0.6358	
Soil × Biochar × Nitrogen	0.311	3	0.104	0.8222	0.4913		0.45830	3	0.15280	0.248	0.8619	
Time	8.017	5	1.603	16.305	0.0000	***	111.99000	5	22.39800	42.388	0.0000	***
Time × Soil	0.689	5	0.138	1.4007	0.2268		4.44460	5	0.88890	1.682	0.1418	
Time × Biochar	1.137	15	0.076	0.7708	0.7083		7.25020	15	0.48330	0.915	0.5491	
Time × Nitrogen	0.185	5	0.037	0.3771	0.8639		1.44310	5	0.28860	0.546	0.7410	
Time × Soil × Biochar	0.444	15	0.03	0.3011	0.9948		5.68250	15	0.37880	0.717	0.7649	
Time × Soil × Nitrogen	0.253	5	0.051	0.5143	0.7652		0.87350	5	0.17470	0.331	0.8939	
Time × Biochar × Nitrogen	1.338	15	0.089	0.9074	0.5572		5.03830	15	0.33590	0.636	0.8422	
Time × Soil × Biochar × Nitrogen	0.735	15	0.049	0.4981	0.9390		3.51230	15	0.23420	0.443	0.9636	
Residuals	15.73	160	0.098				84.54550	160	0.52840			