Biochar amendment and greenhouse gas emissions from agricultural soils

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Declaration

I confirm that this work is my own, except where indicated otherwise. No part of this study has been submitted for any other degree or qualification.

Name: .....  ..... Date: .....15/08/2013.....

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Abstract

The aim of this study was to investigate the effects of biochar amendment on soil greenhouse gas (GHG) emissions and to elucidate the mechanisms behind these effects. I investigated the suppression of soil carbon dioxide (CO_2) and nitrous oxide (N_2O) emissions in a bioenergy and arable crop soil, at a range of temperatures and with or without wetting/drying cycles. More detailed investigation on the underlying mechanisms focused on soil N_2O emissions. I tested how biochar altered soil physico-chemical properties and the subsequent effects on soil N_2O emissions. In addition, ^{15}N pool dilution techniques were used to investigate the effect of biochar on soil N transformations.

Biochar amendment significantly suppressed soil GHG emissions for two years within a bioenergy soil in the field and for several months in an arable soil. I hypothesised that soil CO_2 emissions were suppressed under field conditions by a combination of mechanisms: biochar induced immobilisation of soil inorganic-N (BII), increased C-use efficiency, reduced C-mineralising enzyme activity and adsorption of CO_2 to the biochar surface. Soil CO_2 emissions were increased for two days following wetting soil due to the remobilisation of biochar-derived labile C within the soil. Soil N_2O emissions were suppressed in laboratory incubations within several months of biochar addition due to increased soil aeration, BII or increased soil pH that reduced the soil N_2O: N_2 ratio; effects that varied depending on soil inorganic-N concentration and moisture content.

These results are significant as they consistently demonstrate that fresh hardwood biochar has the potential to reduce soil GHG emissions over a period of up to two years in bioenergy crop soil, while simultaneously sequestering C within the soil. They also contribute greatly to understanding of the mechanisms underlying the effect of biochar addition on soil N transformations and N_2O emissions within bioenergy and arable soils. This study supports the hypothesis that if scaled up, biochar amendment to soil may contribute to significant reductions in global GHG

emissions, contributing to climate change mitigation. Further studies are needed to ensure that these conclusions can be extrapolated over the longer term to other field sites, using other types of biochar.

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BD – bulk density

BII – biochar-induced immobilisation of inorganic N

C – carbon

CH_4 – methane

CO_2 – carbon dioxide

$CO_{2eq.}$ – carbon dioxide equivalent, i.e. the sum of the radiative forcing of carbon dioxide, methane and nitrous oxide relative to carbon dioxide alone

ECD – electron capture detector

FID – flame ionisation detector

GC – gas chromatograph

GHG – greenhouse gas. In this study used as a term to include carbon dioxide, methane and nitrous oxide

GMC – gravimetric moisture content

MDL – minimum detection limit

MS – mass spectrometer

N – nitrogen

N_2 – dinitrogen (nitrogen gas)

N_2O – nitrous oxide

NH_3 – ammonia

NH_4^+ - ammonium

NO_2^- - nitrite

NO_3^- - nitrate

SOC – soil organic carbon

SOM – soil organic matter

WHC – water holding capacity

WFPS – water-filled pore space

1 Literature review and aims

Global surface temperature has increased by 0.8°C in the last 100 years (Hansen et al., 2010). This warming has primarily been caused by increased anthropogenic emissions of long-lived greenhouse gases (GHGs) such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4). Further increases will have large effects on natural cycles and ecosystems and by consequence human activities. This may impose huge adaptation costs on societies worldwide (Parry et al., 2007). Therefore there is a strong incentive to mitigate further increases in temperature by reducing GHG emissions.

Fossil fuel use, agriculture and land use change have been the dominant sources of increased atmospheric GHG concentrations in the last 250 years (Solomon et al., 2007a). Agricultural land occupies 40 to 50% of the world's surface, and in 2005 accounted for 10–12% (5.1 to 6.1 Gt $CO_{2eq.}$ yr^{-1}) of total anthropogenic GHG emissions (Smith et al., 2007). This study focuses on GHG emissions from agriculture and methods to reduce them.

1.1 Climate change

Greenhouse gases are those that adsorb and emit radiation within the thermal infrared range (IPCC, 2007). They include water vapour, ozone and three 'primary' anthropogenic gases (carbon dioxide (CO_2), nitrous oxide (N_2O), methane (CH_4)). A number of other gases such as halogens, hydrocarbons and aerosols may increase or decrease radiative forcing, however this study focuses on the three primary greenhouse gases that together contribute the most to global radiative forcing (henceforth referred to just as GHGs) (Pachauri & Reisinger, 2007). A general summary of each GHG – their lifetime in the atmosphere, their radiative forcing and their global warming potential over 100 years – is presented in Table 1.1 below.

Table 1.1. The global warming potentials of carbon dioxide, nitrous oxide and methane. Adapted from Solomon et al., (2007a).

Greenhouse gas	Lifetime (years)	Radiative forcing (W m^{-2}) (2005)	100-year global warming potential
Carbon dioxide	5-20	1.66	1
Nitrous oxide	114	0.16	298
Methane	12	0.48	25

In order to develop strategies to reduce GHG emissions it is important to have a full understanding of their origins and properties. Carbon dioxide is present in the atmosphere at 396 ppm, compared to 280 ppm in pre-industrial times (Tans & Keeling, 2012). The concentration of CO_2 is increasing at a rate of approximately 3 ppm per year (Tans & Keeling, 2012). Carbon dioxide is produced predominately by human activity, with major sources including fossil fuel combustion and land use change (i.e. the conversion of natural ecosystems into a land use managed by humans, Guo and Gifford, (2002)) (Solomon et al., 2007a). Nitrous oxide is present in the atmosphere at 323 ppb compared to 270 ppb in pre-industrial times (European Environment Agency, 2012). This gas contributes approximately 6% of annual anthropogenic radiative forcing (Davidson, 2009; Canfield et al., 2010). Atmospheric concentrations have increased linearly over the past few decades at approximately 0.8 ppb per year, with 40% of emissions being attributed to human activities (Solomon et al., 2007b). In 2005, CH_4 was present in the atmosphere at an average concentration of 1.8 ppm, concentrations that are unprecedented for 650,000 years (Le Mer & Roger, 2001). Atmospheric CH_4 concentrations increased by ~1% per year in the late 1970s and early 1980s, but more recently this increase has almost ceased (Solomon et al., 2007b). This trend is related to the imbalance between sources and sinks of CH_4 (Wuebbles & Hayhoe, 2002).

Agricultural land occupies 40 to 50% of the world's surface and accounted for 10-12% of anthropogenic GHGs in 2005, the sum total of which is often defined as total CO_2 equivalent emissions (total $CO_{2eq.}$ emissions) (Smith et al., 2007). See Table 1.2 for a summary of the emissions of each GHG from agriculture.

Table 1.2. Total anthropogenic emissions of GHGs compared to those from agriculture. Agricultural emissions do not include CO_2 emissions due to land use change, electricity or fuel use. Units are Gt $CO_{2eq.}$ yr^{-1} and are from 2005 unless specified. Data adapted from (Smith et al., 2007).

Total anthropogenic $CO_{2eq.}$ emissions (2004)	**Total $CO_{2eq.}$ emissions from agriculture**	**CO_2 emissions from agriculture**	**N_2O emissions from agriculture**	**CH_4 emissions from agriculture**
49.0	5.1 – 6.1	0.04	2.8	3.3

Agricultural crops uptake large amounts of CO_2 from the atmosphere during photosynthesis, but this is offset by CO_2 emissions derived from the decomposition of soil microbes and soil organic matter (SOM, accelerated following ploughing or land use change) or biomass burning (Smith et al., 2007). Overall, CO_2 emissions from agriculture (excluding electricity use and fuel) contribute relatively less than 1% to total anthropogenic $CO_{2eq.}$ emissions (Table 1.2).

Soils contain approximately twice as much C as the atmosphere (~ 1,500 compared to ~ 750 Pg C, Smith (2008)). Therefore, small changes in soil C contents can have large implications for climate change mitigation. A historical soil C loss due to anthropogenic soil cultivation and disturbance has been estimated to be between 40 and 90 Pg C (Smith, 2008). There is potential to significantly increase C stocks in depleted soils (Rees et al., 2005), the mitigation potential has been estimated to be up to 4.8 Gt $CO_{2eq.}$ yr^{-1} by 2030 (Smith et al., 2013).

Of the 16 Tg N_2O-N emitted globally from all sources in 2010, approximately 6.4 Tg came from human activities (Reay et al., 2012). Soils account for ~70% of the atmospheric loading of N_2O, with ~4.2 Tg of annual anthropogenic N_2O emissions attributed to agricultural soils (Baggs, 2011). Overall, N_2O emissions from agriculture are projected to rise to 7.6 Tg N_2O-N yr^{-1} by 2030, mostly through increases in the demand for N_2O-intensive products (e.g. meat and biofuels) and new agricultural practices are needed to reduce N_2O emissions from agriculture (Popp et al., 2010; Reay et al., 2012). Emissions of N_2O (direct and indirect) from agricultural soil come primarily from the incomplete denitrification of applied N

from fertiliser applications (both synthetic and organic, such as manure), particularly in wet or saturated soil (Davidson, 2009).

In the UK, N_2O emissions from agriculture are responsible for 78% of overall N_2O emissions, although they have decreased by 23% since 1990 primarily due to reduced N fertiliser application rates and a decrease in livestock numbers (Skiba et al., 2012). The largest emissions of N_2O in the UK come from regions dominated by grasslands and livestock production; fertiliser and manure application are responsible for 23% of N_2O emissions, nitrogen excretion onto pasture range and paddocks accounted for 8%, and manure storage was responsible for 6%, with the rest being put down to indirect emissions of NH_3 and NO_x to the atmosphere and denitrification of the NO_3^- lost to water (Skiba et al., 2012).

The sources and relative contributions of sources of CH_4 emissions are generally well known (Solomon et al., 2007a). Approximately 60% of the ~440 Tg of annual production of atmospheric CH_4 comes from human activity (Heimann, 2010). Significant sources include rice paddies, enteric fermentation from ruminant animals, manure management, landfills, biomass burning as well as fossil fuel burning. Agriculture is responsible for ~47% of total anthropogenic emissions of CH_4, while 30% of this total comes from rice paddies (Neue, 1997; Le Mer & Roger, 2001). Sinks of CH_4 include chemical reactions in the atmosphere and soils, through methanotrophy (Solomon et al., 2007a). It is recognised that there is a great potential to both decrease emissions of and increase sinks of CH_4 from agriculture (Smith et al., 2013).

1.2 Bioenergy and arable systems

Second generation bioenergy crops and arable crops are two examples of typical agricultural management regimes in Europe and the UK, and are significant contributors to agricultural GHG emissions (Paustian et al., 2000; Van Groenigen et al., 2010; Don et al., 2012).

Bioenergy crops may offset energy production from fossil fuels, therefore they have been proposed as a potential solution to mitigate climate change (Whitaker et al., 2010). There are two primary types of bioenergy crops – first and second generation (bioenergy derived from algae is a relatively recent third-generation crop) (Fairley, 2011). First-generation bioenergy is created from the sugars, starches or oils of crops such as corn or sugarcane (de Vries et al., 2010). Production of first-generation biofuel has greatly increased in recent years; however the sustainability and GHG balance of first-generation bioenergy crops has received considerable attention and criticism in the literature (Crutzen et al., 2007; Searchinger et al., 2008; Smeets et al., 2009; Whitaker et al., 2010). First-generation bioenergy is produced from food crops, while second-generation bioenergy is derived from cellulosic, typically woody materials, (Bartle & Abadi, 2010). Second-generation bioenergy crop production is typically responsible for lower GHG emissions over its life cycle than first-generation bioenergy crops due to less intensive management practices (Hillier et al., 2009; Havlík et al., 2011; Rowe et al., 2011). Nevertheless, methods to improve the sustainability of all bioenergy crop-types are being considered (Gopalakrishnan et al., 2009; Thornley et al., 2009).

One of the most promising second-generation biomass energy crops in the UK in terms of environmental sustainability is *Miscanthus* (*Miscanthus X Giganteus*) (Rowe et al., 2009; Whitaker et al., 2010). This crop is a perennial rhizomatous C_4 grass that is planted on approximately 13,500 ha of UK cropland (Don et al., 2012). *Miscanthus* has low nitrogen (N) requirements and generally does not require fertiliser N during the first two years after establishment (Caslin et al., 2011). It is generally known that high yields are maintained after this period (Lewandowski et al., 2000; Rowe et al., 2009), although recent work suggests that additional N inputs in the fourth year could improve yields by up to 40% (Wang et al., 2012a). Therefore soil N_2O emissions are minimal from this land use. In addition, *Miscanthus X Giganteus* is hypothesised to sequester C in the soil over time compared to arable crops due to the accumulation of C within its extensive root and rhizome structure and

decreased extraction of harvested C from the field compared to arable crops (Poeplau et al., 2011; Don et al., 2012).

The second land use considered in this study is arable crops. The 'arable land and permanent crops' land use covers 25% of land area in Europe compared to 37% of agriculture as a whole (European Environment Agency, 2010). Arable soil is typically annually amended with N-based fertiliser: which can consist of inorganic forms such as compounds of ammonium (NH_4^+), nitrate (NO_3^-) or organic fertilisers such as urea, compost, or manure (which can be solid or wet such as slurry) (Akiyama et al., 2004; Jones et al., 2007; Smith et al., 2012). Fertilised arable land is the biggest contributor to N_2O emissions from agriculture globally, contributing approximately 3.3 Tg N_2O-N to annual N_2O emissions compared to 0.8 Tg N_2O-N from grasslands (Stehfest & Bouwman, 2006; Van Groenigen et al., 2010), however, in the UK the greatest contribution to N_2O emissions from agriculture comes from grasslands and livestock production (Skiba et al., 2012). The addition of N-based fertiliser greatly increases soil N_2O emissions and much of the N is wasted from run-off or leaching (Sutton et al., 2011). The percentage of N released as N_2O from N-based fertiliser addition (the N_2O emission factor) is estimated to be between 1 and 4% depending on crop type and form of fertiliser added (De Klein et al., 2007; Crutzen et al., 2007; Davidson, 2009; Lesschen et al., 2011), and the N-use efficiency (i.e. the amount of N that ends up in the final crop compared to the amount of N added) in arable crops has reduced throughout time from ~80% to ~30% (Erisman et al., 2008). It is recognised that there is great potential within arable crop management to reduce the N_2O emission factor and increase the N-use efficiency of agriculture, of which arable soils form a significant part (Reay et al., 2012; Smith et al., 2013).

Both bioenergy and arable crop soils contribute significantly to global fluxes of GHGs. Therefore it is important to consider methods to reduce overall GHG efflux from these soil systems. Greenhouse gas emissions from agriculture may be reduced by targeting either supply or demand-side sources (Smith et al., 2013). Supply-side

agricultural GHG emissions are derived from the efficiency of the agricultural process (i.e. changes in land management practice and/or technology) and have the potential to reduce total $CO_{2eq.}$ emissions by 1.5-4.3 Gt $CO_{2eq.}$ yr^{-1} across the agricultural sector globally, particularly methods that increase agricultural product per unit of input (Smith et al., 2013). Novel supply-side approaches are needed to counteract the trends of growing GHG emissions from agriculture. This study focuses on one particular supply-side method to reduce total $CO_{2eq.}$ emissions from agricultural soils, the amendment of soil with biochar.

1.3 Biochar

Charcoal-rich soils were discovered during the 20th century in the Amazon basin of South America (Lehmann et al., 2004). These 'Amazonian Dark Earths' were the result of human management over many centuries and contained significantly greater amounts of charcoal-derived C, SOC and nutrients than adjacent soils (Glaser et al., 2001; Lehmann et al., 2006). Researchers suggested that soil quality elsewhere could be improved and concurrently contribute to climate change mitigation by the addition of charcoal (Lehmann, 2007; Woolf et al., 2010). 'Biochar' was the term employed to designate charcoal produced in a controlled environment with the intention of adding it to soil (Lehmann et al., 2006).

1.3.1 Biochar production

Biochar is created by heating biomass to between 350 and 600°C in an oxygen-limited environment, a process called pyrolysis (Sohi et al., 2010). It can be made from a wide range of biomass feedstocks, including wood-derived materials, agricultural residues and manures (Singh et al., 2010b). Its physical and chemical properties are similar to those of charcoal, typified by its high C content, low N content, high surface area and cation exchange capacity compared to unheated biomass, discussed in more detail below (Singh, et al., 2010).

The production of biochar is a field of research in itself (Garcia-Perez et al., 2010; Meyer et al., 2011). All production processes produce a variety of gases, bio-oils as

well as biochar. Production methods can be generalised to four main processes that are characterised by different heating temperature, time of heating and the biochar yield. These processes are summarised in Table 1.3.

Table 1.3: Types of biochar production processes, adapted from (Brown, 2009; Brownsort, 2009). Biochar yield refers to the % of initial C in the biomass remaining as biochar C.

Process name	Heating temperature (°C)	Time	Biochar yield (%)
Slow pyrolysis	350 – 400	2– 30 mins	35
Intermediate pyrolysis	350 – 450	1 – 15 mins	20
Fast pyrolysis	450 – 550	1 – 5s	12
Gasification	> 750	10 to 20s (vapours)	10

The biochar production process emits GHGs from the decomposition of the biomass, releasing substances such as water vapour, CO_2 and carbon monoxide (CO). Although much of the remaining C in biochar created via slow pyrolysis is more labile than the remaining C in biochar created via fast pyrolysis (a % content that can vary widely according to process conditions and feedstock), it was concluded in one life cycle assessment paper that the production system of biochar produced via slow pyrolysis had a greater carbon abatement (Hammond et al., 2011). More modern production processes can better minimise or capture waste gases from the pyrolysis process (Brown, 2009).

Biochar can be produced concurrently with energy production from biomass (Laird et al., 2009). Several life cycle assessments have demonstrated that producing bioenergy and biochar concurrently resulted in reductions in total $CO_{2eq.}$ emissions compared to producing bioenergy alone, primarily by increasing long-term C storage in the soil and reducing soil N_2O emissions (Gaunt & Lehmann, 2008; Roberts et al., 2010; Woolf et al., 2010; Hammond et al., 2011). As well as production, another important component of the biochar life cycle in terms of total $CO_{2eq.}$ emissions is the effect of biochar on the soil, which is discussed in the following section.

1.3.2 Biochar properties and effects on soil

Biochar amendment to soil can have a wide range of effects on soil physical, chemical and biological properties. These effects depend significantly on the properties of the biochar itself (Lehmann et al., 2011; Jeffery et al., 2011; Spokas et al., 2012a).

The properties of biochar are determined by its production method. Along with feedstock type, heating temperature is the primary control on resulting biochar properties from pyrolysis (Brownsort, 2009). With increasing pyrolysis temperature, there is a decrease in the proportion of volatile compounds on the biochar surface and an increase in the proportion of recalcitrant (aromatic) C compounds (Joseph et al., 2010; Spokas, 2010). Therefore, biochar from high temperature pyrolysis is more resistant to mineralisation and contains lower amounts of volatile matter on its surface (Spokas, 2010). Spokas et al., (2010) predicted that biochar created at 400°C or above (O: C ratio < 0.6) had a minimum half-life of 100 years, while those created at temperatures of 600°C or above (O: C ratio < 0.2) were predicted to have a half-life of at least 1000 years. These findings suggest that biochar C has a significant residence time in soil and that it can be used to effectively sequester CO_2 from the atmosphere over long time scales (Woolf et al., 2010).

Research into biochar use in agriculture has linked many of its chemical and physical properties to beneficial effects on soil. These effects include adsorbing nutrients or contaminants to the biochar (Beesley et al., 2011; Spokas et al., 2012b), increasing crop yield (Jeffery et al., 2011) and suppressing soil GHG emissions.

Biochar has a much greater surface area than that of soil (Joseph et al., 2010). The surface of biochar is covered in micrometre-scale pores that are large enough to harbour nutrients water, micro-organisms and many other substances (Chan & Xu, 2009). Additionally, the negative surface charge of fresh biochar can attract positively-charged compounds such NH_4^+ (Spokas et al., 2012b). The adsorption of inorganic N could have potential benefits for fertiliser N-use efficiency in agricultural soils, provided the N is available to crops. Increased adsorption of

water could increase moisture retention within a soil system (Karhu et al., 2011). It is currently unclear whether increased retention of polycyclic aromatic hydrocarbons, herbicides, pesticides or other contaminants may increase or decrease their availability to the soil microbial community (Cao et al., 2009; Yang et al., 2010; Quilliam et al., 2012; Lü et al., 2012).

Biochar addition to soil may affect crop yield, depending on crop type, biochar type and the co-amendment of fertiliser. A meta-analysis of 16 studies found that biochar addition to soil overall increased crop yields by ~10% (Jeffery et al., 2011). However, increases in crop yield have yet to be shown in studies lasting longer than two years. Additionally, there have been studies that have shown a negative effect of biochar application on crop yield, particularly when biochar is applied on its own without any other forms of organic or inorganic fertiliser (Spokas et al., 2012a).

Another potential benefit of biochar addition to soil is the suppression of soil CO_2, N_2O and CH_4 emissions (Kimetu & Lehmann, 2010; Van Zwieten et al., 2010b; Wang et al., 2012b). The following section discusses the range of effects of biochar amendment on soil GHG emissions reported in the published literature and the potential mechanisms underlying this effect.

1.4 Greenhouse gas emissions from soil and the effect of biochar amendment

A number of mechanisms have been proposed within the literature to explain the effect of biochar amendment on soil GHG emissions, with limited amounts of evidence to support them. This section discusses these mechanisms for each of the three gases in turn (CO_2, N_2O and CH_4).

1.4.1 Soil carbon dioxide (CO_2) emissions

The primary sources of soil CO_2 emissions are shown in Fig. 1.1. Soil CO_2 emissions can be derived from native SOM, the mineralisation of added C compounds (such as

dead plant material), the mineralisation of root exudates or dead roots and the direct respiration from plant roots (Hanson et al., 2000; Luo & Zhou, 2006).

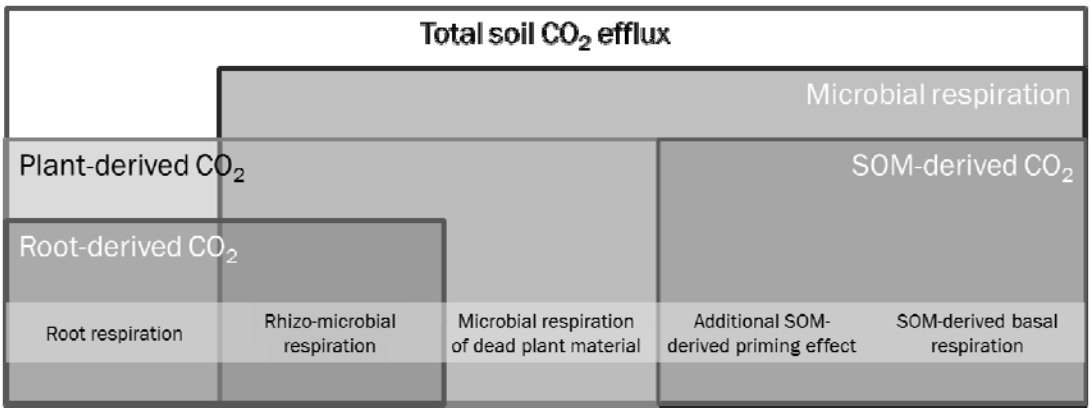

Fig. 1.1. The primary sources of soil CO_2 emissions in soil and plant systems. Adapted from Luo and Zhou, (2006).

Carbon dioxide emissions from soils are primarily controlled by soil temperature, moisture conditions and the availability of substrate (Raich & Tufekciogul, 2000). Soil CO_2 emissions may be affected by biochar amendment. Some authors suggest that a co-benefit of biochar amendment is a reduction in soil CO_2 emissions and associated long-term increases in SOC in the soil (Lehmann et al., 2011). However few long-term studies support this hypothesis. Those that exist are contradictory, with increased (Major et al., 2009), decreased (Kuzyakov et al., 2009), and variable effects observed (Zimmerman et al., 2011). The mechanisms underlying the effects of biochar amendment on soil CO_2 emissions are also uncertain, summarised in Table 1.4 and explained in further detail below.

Table 1.4. Mechanisms suggested within the biochar literature to explain the effect of biochar on soil CO_2 emissions

Number	Mechanism	Effect
1	Biochar reduces the albedo of the soil, increasing soil temperature (Meyer et al., 2012)	Increased CO_2 emissions from soil
2	Addition of labile C, increased substrate for soil C mineralising enzymes (priming). (Zimmerman et al., 2011)	Increased CO_2 emissions from soil
3	Agglomeration of soil C, microbes, nutrients on biochar surface. Increased C-use efficiency (Lehmann et al., 2011)	Reduced CO_2 emissions from soil
4	Reduction of C-mineralising enzyme activity (Jin, 2010; Bailey et al., 2011)	Reduced CO_2 emissions from soil
5	Soil-derived CO_2 precipitation onto the biochar surface as carbonates (Joseph et al., 2010; Lehmann et al., 2011)	Reduced CO_2 emissions from soil

Increasing soil temperature generally results in greater CO_2 emissions up until ~40°C (Fang & Moncrieff, 2001; Luo & Zhou, 2006; Richardson et al., 2012). There is no direct evidence of a significant effect of biochar addition on soil temperature in the field. It has been hypothesised that biochar addition to soil may indirectly increase soil temperature in the field due to decreases in soil albedo with biochar amendment that may increase CO_2 efflux (Genesio et al., 2012; Meyer et al., 2012). However, the influence of a lower soil albedo with biochar on soil CO_2 emissions in the field has not been directly analysed.

Biochar has a lower bulk density (BD) and higher water holding capacity (WHC) than that of soil alone, therefore the addition of this material to soil may affect these soil properties and hence increase soil aeration (Sohi et al., 2010; Karhu et al., 2011; Basso et al., 2012). The relationship between soil CO_2 emissions and soil aeration is unclear; emissions of soil-derived CO_2 may be highest within an 'optimal moisture content' range or increase with soil moisture content up to saturation (Xu et al., 2004; Cook & Orchard, 2008). The effect of biochar addition on soil aeration may be particularly important immediately after mixing biochar into the soil. Mixing soil (e.g. ploughing) can increase CO_2 emissions in the days following disturbance, by re-mobilising soil nutrients, soil microbes and increasing O_2 availability within previously-inaccessible soil layers (Reicosky et al., 1997; Reicosky, 1997). Therefore,

increased soil aeration in saturated soils following biochar incorporation may increase soil CO_2 emissions; however the magnitude of the effect of this mechanism has not been directly quantified.

Soil CO_2 emissions may be increased following the addition of labile C compounds to the soil, which may be derived from mineralisation of the added C itself, or of mineralisation of the native soil organic matter (priming) (Kuzyakov, 2010). Fresh biochar often adds a significant amount of labile C to the soil that can be mineralised to increase soil CO_2 emissions. However, this is likely to be a short-term effect as the labile fraction is rapidly mineralised (Zimmerman et al., 2011). Roots may also add C-based substances to the soil. Root respiration is controlled by temperature and moisture conditions (Bouma et al., 1997). There is currently no evidence to suggest that root-derived CO_2 emissions are directly affected by biochar amendment (Lehmann et al., 2011).

It is not clear whether biochar addition leads to decreased or increased native soil C mineralisation in the long term (Wardle et al., 2008; Lehmann et al., 2011; Spokas, 2012). If biochar addition was consistently proven to 'prime' mineralisation of native soil C in the long term, current estimates of the potential reduced total $CO_{2eq.}$ emissions with large-scale biochar addition could be greatly reduced (Woolf et al., 2010). Biochar amendment may increase soil microbial biomass due to the increase of C-use efficiency of the system following agglomeration of SOC, microbes and nutrients onto the biochar surface, (Lehmann et al., 2011). It is possible that the activity of C-mineralising enzymes may be reduced following biochar amendment, therefore reducing soil CO_2 emissions (Jin, 2010), although this has not been proven (Bailey et al., 2011). Finally, it has been suggested that soil-derived CO_2 may adsorb to the biochar surface as carbonates, reducing its efflux to the atmosphere (Joseph et al., 2010; Lehmann et al., 2011).

1.4.2 Soil nitrous oxide (N_2O) emissions

The pathways by which N_2O is produced from soil and the environmental factors that control them are well understood. However, however the interactions between them are not, particularly following the addition of organic materials to the soil (Chen et al., 2013).

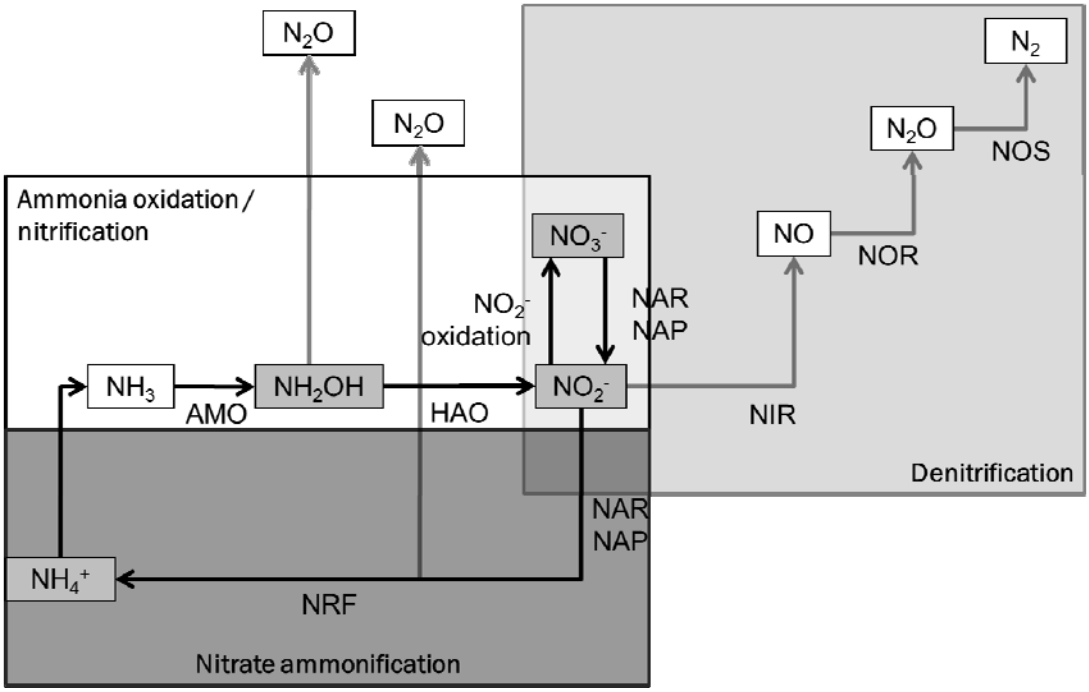

Fig. 1.2. The four primary processes underlying soil N_2O emissions: NH_3 oxidation (nitrification), denitrification, NO_3^- ammonification and nitrifier denitrification. Nitrifier denitrification is the NH_3 oxidation and denitrification pathway without passing through the 'NO_2^- oxidation stage'. Capital letters indicate the enzyme involved in the conversion between two substances, the direction of conversion indicated by the arrow. White boxes indicate a gaseous substance while a grey box indicates a liquid. Adapted from Baggs (2011).

Nitrous oxide is produced in soils primarily by microbial activity via nitrification (De Boer & Kowalchuk, 2001), nitrifier denitrification (Wrage et al., 2005), NO_3^- ammonification (Baggs, 2011) and denitrification (Gillam et al., 2008). These processes are summarised in Fig. 1.2. This study focuses on the two primary N_2O-producing mechanisms – nitrification and denitrification. Nitrification is the oxidation of NH_4^+ or ammonia (NH_3) into nitrite (NO_2^-) and NO_3^-, while denitrification is the conversion of NO_3^- into nitric oxide (NO), N_2O and dinitrogen (N_2).

Biochar amendment to soil can have significant effects on soil N_2O emissions; however the magnitude of effect varies widely between studies. Several short-term laboratory incubations (generally ranging from a few days to a few months in duration) have shown that biochar amendment can suppress soil N_2O emissions (Yanai et al., 2007; Singh et al., 2010a; Van Zwieten et al., 2010b; Stewart et al., 2013). Until now, few studies have demonstrated a similar effect in the field (Zhang et al., 2010; Taghizadeh-Toosi et al., 2011a; Wang et al., 2011). Other short and long-term studies have not observed a suppression of soil N_2O emissions (Clough et al., 2010; Scheer et al., 2011). Furthermore, longer-term field studies concluded soil N_2O emissions were not suppressed with biochar amendment in the long term (up to three years after biochar addition) (Spokas, 2012; Jones et al., 2012).

Biochar amendment causes changes to a range of soil physical and chemical properties that regulate N-cycling processes. However, the mechanisms by which biochar amendment affects soil N cycling processes are unclear (Spokas et al., 2012b). A summary of the five primary mechanisms suggested in the published literature is shown in Table 1.5 below. In the following section we discuss the mechanisms in turn. These relate to changes to physical properties such as soil aeration, biochar-nutrient reactions (immobilisation adsorption of inorganic N), increases in soil pH, the addition of labile C and inhibitive substances in the biochar itself.

Table 1.5: Mechanisms suggested within the literature to explain the effect of biochar amendment on soil N_2O emissions

Number	Mechanism	Effect
1	Increased WHC and decreased BD of the soil, increasing soil aeration (Yanai et al., 2007; Karhu et al., 2011)	Reduced activity of denitrifying micro-organisms
2	Immobilisation of soil inorganic N via adsorption to biochar surface or increased microbial immobilisation (Clough & Condron, 2010; Spokas et al., 2012b)	Reduced N substrate for nitrifying and denitrifying enzymes, therefore reduced enzymatic activity
3	Increased soil pH (Šimek et al., 2002; Van Zwieten et al., 2010b; Baggs et al., 2010)	The N_2O: N_2 emission ratio produced during denitrification is decreased
4	Increased labile C added to the soil (Bruun et al., 2011a)	The N_2O: N_2 product ratio of denitrification is reduced
5	Substances that may inhibit microbial activity, are emitted by the biochar, such as ethylene, α-pinene, PAHs, VOCs (Clough et al., 2010; Spokas et al., 2010; Taghizadeh-Toosi et al., 2011a; Quilliam et al., 2012)	Reduced activity of soil nitrifying/denitrifying organisms

As previously discussed, biochar amendment has been observed to reduce soil BD or increase WHC (Karhu et al., 2011; Basso et al., 2012), therefore increasing soil aeration. This may lead to lower soil N_2O emissions, as nitrifier and denitrifier activity is strongly influenced by soil aeration (Yanai et al., 2007; Van Zwieten et al., 2010b). Nitrifier activity is at a maximum at a moderate water-filled pore space (WFPS) (~ 60%), while denitrifier activity increases greatly with soil WFPS > 70% (Bateman & Baggs, 2005). Soil N_2O emissions are generally enhanced for several days following wetting in both laboratory and field conditions due to greater denitrifier activity (Skiba et al., 1996; Dobbie & Smith, 2001; Khalil & Baggs, 2005; Sänger et al., 2010). Soil mixing may reduce denitrifier activity and resulting soil N_2O emissions by reducing the BD of the soil and increasing soil aeration (Ruser et al., 2006).

Extractable inorganic N contents within the soil may be reduced following biochar addition (Spokas & Reicosky, 2009; Van Zwieten et al., 2010b; Spokas et al., 2012b). The availability of inorganic N is a key factor when considering nitrifier and denitrifier activity (Norton & Stark, 2011; Saggar et al., 2012). Nitrification uses NH_4^+ or NH_3 as a substrate, while denitrification can use NO_3^- or NO_2^-. Adding N-based fertiliser to soil is a standard agricultural practice (Olfs et al., 2005) and significantly

increases nitrifier or denitrifier enzymatic activity in the days following addition (Clayton et al., 1997). Increased immobilisation of extractable inorganic-N following biochar amendment may occur by one of two processes: abiotic-N adsorption to the biochar surface or indirect immobilisation of soil N into microbial biomass (Spokas & Reicosky, 2009; Singh et al., 2010a; Van Zwieten et al., 2010b).

Both NH_4^+ and NO_3^- are known to adsorb abiotically to biochar. Ammonium may adsorb to negatively-charged carboxylic groups on the biochar surface (Kastner et al., 2009; Taghizadeh-Toosi et al., 2011b; Spokas et al., 2012b), while the mechanisms by which NO_3^- is adsorbed to biochar are unclear (Mizuta et al., 2004; Spokas et al., 2012b). However, it is not certain if this mechanism continues into the long term, as it has been hypothesised to be due to the surface of the biochar may become 'clogged' with water, organic and inorganic material (Spokas, 2012). Therefore, after a number of months the adsorption capacity of added biochar may be reduced. Microbial-N immobilisation is generally the predominant form of N immobilisation in soil, and generally cycles more rapidly than abiotic-N immobilisation (Barrett & Burke, 2000). This form of immobilisation may be increased shortly following biochar amendment, as the labile C fraction of fresh biochar is typically quickly mineralised following amendment to soil, increasing the microbial requirement for N (Deenik et al., 2010; Lehmann et al., 2011; Ippolito et al., 2012).

Biochar often has a high pH, and so its addition to soil may increase soil pH (Singh et al., 2010b; Lehmann et al., 2011). Changes in soil pH may result in changes in nitrifier or denitrifier enzymatic activity and therefore soil N_2O emissions. Denitrifier enzyme activity was hypothesised to be at a maximum close to the natural pH of the soil in the short-term, but in the long term, the optimum pH for denitrifier enzyme activity was a maximum between 6.6 and 8.3 (Šimek et al., 2002). Below pH 6, the denitrifier conversion of N_2O to N_2 decreases, as bacterial N_2O reductase (Nos) enzymes are sensitive to low pH (Baggs et al., 2010). Increased soil pH with biochar amendment has been hypothesised to explain differences in soil N_2O emissions by this mechanism (Van Zwieten et al., 2010b). Turning to

nitrification, increasing soil pH up to 5 was shown to decrease the ratio of N_2O: (NO_2^- + NO_3^-) production during nitrification, but this effect was not demonstrated at a higher soil pH (Mørkved et al., 2007).

The addition of fresh biochar may add a significant amount of labile C to soil (Spokas, 2010), and therefore affect soil N_2O emissions by increasing the denitrifier conversion of NO_3^- to N_2 over N_2O (Bruun et al., 2011b). Some studies have suggested that adding labile C to soil affects the production of soil N_2O emissions (Morley & Baggs, 2010; Saggar et al., 2012). Generally, an increase in the availability of labile C in soil increases the denitrification rate, but it may also increase the conversion of N_2O to N_2 via denitrification (and resulting N_2O: N_2 product ratio) as C can be limiting for the final N_2O reduction process (Azam et al., 2002; Morley & Baggs, 2010; Saggar et al., 2012; Senbayram et al., 2012).

Inhibitive substances on or within the biochar may explain the suppression of soil N_2O emissions (Lehmann et al., 2011). Spokas (2012) suggested that nitrification/denitrification inhibitors on the biochar surface may suppress soil N_2O emissions. Other authors have suggested that other substances, such as ethylene, α-pinene, VOCs or ethylene from biochar had a significant suppressive effect on nitrifier and denitrifier activity, therefore reducing soil N_2O emissions; however this was not proven directly (Clough et al., 2010; Spokas et al., 2010, 2011; Taghizadeh-Toosi et al., 2011a). The concentrations of inhibitive substances within biochar vary widely depending on the feedstock and production conditions (Spokas et al., 2010). It has been hypothesised that the concentration of nitrification or denitrification inhibiting compounds reduces on the surface of the biochar with time; however this was not proven directly (Spokas, 2012).

1.4.3 Soil methane (CH_4) emissions

There is limited evidence to suggest that biochar amendment affects soil CH_4 emissions; evidence that comes mostly from studies in rice paddies (Zhang et al., 2010; Wang et al., 2011, 2012b). Methane emissions are generally significant in

saturated soils such as rice paddies but not in other more aerobic crop soils (Le Mer & Roger, 2001).

Wang et al., (2012b) found that soil CH_4 emissions were increased by 37% with biochar amendment in a rice paddy. Zhang et al., (2010, 2012) and Knoblauch et al. (2011) similarly observed an increase in soil CH_4 emissions from the same land use. For other crop types, three studies reported no significant effect of biochar amendment on CH_4 emissions in arable and pasture soils (Castaldi, 2011; Scheer et al., 2011; Wang et al., 2012b), whilst in Finnish agricultural soil a 96% increase in CH_4 uptake was measured in biochar-amended soil (Karhu et al., 2011). The mechanisms underlying changes in soil CH_4 emissions following biochar amendment are unclear and are summarised in Table 1.6 (Lehmann et al., 2011).

Table 1.6. Mechanisms proposed within the literature to explain the effect of biochar on soil methane (CH_4) emissions

Number	Mechanism	Effect
1	Biochar amendment adds labile C to saturated soil; substrate for methanogens (Wang et al., 2012b)	Increased soil CH_4 production
2	Increased soil aeration with biochar amendment (Karhu et al., 2011)	Increased soil CH_4 uptake

Increased availability of labile C substrates for methanogenic bacteria may explain increased CH_4 emissions following the addition of biochar to soil (Wang et al., 2012b). Methanogens produce methane as a metabolic by-product of organic matter mineralisation in anaerobic conditions; the two primary pathways being via CO_2 reduction by H_2 or via acetotrophy (Le Mer & Roger, 2001).

Soil methanotrophs are the only known biological sink for atmospheric CH_4, which oxidise CH_4 and produce CO_2 as a by-product (Topp & Pattey, 1997). Biochar addition has been observed to increase soil methanotrophic activity in one published study; Karhu et al., (2011) observed increased soil CH_4 uptake within an arable soil following biochar amendment that they put down to increased soil aeration. Soil methanotrophs require oxygen as a terminal electron acceptor and

their activity is highest at around 60% WFPS and decreases above this moisture content (Castro et al., 1995; Karhu et al., 2011).

As previously discussed, biochar addition to soil may decrease soil albedo and has been hypothesised to increase soil temperature and typically, high pH biochar increases the pH of the soil it is added to (Lehmann et al., 2011; Meyer et al., 2012). Methanogenic activity increases with temperature (up to 40°C) and is at a maximum at close-to-neutral pH (Topp & Pattey, 1997), while soil methanotrophy increases with temperature up until 10°C, (Castro et al., 1995) and methanotrophic activity is at a maximum at a close-to-neutral pH (Topp & Pattey, 1997). However, the effect of biochar on soil temperature and soil pH has not been suggested as mechanisms to explain differences in overall soil CH_4 emissions (or methanogenic or methanotrophic activity) following biochar amendment.

1.5 Aims and experimental approach

The first part of this review highlighted the need for strategies to mitigate the effects of climate change. Agriculture is a significant source of soil N_2O and CH_4 emissions and is responsible for large fluxes of CO_2. One mitigation technique currently being investigated is the application of biochar to soil.

Many published studies have shown that biochar amendment to soil has the potential to affect soil CO_2, N_2O and CH_4 emissions. The mechanisms underlying these effects are not well understood and existing research has two main shortcomings: studies are often confined to laboratory incubations and short time periods, and the mechanisms underlying the effects on soil GHG emissions are not elucidated. These shortcomings undermine the potential applications in agricultural practices, as one of the primary claims for the benefits of biochar is its potential to reduce total $CO_{2eq.}$ emissions from agricultural systems (Woolf et al., 2010). This research aims to address this issue.

The effects of these shortcomings are highlighted in the only published study that attempted to scale up the potential of biochar amendment to the global scale. Woolf

et al (2010) concluded that reductions of soil GHG emissions following biochar amendment on a large scale, concurrent with increased soil C storage could contribute on a significant scale to offsetting global annual $CO_{2eq.}$ emissions (1.8 Pg CO_2-$C_{eq.}$ yr^{-1} by 2100, equivalent to 12% of annual anthropogenic GHG emissions, or a cumulative total of 130 Pg CO_2-$C_{eq.}$ by 2100) (Woolf et al., 2010). However, these conclusions were based upon broad assumptions that biochar amendment will affect GHG emissions from every soil worldwide in the same way. These assumptions are summarised in Table 1.7 and in the following discussion.

Table 1.7. The assumptions made by Woolf et al., (2010) relevant to soil GHG emissions for global soil $CO_{2eq.}$ mitigation potential of large-scale biochar application. The same assumptions applied for all biochars and soils applied globally unless specified.

Half-life of labile biochar C (years)	Labile biochar C content (%)	Overall change in soil CO_2 emissions due to change in native SOC	Reduction of soil N_2O emissions (%)	CH_4 uptake from atmosphere (mg CH_4-C m^{-2} yr^{-1})
20	15	Increase. Magnitude varies by biochar feedstock (based on data from Powlson et al., (2008)	25	100

Woolf et al., (2010) assumed a 25% decrease in soil N_2O emissions, an uptake of 100 mg CH_4-C m^{-2} yr^{-1} and increased soil CO_2 emissions due to the mineralisation of labile portions of biochar C (a 20-year half-life of the labile portion of biochar, responsible for 15% of the biochar content). They also assumed overall increases in soil CO_2 emissions due to losses in SOC from field spreading of agricultural residue diverted to biochar production, not offset by increased crop yield due to biochar amendment. The authors state that this was decided in order to make a conservative estimate of the mitigation potential of biochar amendment (Woolf et al., 2010).

All of the above assumptions depend heavily on the effect of biochar addition on the soil it is added to, particularly soil GHG emissions. This study investigated the assumptions of Woolf et al., (2010) within bioenergy and arable crops (Table 1.7).

The overall aim of the work presented in this thesis was to investigate the effects of biochar amendment on soil GHG emissions from a typical bioenergy and arable soil and to identify the mechanisms underlying these effects.

In order to address this aim, Chapter 2 focused on the effects of hardwood biochar amendment on soil GHG emissions from a bioenergy soil and particularly the influence of increased soil aeration with biochar amendment. Once the importance of this mechanism had been established, further work was then required to establish that the same effect of biochar amendment on soil GHG emissions occurred in the field in the medium-term (two years) (Chapter 3). Chapter 4 then extended the investigation of the effect of biochar on soil GHG emissions to an arable soil, and considered in-depth the mechanisms behind to effect of biochar amendment on soil N cycling processes.

The experimental approach involved studying GHG emissions under a combination of laboratory (Chapter 2 to 4) and field conditions (Chapter 3). The study conducted short-term laboratory experiments with fresh biochar (Chapter 2 and 4) and medium-term experiments with biochar amendment to the field (Chapter 3) in order to investigate the effect of biochar addition on soil GHG emissions at varying times from addition.

A primary claimed benefit of biochar amendment is that it can improve the sustainability of agricultural practices; therefore the choice of biochar has to be consistent with maximising this benefit. The sustainability of the biochar production and amendment life cycle is maximised when transportation is kept to a minimum and biochar is produced from waste products (Roberts et al., 2010). This research used a hardwood, slow-pyrolysis biochar derived from forest waste products native to the UK (Bodfari Environmental, Bodfari, Wales). As a secondary benefit, hardwood biochar has been used in many biochar amendment studies, which suggests it is potentially applicable to large-scale future uses of biochar and allows us to compare our results with published data (Hartley et al., 2009; Singh et al., 2010b; Beesley et al., 2011; Jones et al., 2012; Hollister et al., 2013).

Introduction to Chapter 2 - The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil – the role of soil aeration

This chapter investigates the effects of biochar amendment on soil GHG emissions in typical environmental conditions from a bioenergy field. It then examines the influence of the mechanism of increased soil aeration with biochar amendment on soil GHG emissions.

We incubated *Miscanthus* soil cores at several temperatures and under two soil moisture regimes in order to represent the range of typical environmental conditions in the field. We added biochar to half of the soil cores in order to investigate its effect on soil GHG emissions. We then conducted a second laboratory incubation with soil under a controlled moisture regime (a constant water holding capacity and water-filled pore space) in order to elucidate the effect of increased soil aeration with biochar addition on soil CO_2 and N_2O emissions. The results from these incubations would lead us towards the incubations conducted in the field (Chapter 3) and within a neighbouring arable field (Chapter 4).

2 The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil – the role of soil aeration

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I conducted the experimental work contained within this chapter. I also performed the analysis and wrote up the paper. Dr Whitaker, Dr McNamara and Dr Reay reviewed and suggested corrections for the manuscript drafts.

2.1 Abstract

Biochar application to soil has significant potential as a climate change mitigation strategy, due to its recalcitrant C content and observed effect to suppress soil greenhouse gas emissions such as nitrous oxide (N_2O). Increased soil aeration following biochar amendment may contribute to this suppression.

Soil cores from a *Miscanthus X. Giganteus* plantation were amended with hardwood biochar at a rate of 2% dry soil weight (22 t ha^{-1}). The cores were incubated at three different temperatures (4, 10 and 16°C) for 126 days, maintained field moist and half subjected to periodic wetting events. Cumulative N_2O production was consistently suppressed by at least 49% with biochar amendment within 48 hours of wetting at 10 and 16°C. We concluded that hardwood biochar suppressed soil N_2O emissions following wetting at a range of field-relevant temperatures over four months. We hypothesised that this was due to biochar increasing soil aeration at relatively high moisture contents by increasing the water holding capacity (WHC) of the soil; however, this hypothesis was rejected.

We found that 5% and 10% biochar amendment increased soil WHC. Also, 10% biochar amendment decreased bulk density of the soil. Sealed incubations were performed with biochar added at 0 to 10% of dry soil weight and wetted to a uniform 87% WHC (78% WFPS). Cumulative N_2O production within 60 hours of wetting was 19, 19, 73 and 98% lower than the biochar-free control in the 1, 2, 5 and 10% biochar treatments respectively. We conclude that high levels of biochar amendment may change soil physical properties, but that the enhancement of soil aeration by biochar incorporation makes only a minimal contribution to the suppression of N_2O emissions from a sandy loam soil. We suggest that microbial or physical immobilisation of NO_3^- in soil following biochar addition may significantly contribute to the suppression of soil N_2O emissions.

2.2 Introduction

2.2.1 Greenhouse gas emissions from soils

Nitrous oxide (N_2O) is a greenhouse gas (GHG) of high importance, with emissions accounting for approximately 6% of total anthropogenic radiative forcing (Davidson, 2009). Agriculture accounts for 58% of anthropogenic emissions of N_2O (Solomon et al., 2007a). A large proportion of N_2O from agriculture comes from the inefficient use of N-based fertiliser, particularly from incomplete denitrification in wet or saturated soils (Davidson, 2009).

N_2O is produced in soils primarily via microbial activity through nitrification (Khalil et al., 2004), nitrifier denitrification (Wrage et al., 2005) and denitrification (Gillam et al., 2008). At high moisture contents, N_2O production from denitrification is thought to be the dominant source (Bateman & Baggs, 2005). Denitrification is known to be strongly affected by soil temperature, nitrate (NO_3^-) content, organic matter availability and lability, redox potential and pH (Hofstra & Bouwman, 2005).

Both nitrification and denitrification are highly moisture sensitive, as increased moisture content reduces oxygen availability to soil microorganisms (Barnard et al., 2005; Gillam et al., 2008). Across soil types, nitrifier activity peaks at around 60% of water holding capacity (WHC) and decreases above this when oxygen becomes more limiting. Denitrifier activity increases above 70% WHC (Linn & Doran, 1984). Considering instead a measure of soil aeration – water-filled pore space (WFPS) – nitrifier activity has been found to peak at 60% WFPS and denitrifier activity increases above 70% WFPS (Bateman & Baggs, 2005). In soils approaching fully waterlogged conditions (and thus fully anoxic conditions) complete denitrification to N_2 may occur resulting in decreased N_2O emissions (Firestone and Davidson, 1989; Clough and Condron, 2010). Nitrous oxide production from soils can also be highly sensitive to intermittent wetting; N_2O emissions are generally enhanced for several days following wetting in both laboratory and field conditions (Skiba et al., 1996; Dobbie & Smith, 2001; Khalil & Baggs, 2005; Sänger et al., 2010).

2.2.2 Biochar

Biochar is created by heating biomass (generally between 350 and 600°C) in an oxygen-limited environment, a process called pyrolysis (Sohi et al., 2010). Its physical and chemical properties are similar to charcoal, typified by its relatively high C content, low nutrient content, high surface area and cation exchange capacity compared to unheated biomass (Singh, et al., 2010). Previous studies have focused on the range of effects that biochar can have on soil condition (Spokas et al., 2012a), crop yield (Laird et al., 2010), uptake of nutrients or contaminants (Cao et al., 2009; Steiner et al., 2010), and soil GHG emissions (Spokas & Reicosky, 2009).

Suppression of N_2O emissions following the wetting of biochar amended soil has been observed both under laboratory conditions (Yanai et al., 2007; Spokas and Reicosky, 2009; Singh, et al., 2010) and in the field (Zhang et al., 2010; Wang et al., 2011). Nitrous oxide emissions have also been suppressed following the addition of urine to biochar amended soils (Van Zwieten et al., 2010b; Taghizadeh-Toosi et al., 2011a). However, there are studies where biochar did not significantly affect soil N_2O emissions in the field (Scheer et al., 2011) and following urine addition in the laboratory (Clough et al., 2010).

Soil N_2O emissions increase with temperature (Bouwman et al., 2002). Previously, laboratory experiments investigating N_2O emissions from biochar amended soils have incubated soils kept at a single temperature (~ 20 °C, Yanai et al., 2007; Singh, et al., 2010; van Zwieten et al., 2010). In this paper we investigate the effect of biochar on soil N_2O emissions at several temperatures relevant to field conditions.

Enhanced soil aeration (Yanai et al., 2007; Van Zwieten et al., 2010b), sorption of NH_4^+ or NO_3^- by biochar (Singh, et al., 2010; van Zwieten et al., 2010) and the presence of inhibitory compounds such as ethylene (Spokas et al., 2010) have all been suggested as mechanisms to explain the suppression of N_2O emissions with biochar addition. In this paper we focus on soil aeration.

Biochar has been observed to affect soil physical properties. With biochar amendment, a field study observed an increase in saturated hydraulic conductivity (Asai et al., 2009); while a pot study observed reduced tensile strength and increased field capacity (Chan et al., 2007). By changing physical properties of the soil, biochar may suppress N_2O production from denitrification by increasing the air content of the soil (Van Zwieten et al., 2010b) or by absorbing water from the soil, thus improving aeration of the soil (Yanai et al., 2007). We aimed to investigate the little-understood interaction between biochar amendment to soil, changes in soil physical properties (WHC, bulk density, BD, and related WFPS) that are linked to increased soil aeration and soil N_2O emissions. To do so we conducted two laboratory studies with the following aims.

2.2.3 Aims

Our primary aim (Experiment 1) was to elucidate any differences in N_2O production from an agricultural soil, with and without biochar amendment, under a range of field-relevant temperatures and subjected to wetting/drying cycles. We hypothesised that biochar amendment would suppress soil N_2O production following wetting at all temperatures. We also hypothesised that this effect would not be seen under field moist conditions, as N_2O production would be too low to observe significant differences between control and biochar amended soil.

The aim of Experiment 2 was to investigate the mechanism(s) behind observed differences in N_2O production with and without biochar. We hypothesised that previously observed suppression of N_2O production was due to biochar increasing soil aeration. By maintaining uniform WHC across several biochar amendment levels (0 – 10%), we would cancel out the effect of increasing soil aeration with biochar addition. Therefore, N_2O production would remain constant with increasing biochar content.

2.3 Materials and methods

2.3.1 Soil and biochar

Bare soil was collected from a *Miscanthus* (*Miscanthus X Giganteus*, a species of elephant grass) field close to Lincoln, Lincolnshire, UK (planted in 2007). The soil is a dense, compacted sandy loam with 53% sand, 32% silt and 15% clay, a BD of 1.68 ± 0.03 g cm^{-3} (n = 3), a low total C (14.7 ± 0.2 g kg^{-1}, n = 105) and total N content (2.70 ± 0.10 g kg^{-1}, n = 105), and low extractable inorganic-N content (NH_4^+-N: 0.6 ± 0.10 mg kg^{-1}, n = 18, NO_3^--N: 1.8 ± 0.35 mg kg^{-1}, n = 18). The crop received an application of 500 kg ha^{-1} PK fertiliser in March 2010 (Fibrophos, UK).

The biochar was produced from thinnings of hardwood trees (oak, cherry and ash greater than 50mm in diameter, Bodfari Charcoal, UK). The feedstock was heated in a ring kiln, first to 180°C to allow the release of volatile gases, and then to approximately 400°C for 24 hours. After sieving and homogenisation, the fresh biochar had a particle size of < 2 mm, a gravimetric moisture content (GMC) of < 5%, a BD of 0.24 g cm^{-3} (n = 1), a total C content of 723 g kg^{-1} ± 15.1 (n = 3), a total N content of 7.12 g kg^{-1} ± 0.10 (n = 3), an extractable NH_4^+ and NO_3^- content below detectable limits (< 1 mg kg^{-1} NH_4^+-N and NO_3^--N < 1.3 mg kg^{-1}, n = 3), a pH of 9.25 ± 0.04 (n = 4, see Section 2.3.2 for description of methods) and a cation exchange capacity (CEC, analysed by ICP-OES) of 145 $cmol^+$ kg^{-1} (n = 1). Further biochar properties such as exchangeable cations, heavy metal content, polyaromatic hydrocarbon (PAH) content and Benzene, Ethylbenzene, Toluene and Xylene (BETX) content are available in the Appendix section (Section 7.1). Metal contents (As, Cd, Ni, Pb, Zn, Hg, Cr) were analysed using ICP-OES. BETX were analysed by HS-GC-MS and PAHs (USEPA 16) were analysed by GC-MS.

2.3.2 Experiment 1: Soil cores undergoing wetting/drying cycles

We assessed the effect of biochar addition on soil N_2O emissions with a fully-factorial experiment (n = 4) at three incubation temperatures (4, 10 and 16°C) and

two moisture conditions (field moist, 23% GMC and wetted, 28% GMC). Environmental conditions were selected based on monthly temperature and moisture sampling at the field site taken over one year from 2008 to 2009 (data not shown).

Soil cores were collected in March 2010. PVC pipes (W 102 mm, H 215 mm) were inserted to a depth between 150 and 180 mm (soil height between these two values, ~ 2 kg dry soil wt.). Soil cores were stored at 4°C for four weeks prior to biochar addition. Biochar (< 2 mm) was added to half of the cores, mixed into the top 7 cm of soil at a rate of 2% dry soil weight (~ 22 t ha^{-1}). Control cores without biochar were also mixed in a similar fashion. Mixed soil BD was determined (1.00 ± 0.01 g cm^{-3}, n = 42) following Emmett et al., (2008). The WFPS in the field moist and wetted treatments (37 ± 1%, n = 24 and 45 ± 1%, n = 19 respectively, assuming uniform distribution of applied water throughout soil core) was calculated assuming a particle density of 2.65 g cm^{-3} (Elliott et al., 1999). As we were interested in the long-term effect of biochar amendment on soil GHG emissions, soil cores were stored at 4°C for a further two weeks prior to the start of the experiment in order to allow the initial flush of CO_2 emissions from newly-mixed soil to equilibrate (Reicosky, 1997; Zimmerman et al., 2011). Throughout the experiment, soil cores were maintained field moist gravimetrically with de-ionised water.

To measure soil GHG emissions, headspace gas samples were taken using the unvented static enclosure method (Livingston & Hutchinson, 1995). A plastic container (Lock & Lock, USA, W 110 mm, H 180 mm) was cut in two widthways and sealed tightly to the outside of the soil core with several layers of duct tape. A 10 mm hole was drilled into the Lock & Lock lid and a rubber septum (Sigma Aldrich, USA) inserted into the hole. This lid was connected to the top of the plastic container during gas sampling. The air tightness of the system was pre-tested. Details regarding the gas sampling method are in Section 2.3.4.

The first gas samples were taken 6 days after the start of incubation, to allow for the initial flush of respiration in response to the warming (Fang & Moncrieff, 2001). Gas

samples were taken from all soil cores at 6, 26, 51, 64 and 127 days. These days were chosen to ensure that all soil cores had dried to the GMC required of 'field moist' conditions in between wetting events. After 51, 72 and 86 days, soil cores were subjected to wetting events. Approximately 120 ml of water was necessary for each core to reach 28% GMC. Headspace gas samples were taken periodically in the 72 hours following wetting (Fig. 2.1, Fig. 2.2). Higher temperature soil cores were subjected to more wetting events due to faster drying rates.

For chemical analyses, soil samples were taken from the top 5 cm of the intact soil cores (the same used for gas sampling) and homogenised. Control soil samples were taken and stored at - 20°C within one week of soil core collection from the field. All other soil samples were taken and frozen at - 20°C the day after the final gas sampling (day 126).

Soil pH (n = 4) was determined using de-ionised water at a ratio of 1:2.5 of dry weight soil or biochar (Emmett et al., 2008), using a Kent-Taylor combination pH electrode (Asea Brown Boveri, Switzerland). Extractable soil NH_4^+ and NO_3^- were extracted using 0.8 M potassium chloride (KCl), and analysed on a Seal AQ2 discrete analyser (Bran and Luebbe, UK) using discrete colorimetric procedures (Maynard and Kalra, 1993). Total C and nitrogen (N) analyses were conducted using 0.1 g oven-dried samples ground and sieved to < 2 mm. Samples were analysed on a LECO Truspec total CN analyser (LECO, USA) with an oven temperature of 950°C (Sollins et al., 1999).

2.3.3 Experiment 2: Soil incubations at uniform water holding capacity

Soil (0 - 10 cm depth, from the same field site as Experiment 1) was collected in October 2010 and stored at 4°C for 8 weeks prior to the experiment. The soil was sieved (< 2 mm) and placed (25 g dry soil wt.) into glass serum bottles (125 ml, Wheaton Science Products, USA). Biochar (< 2 mm, GMC < 5%) was added to bottles at a rate of 0, 1, 2, 5 and 10% of total dry soil weight (n = 4) and thoroughly mixed.

The bottles were then incubated at 16°C in the dark, and left for 3 days to allow for the equilibration of enhanced CO_2 emissions due to mixing and increased soil temperature (Reicosky, 1997; Fang & Moncrieff, 2001).

WHC was determined using a method similar to Ohlinger, (1995). Briefly, 25 - 30g of field moist soil was added to plastic cylinders (W = 40 mm), with the bottom end covered in a fine mesh. These were saturated in water for one hour. The cylinders were covered with plastic film (Parafilm, USA), placed on top of a funnel, and placed in a humid, closed plastic box to limit evaporation. The soil was removed and weighed after three hours, heated to 105°C for 16 hours and re-weighed. The maximum WHC under laboratory conditions was then calculated. Bulk density and WFPS were determined as in Section 2.3.2.

Pilot tests demonstrated (data not shown) that wetting greatly increased soil N_2O emissions in the first 72 hours following water addition when wetted from field moist (34% of WHC) to 87% WHC. Based on this result, de-ionised water was added to all bottles to wet the biochar amended soils to 87% WHC (WFPS = 78 ± 1%, n = 20, assuming a particle density of 2.65 g cm^{-3} as in Section 2.3.2) on day 0 of the experiment. The bottles (with a laboratory air atmosphere) were then sealed for the duration of the experiment with butyl rubber stoppers and aluminium crimp caps. Headspace gas samples were taken at 0, 12, 24, 36, 48, 60 and 168 hours after wetting.

For soil chemical analyses, following completion of Experiment 2, the soils were stored at 4°C for less than one week before analysis for extractable NH_4^+, extractable NO_3^- and pH. Field soil, collected in October 2010 and stored at 4°C until analysis in January 2011, was used as the control. For total C and total N, all soil samples were stored at – 20°C immediately after the end of the experiment until analysis. Bulk density was determined from sub samples of 25 g of fresh soil for each biochar treatment. The methods for physical and chemical analysis were the same as those described in Section 2.3.2.

2.3.4 Headspace gas analysis

Concentrations of CO_2 were analysed on a PerkinElmer (PerkinElmer, USA) Autosystem Gas Chromatograph (GC) fitted with two flame ionization detectors (FID) operating at 130 (FID) and 300°C (FID with methaniser) respectively. Nitrous oxide was analysed on a PerkinElmer Autosystem XL GC using an electron capture detector (ECD) operating at 360°C. Both chromatographs contained a stainless steel Porapak Q 50 - 80 mesh column (length 2 m, outer diameter 3.17 mm), maintained at 100°C and 60°C in the CO_2 and N_2O GC respectively.

Results were calibrated against certified gas standards (Air Products, UK). Minimum detection limits (data not shown) were calculated as in Trace Gas Protocol Development Committee, (2003). For Experiment 1, Headspace gas fluxes were calculated using the linear accumulation of N_2O and CO_2 gas concentrations sampled at 0, 20, 40, 60 minutes using the approach of (Holland et al., 1999). Gas production following wetting was converted into cumulative gas production m^{-2} by summing modelled hourly production for each wetted soil core between 0 and 48 hours. For Experiment 2, gas samples (0.2 ml) were taken with a 1 ml gas-tight syringe from the bottle headspace and immediately injected into the gas chromatograph. Cumulative GHG production was calculated directly from the difference between the sealed headspace gas concentrations at t_0 and the time of sampling.

2.3.5 Statistical analysis

Statistical analyses were conducted using the statistical package R, version 2.14.0 (The R Project, 2013). Dependent variables were log transformed in the form $\log_{10}$ (gas flux + most negative gas flux in dataset + 1) where appropriate (Table 2.1,

Table 2.3 and Table 2.5), as this transformation gave data that better approached normality than the raw data or a square root transformation. For Experiment 1, linear mixed-effects models were run for gas fluxes (Table 2.1). All models were refined and validated following the guidance provided in Zuur et al., (2010).

Cumulative GHG flux within 48 hours of a wetting event (Table 2.2) and all chemical properties (pH, total C, total N, extractable NH_4^+, extractable NO_3^-, Table 2.4) were analysed using a three-way ANOVA.

For Experiment 2, cumulative N_2O and CO_2 production at 60 hours after wetting (the time of peak cumulative N_2O production, Table 2.5), soil extractable NH_4^+, soil extractable NO_3^-, pH, total C content, total N content and WHC (Fig. 2.6) were analysed using a one-way ANOVA with biochar amendment level (0 – 10%) as the independent variable. Tukey's Honestly Significant Difference test was applied to investigate differences between each level of biochar amendment.

2.4 Results

2.4.1 Experiment 1: Soil cores undergoing wetting/drying cycles

Increasing temperature significantly increased N_2O emissions across all field moist treatments ($p < 0.05$, Table 2.1). However, N_2O fluxes in the field moist soil cores were consistently observed to be low, generally less than minimum detection limits (found to be 2.7 μg N_2O-N m^{-2} h^{-1}) at all temperatures (Fig. 2.1). Overall, biochar amendment did not significantly affect N_2O emissions in field moist soil cores ($p > 0.05$, Table 2.1).

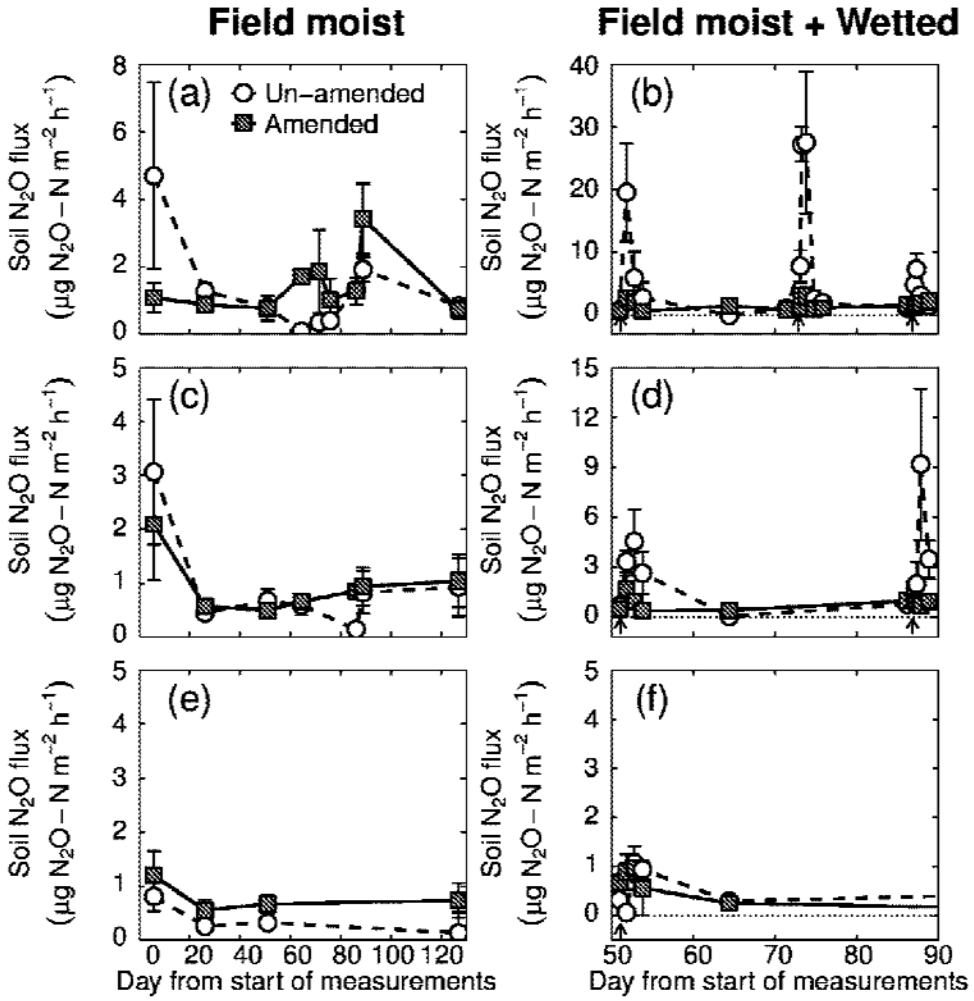

Fig. 2.1. Experiment 1: nitrous oxide (N_2O) emissions from soil cores undergoing wetting/drying cycles. Soil cores are field moist or field moist subject to wetting events (b), d), f), at time indicated by arrow) and incubated at 16 (a), b)), 10 (c), d)) or 4°C e), f)). A horizontal dotted line indicates 0. Data points represent mean ± standard error (n = 4).

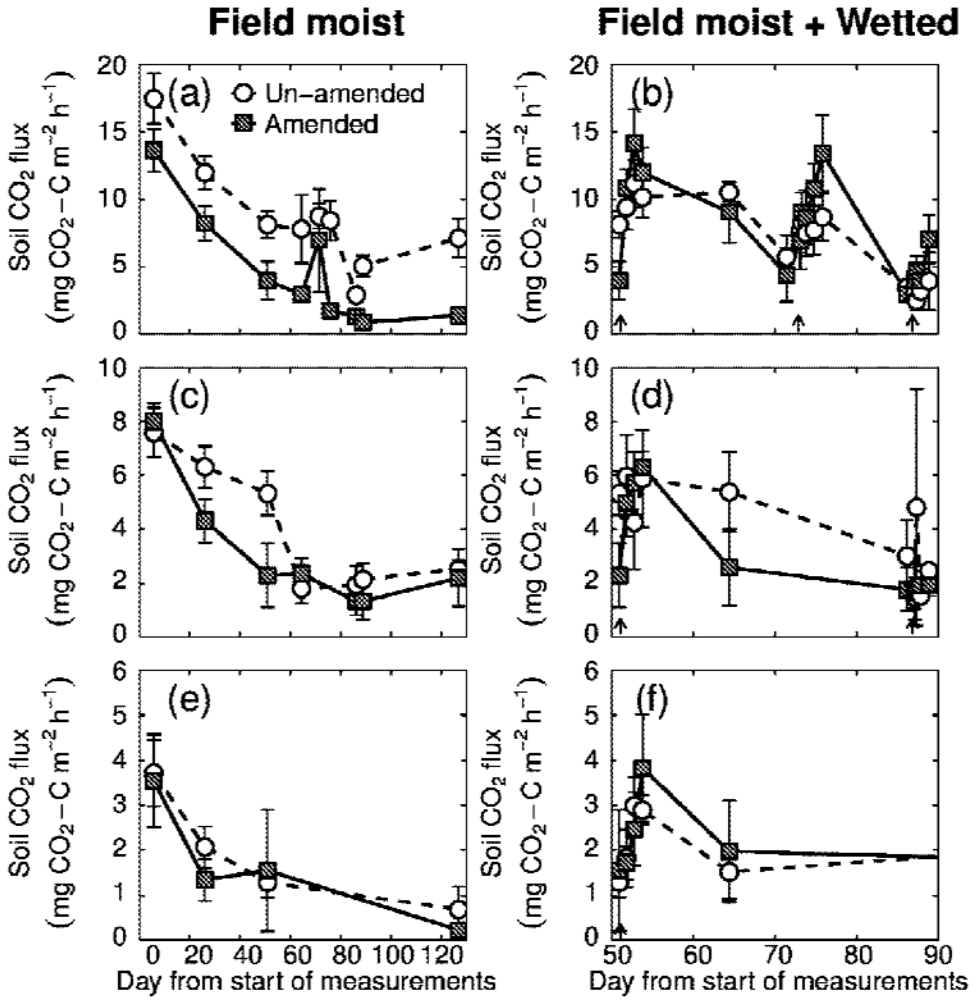

Fig. 2.2. Experiment 1: carbon dioxide (CO_2) emissions from soil cores undergoing wetting/drying cycles. Soil cores are field moist or field moist subject to wetting events ((b), d), f), at time indicated by arrow) and incubated at 16 (a), b)), 10 (c), d)) or 4°C e), f)). A horizontal dotted line indicates 0. Data points represent mean ± standard error (n = 4).

Despite a trend of lower CO_2 emissions at 16°C in field moist soil cores (Fig. 2.2), the effect of biochar was not significant ($p > 0.05$, Table 2.1). Increasing temperature significantly increased CO_2 emissions ($p < 0.001$, Table 2.1). For example, field moist CO_2 emissions on day 6 were 17.5, 7.6 and 3.7 CO_2-C mg m^{-2} h^{-1} for the 16, 10 and 4°C control (un-amended) treatments respectively (Fig. 2.2). Carbon dioxide emissions decreased significantly with incubation time at all temperatures (Fig. 2.2, $p < 0.001$, Table 2.1).

Table 2.1. Experiment 1: The significance of fixed effects in the linear mixed-effects models for N2O and CO2 fluxes. 'Time from start' indicates time from start of the experiment, while 'Time from wetting' indicates time from last wetting event. Gravimetric moisture content is referred to as 'wetting' in the text. Gas efflux data were transformed in the form log10 (gas flux + 1). Symbols indicate the presence of the term within the model or significance of the term: - = not present in refined model, ns = not significant, . = p < 0.1, * = p < 0.05, ** = p < 0.01, *** = p < 0.001

	N_2O emissions field-moist		**CO_2 emissions field-moist**		**CO_2 emissions field-moist at 16°C**		**N_2O emissions wetted, 16 and 10 ˚C**		**CO_2 emissions wetted**	
Effect	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**
Biochar	0.9	ns	-0.3	ns	-	-	1.8	ns	-1.5	ns
Temperature	4.9	***	9.0	***	-	-	3.6	**	5.0	***
Time from start	-2.1	*	-4.0	***	-5.7	***	-1.8	.	-9.0	***
Temperature * Time from start	-	-	-0.9	ns	-	-	-	-	-	-
Time from wetting	-	-	-	-	-	-	-1.8	.	1.1	ns
Biochar * Temperature	-2.2	*	-2.4	*	-	-	1.6	ns	0.9	ns
Biochar * Time from wetting	-	-	-	-	-	-	-2.5	*	2.6	**
Biochar * Time from start	2.4	*	0.4	0.7	-4.2	***	-	-	-	-
Biochar * GMC	-	-	-	-	-	-	-2.1	*	-	-
Biochar * Time from start * Temperature	-	-	-1.26	ns	-	-	-	-	-	-

Cumulative GHG production was calculated for 0 - 48 hours following a wetting event (Table 2.2) and is compared below. Wetting significantly increased soil N_2O emissions in the un-amended cores incubated at 10 and 16°C ($p < 0.01$, Fig. 2.1). After the first wetting event (day 51), wetting increased N_2O fluxes in the un-amended treatment at 10°C from 0.7 to a maximum of 4.6 μg N_2O-N m^{-2} hr^{-1} (Fig. 2.1). Increasing temperature significantly increased soil N_2O emissions ($p < 0.01$, Table 2.1). At 16°C for the same wetting event, N_2O fluxes increased in the un-amended treatment from 0.8 to a maximum of 19.6 μg N_2O-N m^{-2} hr^{-1} (Fig. 2.1).

Table 2.2. Experiment 1: cumulative greenhouse gas production from 0 to 48 hours after wetting from soil cores undergoing wetting/drying cycles. Data indicate mean value (standard error). n = 4.

Wetting event	Temperature (°C)	Treatment	CO_2 production (CO_2-C mg m^{-2})	N_2O production (N_2O-N μg m^{-2})
1	16	Un-amended	451 (72)	516 (129)
	16	Amended	523 (74)	99 (27)
	10	Un-amended	249 (58)	155 (22)
	10	Amended	225 (53)	57 (14)
	4	Un-amended	104 (18)	23 (1)
	4	Amended	127 (24)	42 (4)
2	16	Un-amended	358 (76)	815 (200)
	16	Amended	418 (85)	104 (32)
3	16	Un-amended	156 (32)	177 (35)
	16	Amended	234 (40)	89 (12)
	10	Un-amended	118 (14)	235 (87)
	10	Amended	89 (12)	45 (3)

Cumulative N_2O production was significantly suppressed in all cases by at least 49% by biochar amendment at 10 and 16°C ($p < 0.001$, Table 2.2, Table 2.3). At 4°C, where N_2O emissions were generally below detectable limits, biochar addition did not significantly affect cumulative N_2O production ($p > 0.05$, 2.1).

Table 2.3. Experiment 1: outputs from the three-way ANOVA models for cumulative N_2O and CO_2 production within the first 48 hours of wetting from soil cores undergoing wetting/drying cycles. 'Time from start' indicates time from start of the experiment. Symbols indicate the p significance of the term: ns = not significant, . = < 0.1, * = < 0.05, ** = < 0.01, *** = < 0.001

Effect	Log_{10} (Cumulative N_2O production)	Cumulative CO_2 production
Time from start	ns	***
Biochar	***	ns
Temperature	**	***
Biochar * Time from start	ns	ns
Biochar * Temperature	ns	ns
Temperature * Time from start	ns	.
Biochar * Temperature * Time from start	ns	ns

For the first wetting event (day 51, Table 2.2), cumulative N_2O production in the un-amended treatment at 10°C was 155 μg N_2O-N m^{-2}, while production from the biochar amended cores was 57 μg N_2O-N m^{-2}, a suppression of 63% (Table 2.2). This

suppression was more pronounced in cores incubated at 16°C, where cumulative N_2O production in the un-amended treatment was 516 μg N_2O-N m^{-2}, but in the 2% biochar treatment only 99 μg N_2O-N m^{-2}, a suppression of 81% (Table 2.2). For the second wetting event (day 72), the 16°C un-amended cumulative N_2O-N production was 815 μg N_2O-N m^{-2} but 104 μg N_2O-N m^{-2} with 2% biochar (Table 2.2). For the third wetting event (day 86), the suppression with biochar amendment was most pronounced in the 10°C treatments. Cumulative N_2O production was 177 and 89 μg N_2O-N m^{-2} for the 16°C un-amended and 2% biochar treatments respectively, compared with 234 and 45 μg N_2O-N m^{-2} for the 10°C, un-amended and 2% biochar treatments (Table 2.2).

Increasing temperature significantly increased cumulative CO_2 production ($p < 0.001$, Table 3). Wetting or biochar addition did not significantly increase cumulative CO_2 production at any incubation temperature ($p > 0.05$, Fig. 2.2,Table 2.3).

Biochar amendment significantly increased total C content in both field moist and wetted soil ($p < 0.001$, Table 2.4). Across all treatments, total C contents ranged from 14.1 g kg^{-1} ($n = 24$, ± 0.5) to 21.7 g kg^{-1} ($n = 24$, ± 2.1) for the un-amended and 2% biochar treatments (Fig. 2.3, Fig. 2.4).

Table 2.4. Experiment 1: model outputs from the three-way ANOVA of soil chemical properties from soil cores undergoing wetting/drying cycles. The soil samples were taken from the top 5 cm of the soil cores and sampled at day 126. Results are expressed by dry weight of soil where relevant. Symbols indicate the p significance of the term: ns = not significant, . = < 0.1, * = < 0.05, ** = < 0.01, *** = < 0.001

Effect	Bulk density	log_{10} (Total C + 1)	Total N	log_{10} (C:N + 1)	Extractable NH_4^+	Extractable NO_3^-	pH
Biochar	ns	***	ns	***	ns	***	ns
Temperature	*	ns	ns	ns	*	***	*
Wetting	.	ns	ns	ns	.	ns	ns
Biochar * Temperature	ns	ns	ns	ns	ns	.	*
Biochar * Wetting	ns	ns	ns	ns	ns	.	ns
Temperature * Wetting	ns	ns	ns	ns	*	ns	ns
Biochar * Temperature * Wetting	***	ns	*	ns	ns	.	ns

Biochar, temperature or wetting did not have a significant effect on total N content ($p > 0.05$, Table 2.4, Fig. 2.3, Fig. 2.4), which was an average of 2.09 ± 0.05 g kg^{-1} (n = 48) across all treatments. Soil C to N ratios were significantly increased by biochar addition ($p < 0.001$, Table 2.5), for example, from an average of 6.9 to 14.2 for the un-amended and 2% biochar 16°C wetted treatment respectively (Fig. 2.4).

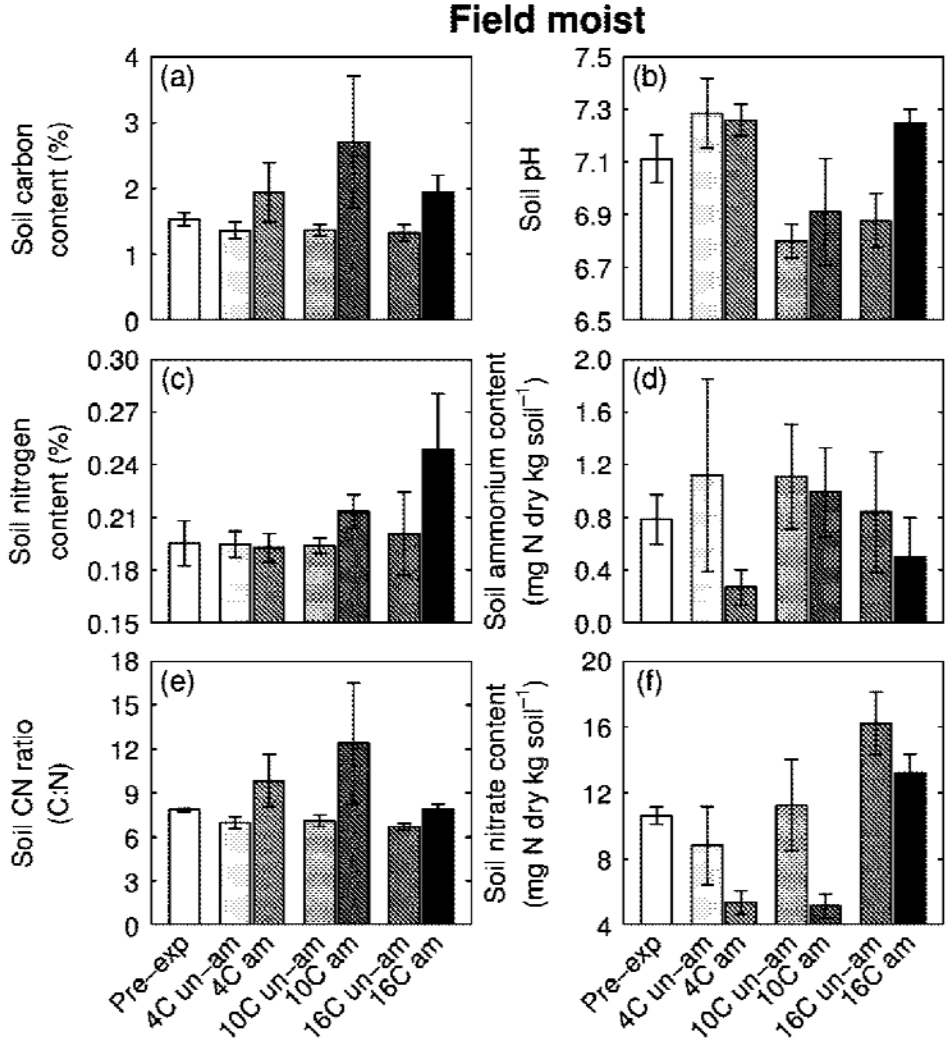

Fig. 2.3. Experiment 1: soil chemical and physical properties from soil cores maintained field moist; (a) soil carbon (C), (b) soil pH, (c) soil nitrogen (N) content, (d) soil NH_4^+ content, (e) soil C: N ratio and (f) soil NO_3^- content. "am" indicates soil amended with biochar, "un-am" indicates without. Treatments were analysed before (pre-experiment control, 'pre-exp') or after (all other treatments) the incubation. Results are expressed by dry weight of soil where relevant. Data indicate mean value (standard error). n = 4.

Extractable NO_3^- contents were significantly lower with biochar content ($p < 0.001$, Table 2.4). Wetted un-amended soil cores incubated at 16°C contained 20.0 mg kg^{-1} of extractable NO_3^--N (Fig. 2.4) compared with 6.8 mg kg^{-1} for 2% biochar soil cores, a reduction of 66%. Reductions also occurred at 10 (61%) and 4°C (34%) between all field-moist 2% and un-amended soil cores (Fig. 2.3).

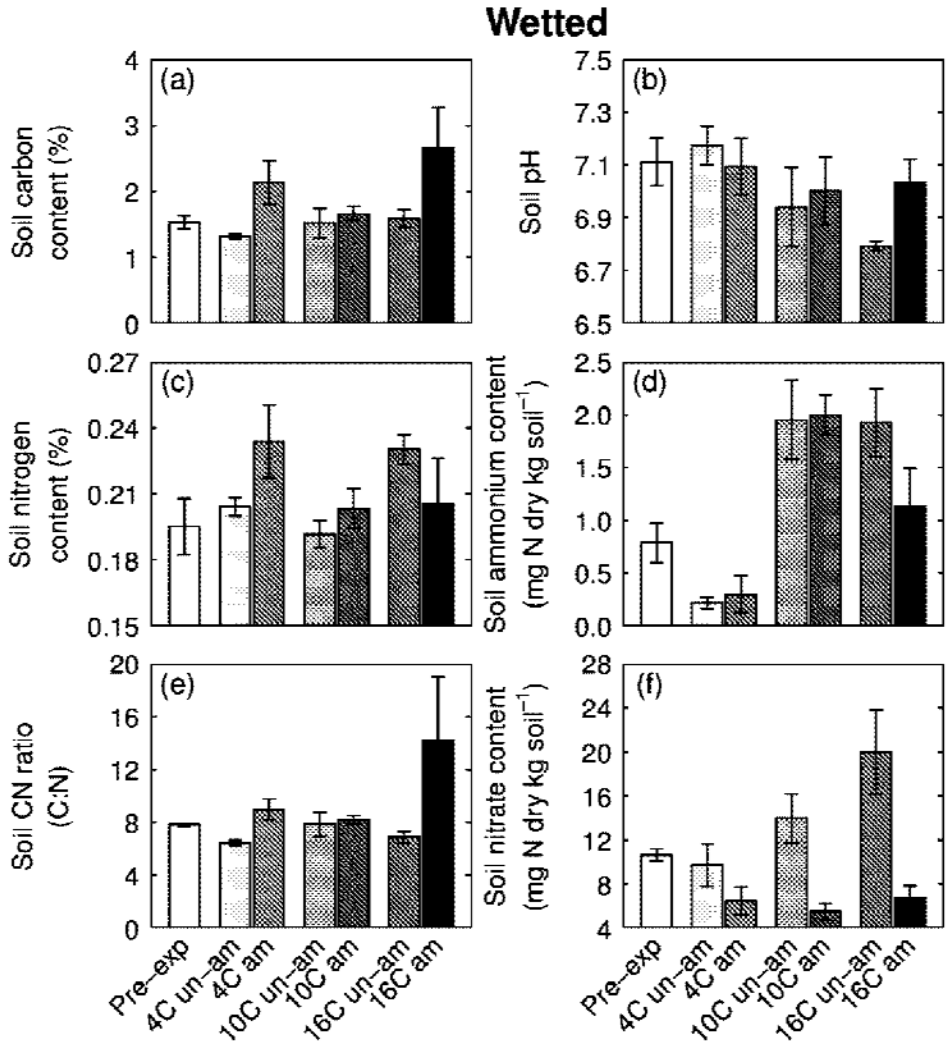

Fig. 2.4. Experiment 1: soil chemical and physical properties from soil cores undergoing wetting/drying cycles; (a) soil carbon (C), (b) soil pH, (c) soil nitrogen (N) content, (d) soil NH_4^+ content, (e) soil C: N ratio and (f) soil NO_3^- content. "am" indicates soil amended with biochar, "un-am" indicates without. Treatments were analysed before (pre-experiment control) or after (all other treatments) the incubation. Results are expressed by dry weight of soil where relevant. Data indicate mean value (standard error). n = 4.

Extractable NH_4^+ contents were low across all treatments (< 2 NH_4^+-N mg kg^{-1}, Fig. 2.3, Fig. 2.4). Biochar addition and wetting did not significantly affect extractable NH_4^+ content ($p > 0.05$, Table 2.4). Biochar amendment significantly affected pH only at 16°C across both field moist and wetted treatments ($p < 0.01$, Table 2.4, from 6.84 ± 0.15, n = 8, to 7.14 ± 0.06, n = 8, Fig. 2.3, Fig. 2.4).

2.4.2 Experiment 2: Soil incubations at uniform water holding capacity

Cumulative GHG production was calculated for 0 - 60 hrs following a wetting event and is compared below (Table 2.5). Biochar addition significantly suppressed cumulative N_2O production in the bottle headspace at all biochar amendment rates (Table 2.5). Cumulative N_2O production was 19, 19, 73 and 98% lower than the un-amended control for the 1, 2, 5 and 10% biochar addition treatments respectively ($p < 0.01$ - 0.001, Table 2.5). Cumulative N_2O production was highly correlated with biochar amendment rate ($r^2 = 0.93$).

Table 2.5. Experiment 2: CO_2 and N_2O cumulative production in the first 60 hours after wetting for soil at uniform water holding capacity (WHC). Data indicate mean value (standard error). Letters indicate grouping by significance following one-way ANOVA and subsequent Tukey's Honestly Significant Difference analysis of 0 to 10% treatments. n = 4.

Treatment	Cumulative CO_2 production (CO_2-C mg g^{-1})	Cumulative N_2O production (N_2O-N μg g^{-1})
Un-amended	12.8 (0.7) a	0.41 (0.01) a
1% biochar	21.0 (0.5) b	0.33 (0.01) b
2% biochar	21.2 (0.4) b	0.33 (0.01) b
5% biochar	17.1 (0.5) c	0.11 (0.01) c
10% biochar	13.3 (0.2) a	0.01 (0.01) d

Net N_2O production rate decreased with time after wetting (Fig. 2.5). Between 60 and 168 hours after wetting, headspace concentration of N_2O decreased (Fig. 2.5). Headspace concentration of N_2O for the un-amended treatment decreased by 52% between these times (Fig. 2.5), similar to the percentage decrease for all other treatments (Fig. 2.5).

Cumulative CO_2 production was poorly correlated with increasing biochar addition rate (r2 = 0.15, Fig. 2.5). Biochar addition significantly increased cumulative CO_2 production compared with control for the 1% and 2% amendments (12.8, 21.0 and 21.2 μg CO_2-C g-1 d. wt. soil respectively, $p < 0.001$, Fig. 2.5, Table 2.5), but significantly decreased relative to this maximum value with greater biochar amendment (Table 2.5). The 5% biochar amendment produced less CO_2 than the 2% biochar treatment, and 10% biochar additions were not significantly different from

the un-amended treatment (17.1 and 13.3 μg CO_2-C g-1 dry soil, $p < 0.05$ and > 0.05 respectively, Table 2.5, Fig. 2.5).

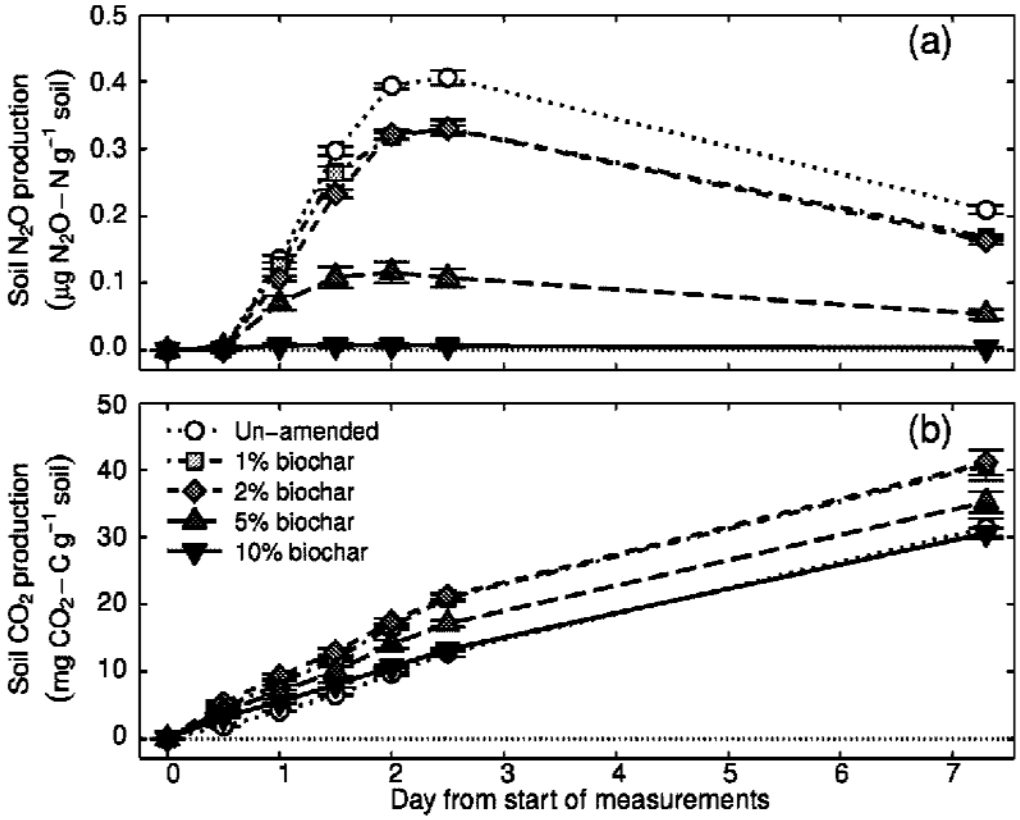

Fig. 2.5. Experiment 2: Effect of biochar amendment rate on (a) nitrous oxide (N_2O) and (b) carbon dioxide (CO_2) cumulative production for soil incubations at uniform water holding capacity (WHC). All treatments were wetted to 87% of WHC (at t_0 on graph). A horizontal dotted line indicates 0. Data points represent mean ± standard error (n = 4).

Biochar alone had a significantly higher WHC (146 ± 4%, n = 3) than all the biochar amended soils ($p < 0.001$, stats not shown). Biochar amendment significantly increased total soil WHC. The WHC of the 5 and 10% biochar addition rates (68 and 73% respectively, Fig. 2.6) were significantly higher than the un-amended control (61%, $p < 0.05$ and 0.01 respectively, Fig. 2.6). The results were not additive, with the predicted WHC of 5 and 10% biochar addition (65 and 69% respectively, based on the WHC of biochar alone) being lower than their actual measured value (Fig. 2.6).

The BD of 10% biochar addition post-wetting was significantly lower than the un-amended control ($p < 0.05$, Fig. 2.6).

Total soil C content increased with biochar amendment ($p < 0.001$, Fig. 2.6). For example, between un-amended to 10% biochar addition there was an increase from 14.4 to 90.3 g kg^{-1} (Fig. 2.6). Total soil N content did not significantly change between treatments, despite an apparent decrease following between field moist and wetted controls and an increasing trend with biochar content (Fig. 2.6). Wetting significantly affected extractable soil NH_4^+ and NO_3^- concentrations, while biochar amendment did not (Table 2.4). Extractable NH_4^+-N decreased significantly from 6.4 to 1.4 mg kg^{-1} at the end of the experiment compared to un-wetted, un-amended soil (or 'field moist control', $p < 0.001$, Fig. 2.6). In contrast, extractable NO_3^- significantly increased from 1.5 with the field moist control to 1.9-2.0 mg kg^{-1} with all wetted treatments ($p < 0.001$, Fig. 2.6). Biochar amendment between 0 to 10% did not significantly affect soil extractable NH_4^+ or NO_3^- concentrations at the end of the experiment ($p > 0.05$, Fig. 2.6). Biochar significantly increased control soil pH from 7.56 to 8.02 - 8.22 depending on biochar addition rate ($p < 0.05$ for 1% biochar addition, $p < 0.001$ for all higher additions, Fig. 2.6).

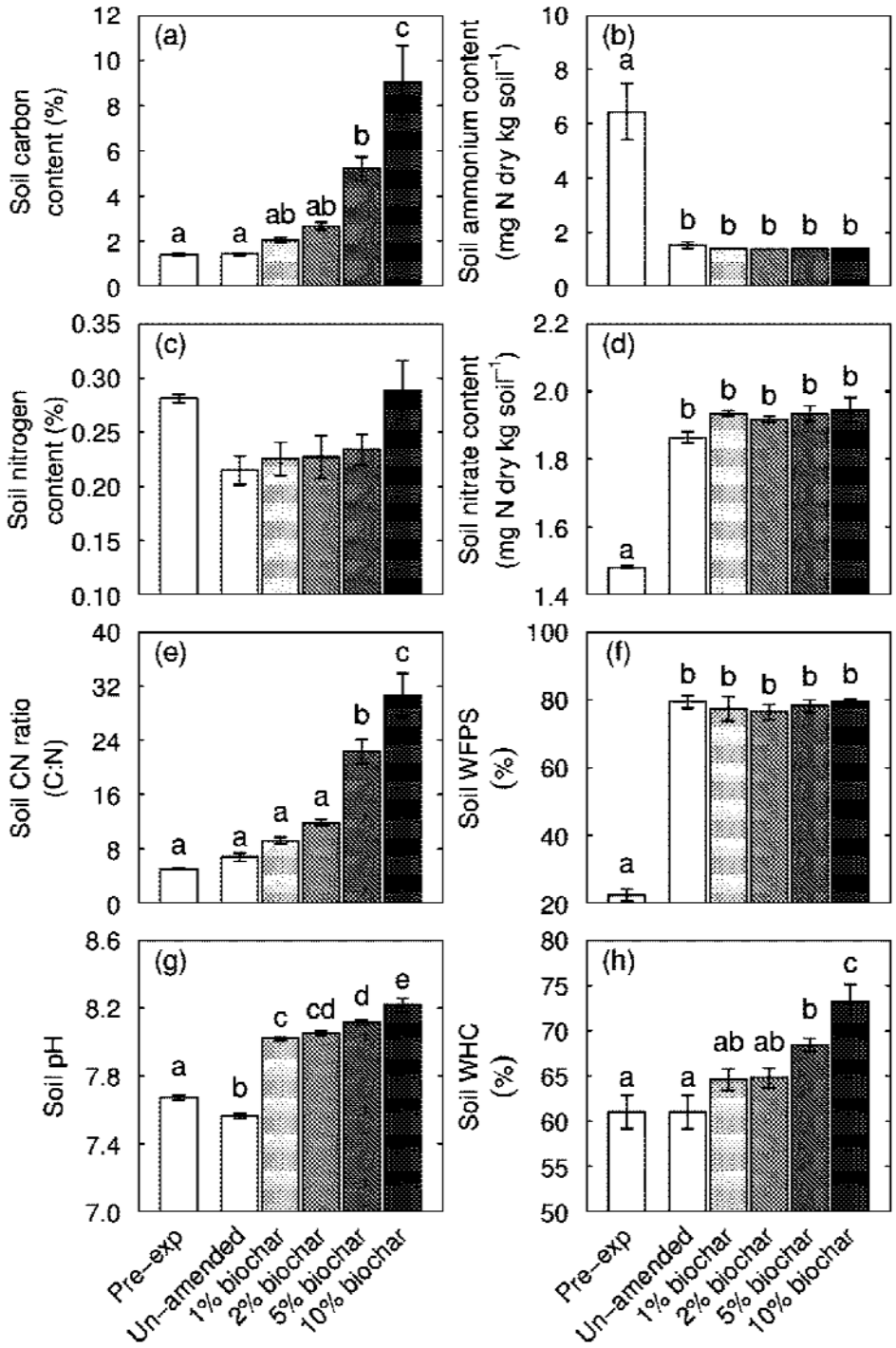

Fig. 2.6. Experiment 2: chemical and physical properties of biochar amended soil for soil at uniform water holding capacity. Treatments were analysed before (0% field-moist treatment) or after (all other treatments) the wetting event incubation. Data indicate mean value (standard error). Letters indicate grouping of treatments by significant difference ($p < 0.05$) following a Tukey's Honestly Significant Difference test on a one-way ANOVA with treatment as the fixed variable.

2.5 Discussion

Wetting significantly increased N_2O emissions from the control soil cores at 10 and 16°C and this 'spike' in soil N_2O emissions observed after wetting was significantly suppressed in biochar amended cores. These results support the first hypothesis that biochar amendment suppresses N_2O emissions following wetting (Aims, Section 2.2.3). This also demonstrates that biochar may suppress N_2O emissions over a range of field-relevant temperatures (10 – 16°C).

In Experiment 2, cumulative N_2O production (0 – 60 hr) reduced with increasing biochar content despite uniform WHC across all biochar amendments (Fig. 2.5), again supporting our first hypothesis. These results did not support the second hypothesis that lower N_2O emissions following biochar amendment and wetting are due to the increased soil aeration of biochar amended soil compared to soil alone, so we rejected hypothesis 2. At 10% biochar addition, N_2O emissions were suppressed by 98%, despite soil aeration being the same (with all treatments the same WHC and WFPS). Therefore the effect of increased soil aeration with biochar appears to be minimal. The observed decline in accumulated headspace concentration of N_2O production between 60 and 168 hours was likely due to N_2O reduction to N_2 by soil denitrifiers in response to the high water content in the soil following wetting (Chapuis-Lardy et al., 2007).

Our results contradict findings from another study, where N_2O emissions from biochar treatments after wetting to very high water contents (83% WFPS) did not differ significantly from controls (Yanai et al., 2007). The authors hypothesised that this was due to the increased pH caused by biochar addition, which increased the activity of denitrifying organisms (Cavigelli & Robertson, 2000). From another study, it has been hypothesised that increased pH following biochar addition to the soil could enhance the activity of N_2O to N_2 reducing enzymes (Taghizadeh-Toosi et al., 2011a). However, results from our experiments do not provide evidence that pH has a strong role in biochar suppression of soil N_2O emissions.

In both Experiment 1 (at 16°C), and Experiment 2 (across all biochar treatments), biochar amended soils had higher soil pH than non-amended soils. The optimum pH for denitrifier activity is generally around the natural pH of the soil, but is generally highest between 6.6 and 8.3 (Šimek et al., 2002). The pH of all biochar amended soils following biochar addition in Experiment 1 (~ 7) and in Experiment 2 (8.02 – 8.22) was less than 0.6 points from the natural soil pH for any biochar treatment. In Experiment 2, cumulative N_2O production (0 - 60 hr) decreased 92% from 1 to 10% biochar amendment, soil pH only increased from 8 to 8.2. We therefore consider it unlikely that pH explains the suppression of soil N_2O production observed. Further studies should confirm the influence of this mechanism, with more frequent pH sampling, perhaps using ^{15}N addition (Baggs, 2008), or bio-inhibitors such as acetylene in order to measure N_2 flux (Groffman et al., 2006).

Soil saturation lowers soil redox potential (Andersen & Petersen, 2009). The decomposition of added organic C to soil from biochar amendment could further decrease soil redox potential by increasing the availability of electrons to soil microorganisms for reduction processes (Paul & Beauchamp, 1989; Joseph et al., 2010). However, microbial activity (if we use CO_2 emissions from both experiments as a proxy), did not increase consistently with biochar amendment, suggesting that biochar did not provide significant amounts of mineralisable organic C during our measurements following biochar addition (three weeks after addition for Experiment 1, 6 days for Experiment 2). Based on this evidence we do not believe this to be the primary mechanism behind N_2O suppression with biochar, at least during our observations. Despite our findings, further and more detailed research is required to investigate the influence of redox potential on N_2O emissions from soil following biochar addition.

The availability and form of N in the soil can strongly affect N_2O production. In Experiment 1, extractable NO_3^- contents at the end of the experiment were lower in biochar amended soils. This finding is similar to studies investigating low N

content, un-pyrolysed green waste (Van Zwieten et al., 2010b), and low N-content, fast-pyrolysis biochar (Bruun et al., 2011b). This could be due to increased immobilisation of NO_3^- within microbial biomass as a result of the increased CN ratio of the soil (Burgos et al., 2006; Andersen and Petersen, 2009). Alternatively, NO_3^- may have been directly sorbed onto the biochar surface by physical means (Joseph et al., 2010; Prendergast-Miller et al., 2011). Our data does not allow us to discriminate between these two processes. In either case, with a lower amount of available NO_3^- in wetted, biochar amended soil, denitrifier activity would be reduced, resulting in decreased N_2O emissions from the soil following wetting.

We hypothesise that lower available NO_3^- in biochar amended soils could also explain the N_2O suppression with increasing biochar amendment in Experiment 2. However, biochar amendment did not change extractable NH_4^+ or NO_3^- concentrations after seven days compared to the wetted control. We propose that there are two processes working simultaneously. Even though net NO_3^- loss between wetting and the end of the experiment was the same, the loss of extractable NO_3^- through denitrification into N_2O or N_2 was much greater in the un-amended treatment compared to the 10% biochar treatment. We hypothesise that biological or physical immobilisation of NO_3^- was greater in the 10% biochar treatment compared to the un-amended treatment, removing significant amounts of NO_3^- from the extractable pool that could not be utilised by soil nitrifiers or denitrifiers that would produce N_2O. More frequent sampling of inorganic-N would be needed for both experiments in order to effectively account for the effect of biochar on N cycling processes.

There are a number of other significant findings from both of our experiments. For Experiment 1, field moist N_2O emissions were generally very low, close to or below the minimum detection limit, which is probably due to the high soil aeration of the field-moist soil throughout the experiment, resulting in low activity of denitrifying enzymes (Bateman & Baggs, 2005). For the wetted cores, WFPS was only increased to 45% following wetting, a value lower than what is normally needed to stimulate

denitrifier activity. However, this measure is of the whole soil core, while water was only applied to the top surface of the soil. We hypothesise that water content was higher than the reported value near the soil surface following wetting, high enough to activate the activity of denitrifying enzymes.

In Experiment 1, CO_2 emissions were not significantly different with biochar amendment or wetting at any temperature. For Experiment 2, cumulative CO_2 production was significantly higher than the control with 1 and 2% biochar amendment, but not with 5 and 10%. We cannot fully explain this inconsistent trend in soil CO_2 emissions with biochar amendment, however, a range of responses for CO_2 emissions have been reported in other studies. Biochar has been observed to increase soil CO_2 emissions, with the effect attributed to mineralisation of the labile biochar C fraction by biotic or abiotic means (Kolb et al., 2009; Zimmerman et al., 2011). Non-significant differences in CO_2 emissions between control and biochar amended soils have been reported elsewhere in the literature (Kuzyakov et al., 2009; Spokas and Reicosky, 2009; Singh, et al., 2010; van Zwieten et al., 2010), with authors attributing these results to a lack of micro-nutrient input to the soil by biochar (Spokas & Reicosky, 2009) or sorption of soil nutrients and organic C onto the biochar (Kuzyakov et al., 2009). Another explanation may be that by storing the mixed soil and biochar for several days/weeks before commencing GHG sampling as we have done in our experiments, the initial burst of CO_2 emissions that may occur with biochar addition is missed (Zimmerman et al., 2011).

Biochar significantly increased the WHC of soil at 5 and 10% amendment. These findings confirm those of other studies that have found that biochar can affect soil physical properties (Chan et al., 2007; Asai et al., 2009). However, these results were found to be slightly higher than predicted WHC based on the WHC of biochar alone. We hypothesise that the interaction between biochar and soil may increase WHC compared to biochar and soil separately, but we have no adequate explanation for the mechanism behind this effect.

Our results are applicable to low inorganic-N content, sandy loam soils amended with slow-pyrolysis, hardwood biochar. Further research is needed to investigate the effect of a range of biochars on soil aeration, N_2O production and N cycling in other soils. Also, studies are needed to intensively measure extractable NH_4^+ and NO_3^-, total C, redox potential and pH with and without biochar amendment at several time points in the hours after wetting, perhaps combined with ^{15}N stable isotope techniques (Rütting & Müller, 2007; Baggs, 2008) and analyses of microbial biomass or organic N (Brookes et al., 1985; Recous et al., 1998).

2.6 Conclusion

Our experiments demonstrated that nitrous oxide (N_2O) emissions from a sandy loam soil were consistently suppressed by hardwood biochar amendments of 2% and above (wt: wt) within 48 hours of wetting, although N_2O emissions were generally low. Carbon dioxide (CO_2) emissions were slightly increased or unaffected by biochar addition.

The enhancement of soil aeration resulting from biochar addition, as measured by soil WHC, did not explain suppressed N_2O emissions following wetting of biochar amended soil compared to controls. We hypothesise that physical or biological immobilisation of NO_3^- may explain the suppression of N_2O emissions with biochar amendment. However, our data are not conclusive, and further research is needed to investigate other potential mechanisms.

These results support the hypothesis that biochar addition to the soil decreases soil N_2O emissions, and has a small or insignificant effect on CO_2 emissions. Thus this paper adds to the evidence that biochar amendment to soil may serve as a potential tool for climate change mitigation.

2.7 Acknowledgements

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Introduction to Chapter 3 - Can biochar reduce soil greenhouse gas (GHG) emissions from a *Miscanthus* bioenergy crop?

Chapter 2 demonstrated that biochar amendment to a *Miscanthus* crop soil suppressed soil N_2O emissions under two soil temperatures (11 and 17°C) and within 48 hours of wetting in the laboratory. We concluded that increased soil aeration could not fully explain this effect.

In order to examine whether these findings could be replicated in field conditions and over a longer time period, we designed a 2-year field experiment to test the hypothesis that biochar amendment to soil could suppress soil GHG in a bioenergy field over the medium term. Additionally, we collected soil cores from the field experiment 10 months after biochar addition to the field to analyse soil GHG emissions more frequently under controlled conditions.

3 Can biochar reduce soil greenhouse gas (GHG) emissions from a *Miscanthus* bioenergy crop?

Running title: Biochar and *Miscanthus* soil GHG emissions

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I conducted the experimental work contained within this chapter. I also performed the analysis and wrote up the paper. Dr Whitaker, Dr McNamara and Dr Reay reviewed and suggested corrections for the manuscript drafts.

3.1 Abstract

Energy production from bioenergy crops may significantly reduce greenhouse gas (GHG) emissions through substitution of fossil fuels. Biochar amendment to soil may further decrease the net climate forcing of bioenergy crop production, however this has not yet been assessed under field conditions. Significant suppression of soil nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions following biochar amendment has been demonstrated in short-term laboratory incubations by a number of authors, yet evidence from long-term field trials has been contradictory. This study investigated whether biochar amendment could suppress soil GHG emissions under field and controlled conditions in a *Miscanthus X Giganteus* crop and whether suppression would be sustained during the first two years following amendment.

In the field, biochar amendment suppressed soil CO_2 emissions by 33% and annual net soil CO_2 equivalent ($_{eq.}$) emissions (CO_2, N_2O and methane, CH_4) by 37% over two years. In the laboratory, under controlled temperature and equalised gravimetric water content, biochar amendment suppressed soil CO_2 emissions by 53% and net soil $CO_{2eq.}$ emissions by 55%. Soil N_2O emissions were not significantly suppressed with biochar amendment, although they were generally low. Soil CH_4 fluxes were below minimum detectable limits in both experiments.

These findings demonstrate that biochar amendment has the potential to suppress net soil $CO_{2eq.}$ emissions in bioenergy crop systems for up to two years after addition, primarily through reduced CO_2 emissions. Suppression of soil CO_2 emissions may be due to a combined effect of reduced enzymatic activity, the increased C-use efficiency from the co-location of soil microbes, soil organic matter and nutrients and the precipitation of CO_2 onto the biochar surface. We conclude that hardwood biochar has the potential to improve the GHG balance of bioenergy crops through reductions in net soil $CO_{2eq.}$ emissions.

3.2 Introduction

The EU has a target for 20% of all energy to come from renewable sources by 2020 (The European Commission, 2009). Bioenergy combustion currently makes up 2% of primary energy generation in the UK and is expected to increase to 8 - 11% of the UK's primary energy to help meet this 2020 target (Committee on Climate Change, 2011; The Department of Energy and Climate Change, 2012).(Rowe et al., 2009; Committee on Climate Change, 2011) The sustainability and greenhouse gas (GHG) balance of first-generation bioenergy crops has received considerable attention and criticism in the literature (Crutzen et al., 2007; Searchinger et al., 2008; Smeets et al., 2009; Whitaker et al., 2010). Second-generation bioenergy crop production is typically responsible for lower GHG emissions over its life cycle than first-generation bioenergy crops due to less intensive management practices (Hillier et al., 2009; Rowe et al., 2011). Nevertheless, methods to improve the sustainability of all bioenergy crop-types are being considered (Gopalakrishnan et al., 2009; Thornley et al., 2009).

One of the most promising biomass energy crops in the UK in terms of environmental sustainability is *Miscanthus* (*Miscanthus X Giganteus*) (Rowe et al., 2009; Whitaker et al., 2010). This crop is a perennial rhizomatous C_4 grass that is planted on approximately 13,500 ha of UK cropland (Don et al., 2012). *Miscanthus* requires minimal soil preparation and common management practices involve adding a relatively small amount of nitrogen (N), if any, during the first few years to benefit rhizome development. It is generally known that high yields are maintained after this period (Lewandowski et al., 2000; Rowe et al., 2009), although recent work suggests that additional N inputs in the fourth year could improve yields by 40% (Wang et al., 2012a).

Biochar is a carbon (C)-rich substance produced from biomass and applied to soils. It is being promoted as a climate change mitigation tool as it has the potential to increase soil C sequestration and reduce soil GHG emissions when applied as a soil amendment (Woolf et al., 2010). For this reason, combining bioenergy cultivation

with biochar application to improve the GHG balance of bioenergy crops is an attractive proposition. Biochar is created by heating biomass in a low-oxygen environment (a process called pyrolysis, typically heated to between 350 and 600°C). One option for biochar production is to produce it concurrently with energy (Laird et al., 2009).

Several life cycle assessments (LCAs) demonstrated that producing energy and biochar concurrently from biomass and subsequently applying the biochar to arable crop soil resulted in greater C abatement than producing energy alone from biomass or fossil fuel energy production (Gaunt & Lehmann, 2008; Roberts et al., 2010; Hammond et al., 2011). Carbon abatement primarily consisted of increased soil stable C content (40 – 66%) and offsetting fossil fuel energy (14 – 48%). The remainder was attributed to indirect effects of biochar on the soil, such as increased fertiliser use efficiency, reduced soil GHG emissions and increased soil organic carbon (SOC) stocks. According to one LCA study, a 30% increase in SOC following biochar amendment would reduce net GHG emissions from small-scale bioenergy/biochar production by up to 60% (Hammond et al., 2011). Suppressed soil N_2O emissions of 25 – 50% contribute only 1.2 – 4.0% of the total emission reduction following biochar amendment (Roberts et al., 2010; Hammond et al., 2011). However, this figure may be an underestimate; one study on first generation biofuels has suggested that the conversion factor of newly-fixed N to N_2O production may be 3–5% as opposed to the default conversion factor from agricultural lands of 1% used by the Intergovernmental Panel on Climate Change (Crutzen et al., 2007).

It is important to fully understand the mechanisms by which biochar amendment to soil may affect soil C and N cycling in order to estimate soil GHG fluxes from such systems. Carbon dioxide (CO_2) emissions from soil organic matter (SOM) result from the mineralisation of resident soil C and are strongly affected by soil temperature, the form and lability of soil C and soil moisture conditions (Rustad et al., 2000; Cook & Orchard, 2008). Nitrous oxide (N_2O) from soil is produced via

three primary pathways, nitrification, nitrifier denitrification and denitrification (Khalil et al., 2004; Wrage et al., 2005; Gillam et al., 2008). Nitrification is dominant under aerobic conditions, whereas under increasingly anaerobic conditions (e.g. at high water filled pore space, WFPS, > 70%), denitrification is the dominant pathway (Bateman & Baggs, 2005). Nitrous oxide production is also constrained by temperature, inorganic-N content, pH and the form and concentration of labile C (Hofstra & Bouwman, 2005).

We have found from previous work that soil CH_4 fluxes are negligible from this *Miscanthus* site (Case et al., 2012). Methane fluxes are mediated by processes known as CH_4 oxidation under aerobic and methanogenesis under anaerobic conditions, and are primarily affected by temperature, substrate availability and the form and content of organic matter (Castro et al., 1995; Le Mer & Roger, 2001).

There is evidence to suggest that a co-benefit of biochar amendment is a reduction in soil CO_2 emissions (Lehmann et al., 2011), however there are few long-term studies available to support this. Those that exist are contradictory, with increased, decreased and variable effects observed (Major et al., 2009; Kuzyakov et al., 2009; Zimmerman et al., 2011). It is known that fresh biochar addition may add a large amount of labile C to the soil, therefore increasing soil CO_2 emissions. However, this is likely to be a short-term effect (Zimmerman et al., 2011). In the longer term, biochar is hypothesised to increase recalcitrant soil C and may even increase soil microbial biomass by agglomeration of SOM and nutrients onto the biochar surface (Lehmann et al., 2011). It is not yet clear whether this leads to decreased or increased native soil C mineralisation in the long term (Lehmann et al., 2011; Spokas, 2012). Biochar amendment may also reduce the activity of multiple C-mineralising enzymes, therefore reducing soil CO_2 emissions (Jin, 2010), although this has not yet been confirmed in a published study (Bailey et al., 2011).

Biochar is also hypothesised to have suppressive effects on soil N_2O emissions. This has been observed in short-term laboratory studies (Spokas & Reicosky, 2009; Singh et al., 2010a; Case et al., 2012), but has yet to be demonstrated in a long-term field

study (e.g. Jones et al., 2012). Several studies have demonstrated that biochar amendment can modify soil physical properties, particularly by increasing the water holding capacity (WHC) and decreasing the bulk density (BD) of soil, leading to a reduced WFPS of soil with biochar amendment and therefore lower soil N_2O emissions (Van Zwieten et al., 2010b; Karhu et al., 2011; Case et al., 2012). Also, in low inorganic-N soils, fresh biochar may immobilise significant amounts of inorganic-N, limiting the substrate available to soil nitrifiers and denitrifiers for N_2O production (Clough & Condron, 2010; Taghizadeh-Toosi et al., 2011a). Biochar amendment may also affect enzyme activity relevant to N_2O production (Anderson et al., 2011).

The authors have shown previously that biochar amendment significantly suppressed soil N_2O emissions from *Miscanthus* soils incubated under standardised conditions in short-term experiments (four months), but had no effect on soil CO_2 emissions (Case et al., 2012). The aims of this study were to investigate whether biochar amendment would significantly reduce soil GHG emissions from a *Miscanthus* crop under field conditions and over the medium term (up to two years from biochar amendment) and to determine the effect of biochar amendment on net soil CO_2 equivalent ($_{eq.}$) emissions from *Miscanthus* soils.

To address these aims, we monitored GHG emissions from biochar-amended and un-amended soils in the field for two years. Given that changes in temperature and moisture over time will affect biochar-amended soils differently from un-amended soil, due to higher WHC (Case et al., 2012) and differing thermal properties (Genesio et al., 2012; Meyer et al., 2012), we also investigated GHG fluxes from biochar-amended soils under standardised environmental conditions (10 – 14 months after amendment). This was done to control for environmental factors known to influence C and N cycling in soils (Reichstein et al., 2000; Dobbie & Smith, 2001; Cook & Orchard, 2008). We hypothesised that under field and standardised conditions, biochar amendment would suppress soil CO_2, N_2O and net soil $CO_{2eq.}$

emissions. We also hypothesised that soil CH_4 fluxes would be too low to detect any significant differences with biochar amendment.

3.3 Materials and methods

3.3.1 Biochar and field site description

The biochar used in this study was the same as that used in Case et al., (2012). Briefly, biochar was produced from thinnings of hardwood trees (oak, cherry and ash, Bodfari Charcoal, UK). The feedstock was heated in a ring kiln, first to 180°C to allow the release of volatile gases, and then to approximately 400°C for 24 hours. The biochar was subsequently 'chipped' to achieve a post-production size of up to 15 mm. The biochar had a total C content of 72.3 ± 1.5 % (n = 3), a total N content of 0.71 ± 0.01 % (n = 3), an extractable NH_4^+ and NO_3^- content below detectable limits (< 1 mg kg^{-1} NH_4^+-N and < 1.3 mg kg^{-1} NO_3^--N, n = 3), a pH of 9.25 ± 0.04 (n = 4), a gravimetric moisture content (GMC) of 3.1 ± 0.4 % and a cation exchange capacity of 145 $cmol^+$ kg^{-1} (n = 1, analysed by ICP-OES). Further biochar properties are available in the supplementary material of Case et al., (2012).

The field site used for this study was a *Miscanthus* plantation close to Lincoln, Lincolnshire, UK. Prior to *Miscanthus* planting in 2006, the field had followed a rotation of one year oilseed rape, three years wheat. The crop was planted at a density of 10,000 rhizomes ha^{-1} without N fertilisation during or subsequent to establishment (Drewer et al., 2012). The soil was a dense, compacted sandy loam with 53 % sand, 32 % silt and 15 % clay, a BD of 1.51 ± 0.02 g cm^{-3} (n = 10), chemical properties of which are shown in Fig. 3.1 (May 2010 control). The crop received no N fertiliser before or during the field experiment.

3.3.2 Effects of biochar on GHG fluxes in the field

Five random sampling blocks were established within the *Miscanthus* field in May 2010. In each of these blocks, three circular plots of 2 m diameter were created, at least 5 m apart, in between the *Miscanthus* shoots to prevent rhizome damage. In

each block, one plot was an un-mixed 'control' plot. Litter was removed from the remaining ten plots and the soil was mixed to 10 cm depth using hand tools. Biochar was applied to the second plot at a rate of 49 t ha^{-1} and mixed into the top 0 - 10 cm using hand tools (amended), while the remaining plot was also mixed to 10 cm but had no biochar applied (un-amended). Litter was then evenly re-applied. To monitor soil GHG emissions from the field plots, PVC chamber collars were permanently installed in the centre of each plot and pushed into the soil to a depth of 2 cm. The chambers had an average height of 16 cm from the soil surface, an internal diameter of 39 cm and a headspace volume of 19 l. At the start of gas measurements, the chambers were covered with a metal lid and connected to the chamber with metal bulldog clips. The lid contained a central septum for gas collection and a plastic tube connected to a partially-filled, open Tedlar bag (DuPont, USA) in order to equilibrate the chamber atmosphere with air pressure changes outside of the chamber (Nakano et al., 2004). Headspace atmospheric samples (10 ml, 0.05% of the total chamber headspace volume) were taken at 0, 10, 20 and 30 minutes following enclosure and injected into 3 ml gas-tight sample vials (Labco, UK) using the static chamber method (Livingston & Hutchinson, 1995). Gas samples were taken at 19, 112, 238, 362, 427, 503, 602 and 713 days from biochar addition. Gas samples were taken seasonally as distance to the field site prevented more frequent measurements.

Soil temperature was monitored in each plot with a Tiny Tag temperature logger with integral stab probe (Gemini Data Loggers, UK) and volumetric soil moisture content (VMC, 0 – 6 cm depth) was measured using a hand-held ML2x Theta Probe (Delta T Devices, UK). The probes were calibrated by creating a linear calibration of measured VMCs from un-amended and amended soil at a range of known GMCs (from 15 – 35%, Appendix). Volumetric moisture contents were converted into GMC using soil BD measurements from May 2012 (Fig. 3.1). Further environmental conditions at the field site (air temperature, rainfall, Fig. 3.2) were obtained through the British Atmospheric Data Centre, using data from a Met Office weather station

situated 2 km away from the field site (Natural Environment Research Council, 2012; The Met Office, 2013).

Soil samples were taken to 10 cm depth. Before biochar amendment to the field plots in May 2010, soil samples were taken from the five control plots. In March 2011, three soil samples were taken from each of the five un-amended and amended field plots and in May 2012 one soil sample was taken from each of the control, un-amended and amended plots. Soil samples were analysed for soil pH, extractable NH_4^+ and NO_3^-, total C and N, GMC and BD. All were frozen at - 20°C for up to four weeks until analysis apart from for GMC and BD, for which analysis was conducted immediately. Water-filled pore space was calculated from the GMC at each time point and the BD of the soil from May 2012 (two years after amendment), using a particle density of 2.65 g cm^{-3} (Ohlinger, 1995b).

3.3.3 Effect of biochar on GHG fluxes under controlled conditions 10 - 14 months after amendment

In order to assess the effects of biochar on soil GHG fluxes, soil cores were collected from the field plots in March 2011, ten months after biochar application. Two intact soil cores were taken from each of the five amended and un-amended plots following the same procedure described in Case et al., (2012). PVC pipes (W 102 mm, H 215 mm) were inserted into the soil as deep as possible using hand tools (150 – 180 mm) and excavated from the surrounding soil. The soil cores were stored at 4°C for 40 days following collection, then placed at 16°C (mean soil temperature of the field site June - September 2009) in the dark for three days before gas sampling to allow any initial flush of soil CO_2 emissions induced by warming to pass (Reichstein et al., 2000). Soil cores were maintained at field moist conditions (23 % GMC) for the duration of the experiment. The chosen soil GMC was based on the mean monthly soil VMC measured directly at the site over one year (Feb 2009 to Feb 2010). Surplus water was allowed to drain into a removable container on the base of the core, which was airtight when connected to the rest of the apparatus.

To analyse soil GHG fluxes, headspace gas samples were taken (10 ml, 1% of the chamber headspace volume of 0.9 l) and injected into 3 ml sample vials (Labco, USA) using the unvented static enclosure method (Livingston & Hutchinson, 1995). The headspace atmosphere was sampled at 0, 20, 40 and 60 minutes following enclosure. Details regarding headspace design are available in Case et al., (2012). Gas samples were taken from all soil cores at seven time points, at day 4, 17, 31, 46, 67, 116 and 120. After the final gas sampling, the soil cores were stored at 4°C and soil samples were collected within four days (10 cm depth). Soil samples were homogenised and analysed for soil pH, extractable NH_4^+, NO_3^-, total C and N. Soil samples were frozen at – 20°C for up to four weeks until analysis.

3.3.4 Soil chemical and physical analyses

Soil pH was determined using deionised water (soil/biochar:H_2O, 1:2.5 w:v), using a Kent-Taylor combination pH electrode (Asea Brown Boveri, Switzerland) (Emmett et al., 2008). Soil NH_4^+ and NO_3^- were extracted using 0.8 M (6%) potassium chloride (KCl), and analysed on a Seal AQ2 discrete analyser (Bran and Luebbe, UK) using discrete colorimetric procedures (Maynard & Kalra, 1993). Total C and N content of 0.1 g oven-dried soil (from a 5 g sample ground and sieved to < 2 mm) was analysed on a LECO Truspec total CN analyser (LECO, USA) with an oven temperature of 950°C (Sollins et al., 1999). Gravimetric moisture content and BD were conducted according to standard methods (Ohlinger, 1995b; Emmett et al., 2008) and soil WFPS derived from these values as described in Section 3.3.2.

3.3.5 Headspace gas analyses

Two different gas chromatograph (GC) systems were used to analyse headspace GHG concentrations. For the first year of the field experiment, CO_2 and CH_4 concentrations were analysed on a PerkinElmer Autosystem GC (PerkinElmer, USA) fitted with two flame ionization detectors (FID) operating at 130 (FID alone) and 300°C (FID with methaniser) respectively. Nitrous oxide concentrations were analysed on a PerkinElmer Autosystem XL GC using an electron capture detector

(ECD) operating at 360°C. Both GCs contained a stainless steel Porapak Q 50 - 80 mesh column (length 2 m, outer diameter 3.17 mm), maintained at 100°C and 60°C for the CO_2/CH_4 and N_2O GCs respectively. For the second year of the field experiment and the laboratory experiment, concentrations of N_2O, CO_2 and CH_4 were analysed on a PerkinElmer Autosystem XL GC. The GC was fitted with an FID with methaniser operating at 300°C and an ECD operating at 360°C. The same column was used for this GC as described above, maintained at 60°C.

Results were calibrated against certified gas standards (Air Products, UK). The minimum detection limits (MDLs) of the GC systems were calculated based on chamber deployment time, number of samples taken per hour and the analytical precision of the instrument (co-efficient of variation %) following (2010). The MDLs were 6.7 CO_2-C mg m^{-2} h^{-1}, 8.0 μg CH_4-C m^{-2} $h^{-1}$and 12.4 μg N_2O-N m^{-2} h^{-1} for the field experiment and 3.7 mg CO_2-C m^{-2} h^{-1}, 4.4 μg CH_4-C m^{-2} h^{-1} and 8.6 μg N_2O-N m^{-2} h^{-1} for the laboratory experiment. Headspace gas fluxes were calculated from the linear flux of CO_2, N_2O or CH_4 concentration in the chamber headspace following enclosure according to the approach of Holland et al., (1999). We used the linear accumulation of headspace CO_2 concentrations to eliminate vials from analysis that had their air-tightness compromised during sampling or subsequent storage. We found that CH_4 fluxes from the soil were below the MDL of the GC throughout both experiments, and N_2O fluxes were below the MDL except for the first gas sampling time point in the field (June 2010). Regardless of whether fluxes were below the MDL or not, we used them in subsequent analysis (Sjögersten & Wookey, 2002; McNamara et al., 2008).

Nitrous oxide and CH_4 fluxes were converted into net soil $CO_{2eq.}$ emissions using the global warming potential over a 100 year period of 298 (N_2O) and 25 (CH_4) given by Solomon et al., (2007a). Net soil $CO_{2eq.}$ emissions per year (kg CO_{2eq} ha^{-1} yr^{-1}) were derived by calculating the mean daily GHG flux of the un-amended and amended treatments over the two-year time period, and multiplying this value by 365 days. Laboratory experiment conditions were representative only of field conditions in

summer. Therefore, to compare net soil $CO_{2eq.}$ emissions from the field and laboratory experiment, we converted fluxes into kg CO_{2eq} ha^{-1} $summer^{-1}$, where 'summer' was defined as the length of the summer months (92 days, the number of days in June, July and August).

3.3.6 Statistical analyses

Statistical analyses were conducted using R version 2.15.2 (The R Project, 2013). Data exploration was conducted following the procedure in Zuur et al., (2010a). Linear mixed-effects models were run using NLME package version 3.1-105, with GHG fluxes, GMC or WFPS as the response variable and 'plot' or 'soil core' as the random factor for the field and laboratory experiments respectively. The models were refined taking into account independent variable heterogeneity and correlation, and validated following the guidance provided in Zuur et al., (2010b).

T-test comparisons were used for chemical and physical soil properties and the comparison of soil N_2O fluxes from un-amended and amended plots at the first time point in the field. Levene's test was initially used to determine whether there was a significant difference in response variable variance for the un-amended and amended soil. If a significant difference was found ($p < 0.05$), Welch's t-test was used for unequal variances; otherwise an unpaired, two-sample t-test was used.

3.4 Results

3.4.1 Effects of biochar on soil GHG fluxes in the field

Over the two year measurement period, soil CO_2 emissions were significantly lower with biochar amendment ($p < 0.05$, Table 3.1). Mean soil CO_2 emissions in the un-amended plots were 43.2 ± 5.5 compared with 28.8 ± 3.4 mg CO_2-C m^{-2} h^{-1} in the amended plots, a suppression of 33% (Fig. 3.2, n = 37). At times of lower soil temperature, soil CO_2 fluxes were low ($p < 0.001$, Table 3.1); in winter and spring of 2011 and 2012, both un-amended and amended plots emitted less than 20 mg CO_2-C m^{-2} h^{-1} (Fig. 3.2).

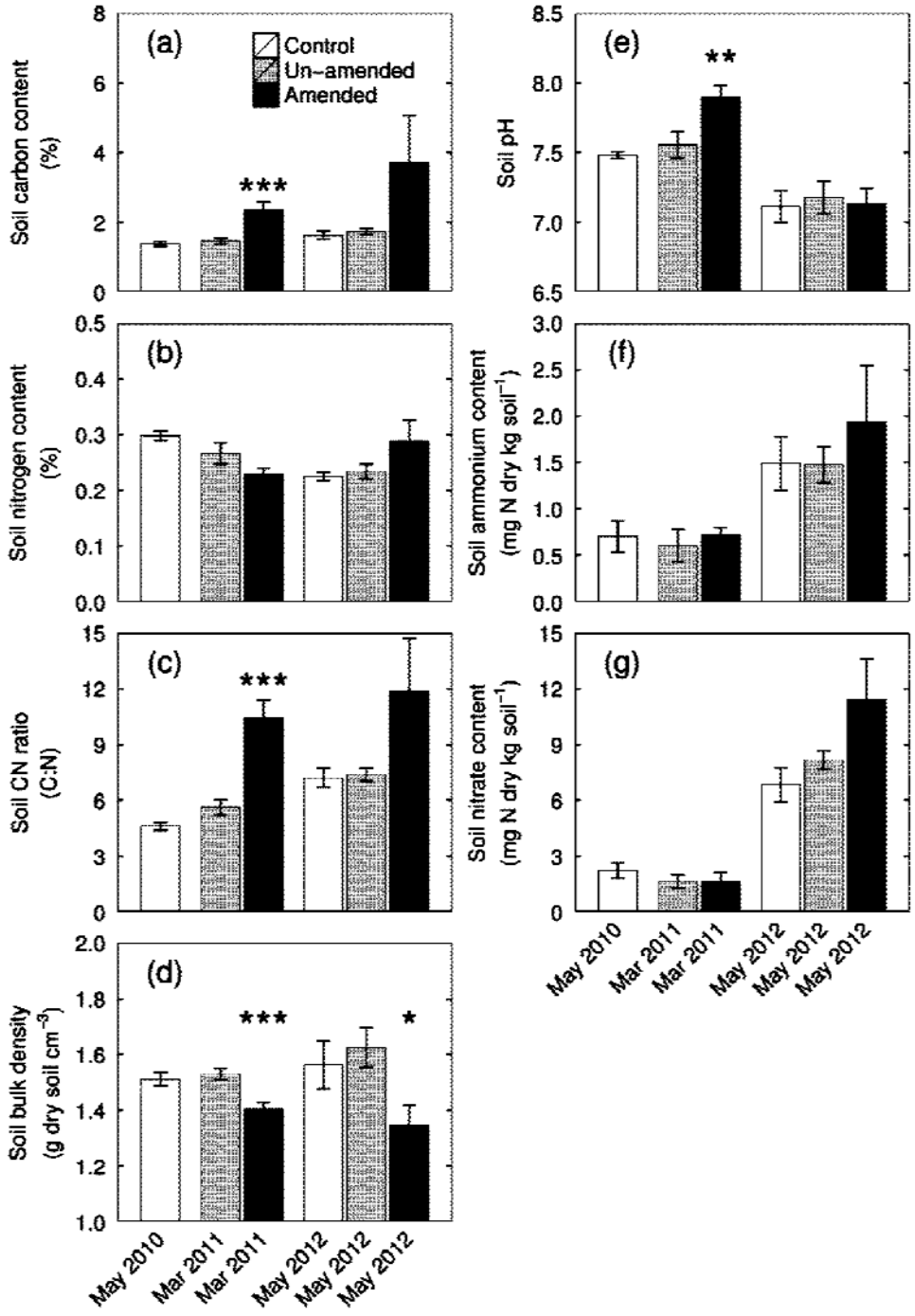

Fig. 3.1. The effect of biochar amendment on physico-chemical properties of soil (0 – 10 cm depth) taken from un-mixed control plots in May 2010 (n = 5), and from un-amended and amended plots 10 months (March 2011, n = 15, 3 replicates per plot) and 24 months after biochar addition in (May 2012, n = 5): soil (a) total C content (%); (b) total N content (%); (c) CN ratio; (d) pH; (e) NH_4^+ content; (f) NO_3^- content and (g) bulk density. Bar plots represent mean ± standard error (n = 5). Annotations above bars indicate significant difference between un-amended and amended soil cores at the same time point: ** = $p < 0.01$, *** = $p < 0.001$.

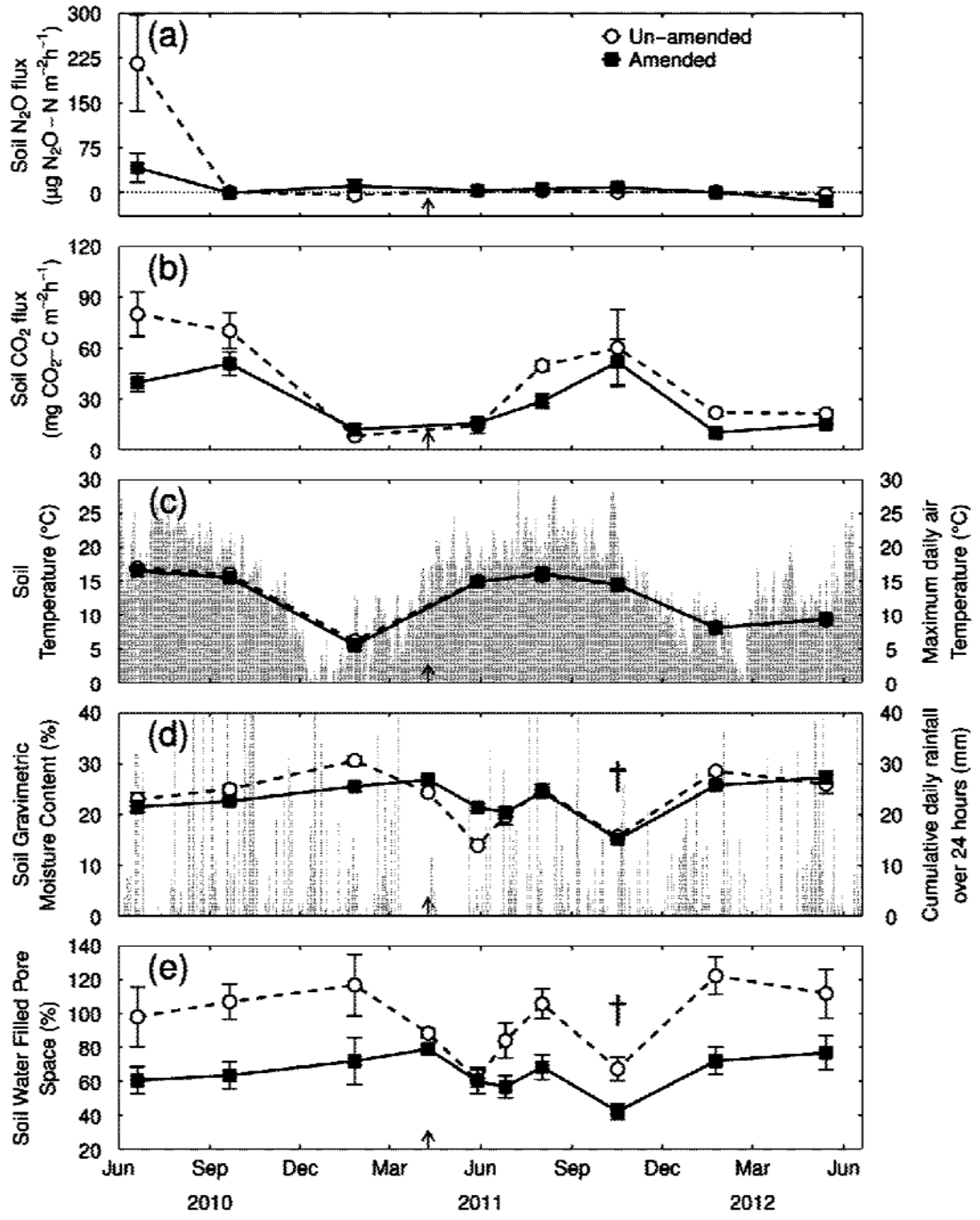

Fig. 3.2. The effect of biochar amendment on soil fluxes of (a) N_2O and (b) CO_2 from *Miscanthus* field plots (June 2010 - May 2012), and environmental conditions (c-e) over the same period: (c) soil temperature and daily maximum air temperature (°C); (d) soil gravimetric moisture content (%) and cumulative daily rainfall (mm day^{-1}); and (e) soil water-filled pore space (%). Arrow indicates time of soil core collection for the laboratory incubation (30th March 2011). The horizontal dotted line in graph (a) indicates 0. The † symbol indicates missing probe values due to the soil being too dry to analyse (replaced with assumed 18 % volumetric moisture content for both treatments). Data points represent mean ± standard error (n = 5). Biochar was added to plots May 20th 2010.

Table 3.1. Variables affecting carbon dioxide (CO_2) fluxes, soil gravimetric moisture content (GMC) and Water-filled pore space (WFPS) in *Miscanthus* field plots, either un-amended or amended with biochar, over two years of seasonal measurements. Data outputs presented are those from refined linear mixed-effects models using plot as the random factor and accounting for independent variable heterogeneity where necessary following the procedure in Zuur et al., (2010b). n = 5. Symbols indicate p-value significance of the term: - = not present in refined model, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Refer to Fig. 3.2 for the data underlying these statistical outputs.

	Independent variable							
Response variable	**Biochar**		**WFPS**		**Soil temperature**		**Biochar * Soil temperature**	
	t	**p**	**t**	**p**	**t**	**p**	**t**	**p**
Soil N_2O emissions	- 1.5	ns	- 1.0	ns	- 0.1	ns	0.4	ns
Soil CO_2 emissions	2.3	*	-	-	10.3	***	- 4.1	***
Soil CH_4 emissions	-	-	-	-	-	-	-	-
Total $CO_{2eq.}$ emissions	2.5	*	-	-	9.5	***	- 3.7	***
GMC	- 2.1	ns	-	-	- 5.9	***	1.8	ns
WFPS	- 3.2	*	-	-	- 3.4	**	1.7	.

Soil N_2O emissions were 216.4 ± 80.8 in un-amended soil compared with 41.8 ± 24.1 μg N_2O-N m^{-2} h^{-1} at the first time point in the field (June 2010, Fig. 3.2, n = 5). Although soil N_2O emissions were lower in biochar-amended soils, at the first time point, this result was not significant (two-sample t-test, t = 2.2, df = 8.0, $p > 0.05$). Nitrous oxide fluxes were very much lower thereafter, with a mean of 0.4 ± 1.9 and 1.8 ± 2.0 N_2O-N μg m^{-2} h^{-1} (n = 33, Fig. 3.2) for the un-amended and amended treatments respectively. Soil CH_4 fluxes were below MDL throughout the experiment, with an overall average of -1.2 ± 3.6 and 5.2 ± 4.4 CH_4-C μg m^{-2} h^{-1} respectively for the un-amended and amended treatments (n = 37).

Net soil $CO_{2eq.}$ emissions were reduced by 37% with biochar amendment (averaged over 2 years, Table 3.2). In un-amended soils, 8% of net soil $CO_{2eq.}$ emissions came from N_2O emissions while for the amended plots, 3% came from N_2O emissions (Table 3.2). High N_2O emissions contributed disproportionately to net soil $CO_{2eq.}$ emissions in June 2010 compared to the other months of the measurement period, contributing 26% of net soil $CO_{2eq.}$ emissions for un-amended soil compared with

11% for amended soil (Table 3.2). When this time point was removed from the dataset (June 2010), the contribution of N_2O fluxes to net soil $CO_{2eq.}$ emissions over two years reduced to 0.1 and 0.9% in un-amended and amended soil respectively (Table 3.2). In the summer of 2010 and 2011, biochar amendment to soil suppressed net soil $CO_{2eq.}$ emissions by 55% and 41% respectively (Table 3.2).

Table 3.2: The effect of biochar amendment on net soil $CO_{2eq.}$ emissions from field plots or soil cores placed under controlled environmental conditions. Mean $CO_{2eq.}$ emissions were calculated from the mean soil GHG emissions sampled during the period specified by the 'Sample dates included' column, and mean $CO_{2eq.}$ production was calculated by multiplying this value by the number of days specified by the column 'Time Period'. The time period 'Year' indicates 365 days, 'Year (-1st)' indicates 365 days with the first, high N_2O measurement sampling date (June 2010) taken out of the calculation. The time period 'Summer' indicates 92 days (the number of days in June, July and August) and the sample date 'Lab incubation' indicates that gas sampling data was used from the whole 120-day laboratory incubation). 'U' indicates 'un-amended', 'A' indicates 'amended' treatments. Data indicate mean (standard error). Sample n indicates the number of individual gas analyses included in the calculation.

Experiment	Time period	Sample dates included	Biochar	Mean $CO_{2eq.}$ emissions (net soil $CO_{2eq.}$ μg m^{-2} h^{-1})	Mean $CO_{2eq.}$ production (net soil $CO_{2eq.}$ t ha^{-1} time $period^{-1}$)	Sample n
Field	Year	2010-2012	U	172.2 (23.5)	15.0 (2.4)	37
	Year	2010-2012	A	108.9 (13.0)	9.5 (1.3)	37
Field	Year (-1st)	2010-2012	U	137.3 (20.0)	12.0 (1.8)	33
	Year (-1st)	2010-2012	A	100.8 (13.8)	8.8 (1.3)	32
Field	Summer	2010/2011	U	289.4 (43.1)	6.4 (1.2)	10
	Summer	2010/2011	A	138.3 (16.1)	3.1 (0.5)	9
Field	Summer	2010	U	395.1 (51.5)	8.7 (1.9)	5
	Summer	2010	A	175.9 (16.3)	3.9 (0.7)	4
Field	Summer	2011	U	183.6 (11.2)	4.1 (0.3)	5
	Summer	2011	A	108.2 (16.2)	2.4 (0.4)	5
Laboratory	Summer	Lab incubation	U	120.2 (9.7)	2.7 (0.2)	45
	Summer	Lab incubation	A	54.6 (6.0)	1.2 (0.1)	41

Monitoring of soil physical properties for two years revealed that biochar amendment did not significantly affect soil GMC (Fig. 3.2, Table 3.1). Soil GMC in both treatments was higher at times of lower soil temperature ($p < 0.001$, Table 3.1, Fig. 3.2). Biochar amendment significantly decreased soil BD. For example, 24 months after amendment (May 2012) BD was reduced from 1.62 ± 0.07 g cm^{-3} to 1.35

± 0.07 g cm^{-3} (n = 5, $p < 0.05$, Fig. 3.1,Table 3.3). Soil WFPS over the two years was reduced with biochar amendment ($p < 0.05$, Fig. 3.2, Table 3.1).

Table 3.3: The effect of biochar amendment on physico-chemical properties of soils sampled 10 months (March 2011, also day 0 of laboratory experiment) and 24 months (May 2012) after biochar addition to field plots (0 – 10 cm depth). Variability between the two groups was determined with Levene's test, the resulting outputs in the table are either from two-sample t-tests for equal variance (Levene's test $p > 0.05$), or Welch's t-test for unequal variance (Levene's test $p < 0.05$). n = 14 for un-amended, n = 15 for amended samples (3 replicates per plot). Symbols indicate the p-value significance of the term: ns = not significant, * = < 0.05, ** = < 0.01, *** = < 0.001. Refer to Fig. 3.1 for the data underlying these statistical outputs.

	10 months after amendment			24 months after amendment		
Response variable	**t**	**df**	**p**	**t**	**df**	**p**
Total C	- 4.2	18.7	***	- 1.5	8.0	ns
Total N	1.8	26.0	ns	- 1.4	8.0	ns
CN ratio	- 4.9	18.7	***	- 1.6	4.1	ns
NH_4^+	- 0.7	8.0	ns	- 0.7	8.0	ns
NO_3^-	0.1	27.0	ns	- 1.4	8.0	ns
pH	- 2.8	27.0	**	0.3	8.0	ns
Bulk density	- 4.0	18.0	***	2.3	8.0	*

Biochar amendment significantly affected soil chemical properties. Ten months after amendment (March 2011), biochar-amended soils had significantly higher total C content, CN ratio and pH relative to un-amended soils ($p < 0.001$, $p < 0.001$, $p < 0.01$, Fig. 3.1, Table 3.3, n = 15). Soil total N, NH_4^+ and NO_3^- contents were not significantly affected by biochar amendment at any time point ($p > 0.05$, Fig. 3.1, Table 3.3, n = 15).

3.4.2 Effects of biochar on soil GHG fluxes under controlled conditions

During a four-month laboratory incubation under controlled environmental conditions (10 months after biochar amendment to the field), biochar amendment had significant effects on soil GHG emissions. Averaging over the 120 days, biochar amendment significantly decreased soil CO_2 emissions by 53%, from 30.2 ± 2.1 to 14.1 ± 1.5 mg CO_2-C m^{-2} h^{-1} ($p < 0.001$, Table 3.4, Fig. 3.3, n = 41). Carbon dioxide emissions also decreased significantly with time in biochar-amended and un-amended soils ($p < 0.001$, Table 3.4).

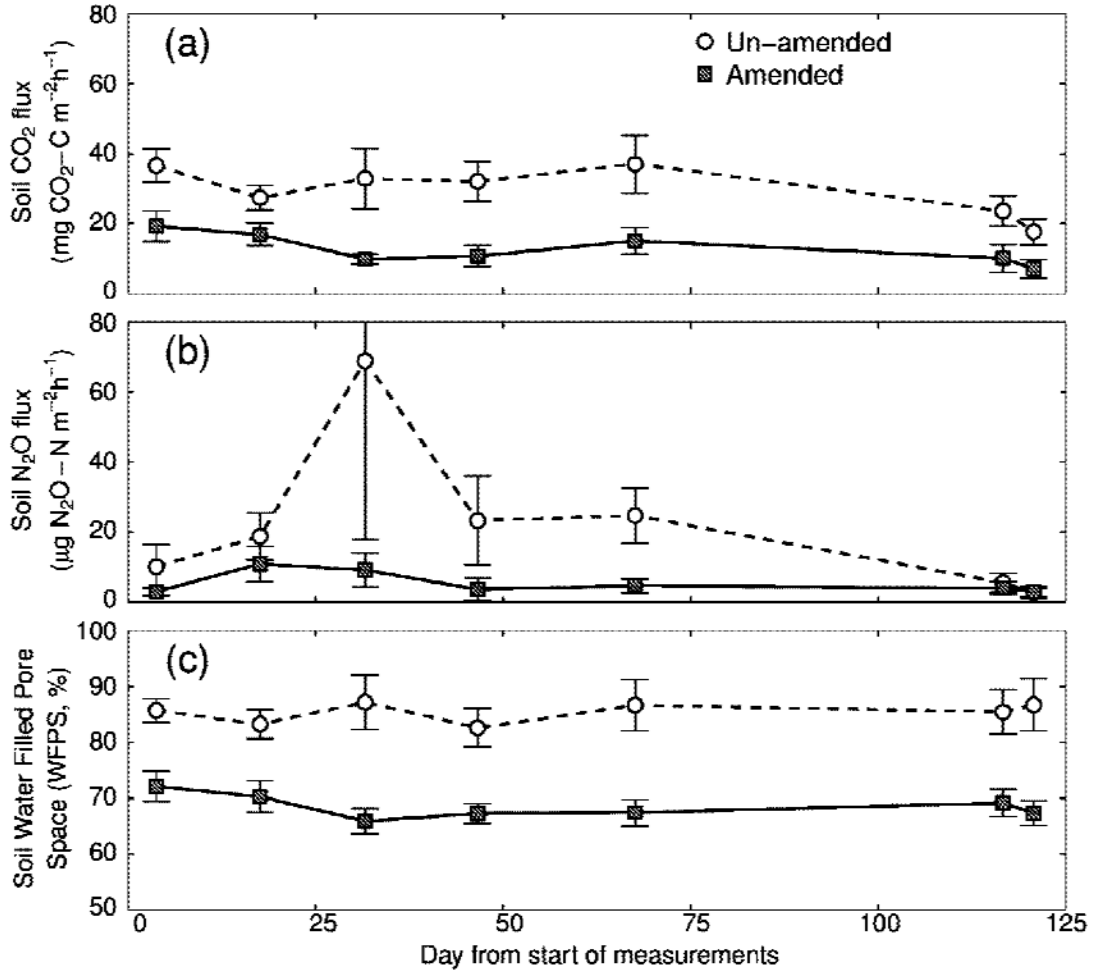

Fig. 3.3. The effect of biochar amendment on soil fluxes of (a) N_2O, (b) CO_2 and (c) the controlled WFPS of Miscanthus soil cores incubated in the laboratory. Soil cores were collected from field plots 10 months after biochar addition (30th March 2011). Data points represent mean ± standard error (n = 5). Statistical model outputs underlying these results are presented in Table 3.4.

Biochar amendment had no significant effect on soil N_2O fluxes ($p > 0.05$, Table 3.3). Nitrous oxide emissions from soil cores were generally low, on average 20.3 ± 6.4 compared to 5.8 ± 1.4 N_2O-N μg m^{-2} h^{-1} in the un-amended and amended soil cores respectively (Fig. 3.3, n = 41).

Table 3.5: The effect of biochar amendment and incubation time on greenhouse gas fluxes from soil cores incubated under controlled environmental conditions. 'Time' represents the number of days from the start of the laboratory experiment. Data outputs presented are those from refined linear mixed-effects models using plot as the random factor and accounting for independent variable heterogeneity where necessary following the procedure in Zuur et al., (2010b). Symbols indicate the p-value significance of the term: - = not present in refined model, ns = not significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Refer to Fig. 3.3 for the data underlying these statistical outputs.

	Independent variable					
	Biochar		Time		Biochar * Time	
Response variable	t	p	t	p	t	p
Soil N_2O emissions	0.9	ns	- 0.6	ns	-1.2	ns
Soil CO_2 emissions	2.8	*	- 3.6	***	-	-
Soil CH_4 emissions	-	-	-	-	-	-
Total $CO_{2eq.}$ emissions	2.7	*	- 3.2	**	-	-

Methane fluxes from soil cores were similarly low, on average 0.3 ± 1.1 compared to 1.8 ± 1.3 CH_4-C μg m^{-2} h^{-1} in the un-amended and amended soil cores respectively (n = 41). Biochar amendment reduced net soil $CO_{2eq.}$ emissions by 55% (Table 3.2). Nitrous oxide fluxes contributed 8% and 5% to net soil $CO_{2eq.}$ emissions for the un-amended and amended soils respectively over the whole experiment (Table 3.2). Biochar amendment had no significant effect on soil chemical properties (Fig. 3.4, Table 3.6, n = 5).

Table 3.6: The effect of biochar amendment on soil chemical properties (0 - 10 cm) at the end of a four-month laboratory incubation. Variability between the two groups was determined with Levene's test, the resulting outputs in the table are either from two-sample t- tests for equal variance (Levene's test $p > 0.05$), or Welch's t-test for unequal variance (Levene's test $p < 0.05$). Symbols indicate the p-value significance of the term: ns = not significant. Refer to Fig. 3.4 for the data underlying these statistical outputs.

Response variable	t	df	p
Total C	- 1.5	8.0	ns
Total N	- 1.5	8.0	ns
CN ratio	- 1.3	8.0	ns
NH_4^+	1.2	8.0	ns
NO_3^-	1.8	8.0	ns
pH	- 0.5	8.0	ns

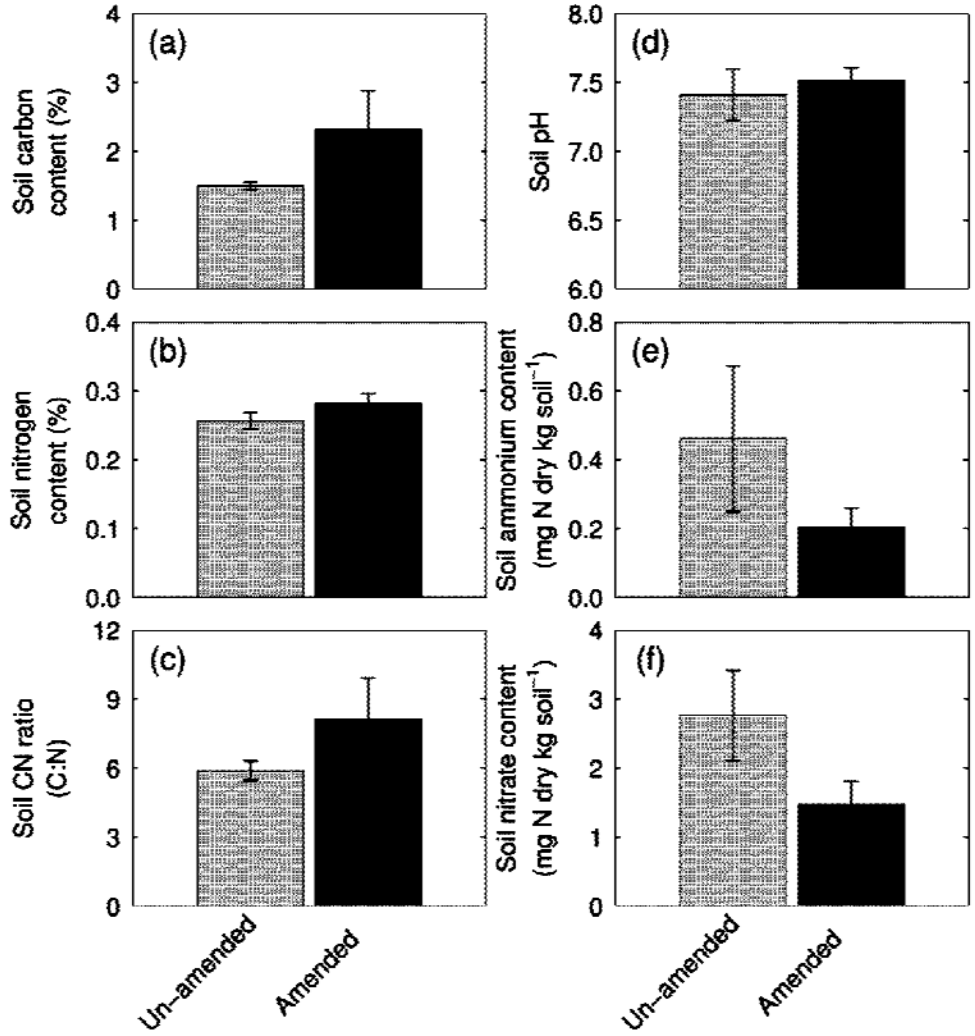

Fig. 3.5. The effect of biochar amendment on physico-chemical properties of soil cores (0 – 10 cm depth) taken from un-amended and amended cores at the end of the four-month laboratory experiment (n = 5): soil (a) total C content (%); (b) total N content (%); (c) CN ratio; (d) pH; (e) NH_4^+ content; and (f) NO_3^- content. Bars represent mean ± standard error (n = 5). Pre-laboratory experiment chemical and physical data are presented in Fig. 3.1 (March 2011).

3.5 Discussion

Suppression of soil GHG emissions from *Miscanthus* soils due to biochar amendment has been shown previously in short-term experiments by the authors, conducted under controlled-environment conditions (Case et al., 2012). The aim of

this present study was to investigate whether the suppressive effect of biochar amendment would be detected under field conditions over a longer time period of two years. In addition, to control for environmental factors known to influence C and N cycling in soils, we monitored GHG fluxes from field-amended soil under controlled "summer" conditions (constant temperature and GMC). We have demonstrated that biochar amendment may have the potential to reduce net soil $CO_{2eq.}$ emissions from a *Miscanthus* crop soil. However, we did not analyse soil CO_2 emissions in the first 19 days following biochar amendment, when soil CO2 emissions may have been greater than later on (Zimmerman et al., 2011). For the purpose of this discussion, we assume that extra soil CO_2 emissions from biochar-amended plots were negligible when considering overall CO_2 production over two years.

Over 2 years in the field, soil CO_2 emissions were suppressed by 33% on average and net soil $CO_{2eq.}$ emissions were 37% lower with biochar amendment. In the summer, biochar amendment reduced net soil $CO_{2eq.}$ emissions in the field by 55 and 41% in 2010 and 2011 respectively. In a four-month laboratory incubation under controlled "summer" conditions the effect was similar; net soil $CO_{2eq.}$ emissions were reduced by an average of 55%.

In the few medium-term studies published (up to three years from biochar amendment, almost all in non-bioenergy crops), biochar amendment has been shown to suppress or have negligible effects on soil CO_2 emissions, with a few notable exceptions (Wardle et al., 2008; Major et al., 2009; Spokas, 2012). There are several theories to explain why biochar amendment to soil may decrease soil CO_2 emissions. It has been hypothesised that biochar may increase microbial biomass in soil by the complexation of SOM with biochar particles and yet simultaneously induce 'negative priming' of native soil C mineralisation (Liang et al., 2010; Woolf & Lehmann 2012). The agglomeration of SOC on the biochar surface may result in a co-location of substrate, nutrients and micro-organisms and therefore promote greater C-use efficiency by the microbial community (Lehmann et al., 2011). Also,

biochar amendment may reduce the activity of carbohydrate-mineralising enzymes such as glucosidase and cellobiosidase and increase the activity of others such as alkaline phosphatase (Jin 2010). However, the effect of biochar on soil enzyme activity is reported to be highly variable due to reactions between at least one type of biochar (switchgrass) and the target substrate (Bailey et al., 2011).

Abiotic reactions may also contribute to the suppression of soil CO_2 emissions. Soil-derived CO_2 may precipitate onto the biochar surface as carbonates, aided by the high pH of the biochar and high content of alkaline metals (Joseph et al., 2010; Lehmann et al., 2011). The biochar used in this study had a high pH and relatively high content of alkaline metals compared to other biochars (Appendix, Section 7.1) and may therefore have caused significant precipitation onto the biochar surface. We conclude that a combination of the biotic and abiotic mechanisms mentioned above may explain the suppression of soil CO_2 emissions observed during this study.

It has been shown in forest ecosystems that low soil inorganic-N content may limit soil C mineralisation and resulting soil respiration (Norby et al., 2010). The *Miscanthus* soil in our study was initially very low in inorganic-N and this was unaffected by biochar amendment, indicating that biochar did not increase soil inorganic-N immobilisation. This is contrary to published data from other studies (van Zwieten et al., 2010; Dempster et al., 2012; Case et al., 2012). Based on this finding, we cannot explain lower soil CO_2 emissions by an effect of biochar amendment on N immobilisation.

Soil CO_2 emissions consist of both soil and root respiration (Sulzman et al., 2005). It is possible that biochar additions in the field may have affected the growth of *Miscanthus* above and below ground, feeding back into effects on root respiration. Whilst we did not directly measure the yield of the *Miscanthus* shoots surrounding the field plots, we did not observe any difference in shoot height from visual observation. Although the 2 m diameter field plots were placed entirely in between the *Miscanthus* where no shoots were growing, it is certain that the root system of

the *Miscanthus* was present underneath the plots. Soil CO_2 emissions from control (un-mixed) plots in the field were not significantly different from un-amended (mixed) plots over the course of the two-year field study (data not shown), indicating that mixing the soil did not significantly affect root activity or growth.

Biochar amendment could reduce root respiration either by reducing root activity or growth, or by killing existing roots. In the laboratory using soil collected 10 months after biochar amendment, we observed suppression of soil CO_2 emissions with biochar amendment despite the absence of live roots, indicating that differences in live root activity could not explain the suppression of soil CO_2 emissions. It is possible that biochar amendment may have significantly reduced root growth and/or increased root necromass underneath the plots in the 10 months following amendment. However, we are not aware of any specific mechanism to explain why biochar would reduce root growth or kill roots apart from increased nutrient limitation, which was not an issue in our study (Lehmann et al., 2011), or the presence of toxic substances on the biochar itself, which we have shown in a previous study not to be the case with this biochar (Case et al., 2012). The evidence therefore suggests that biochar amendment did not significantly affect root growth or activity in this study.

Soil CO_2 emissions in the field were unexpectedly low in May 2011 and May 2012 compared to other months of relatively high soil temperature (Fig. 3.2). Low soil CO_2 emissions of similar magnitude were observed on the same day at the field site (Bottoms, Robertson, pers. comm.). This may be explained by the fact that our May samplings occurred less than one month following the annual *Miscanthus* harvest, a time when there is likely to be minimal contribution from plant/root respiration as plant shoots have not yet emerged from the soil.

In both the field and the laboratory experiment, soil WFPS was lower with biochar amendment. However, as soil WFPS with biochar amendment was closer to the ideal range for soil CO_2 emissions (above 60%), we conclude that the physical effects of biochar amendment on the soil do not explain the suppression of soil CO_2

emissions (Linn & Doran, 1984). Biochar amendment increased soil pH 10 months after amendment. However, as pH levels were close to seven in both the un-amended and amended soils and were not significantly different 14 or 24 months after amendment, we cannot say conclusively that increased pH due to biochar amendment can explain lower soil CO_2 emissions.

Our observations of reduced soil CO_2 emissions following biochar addition are particularly relevant within the context of the overall GHG balance of bioenergy crops. If lower soil CO_2 emissions were to continue into the long-term, there would be a relative increase in SOC in amended compared to un-amended soil. The authors of one LCA study concluded that if there is no change in SOC stocks following biochar amendment then biochar production gives only a small C abatement benefit compared to gasification, whereas an increase in SOC makes pyrolysis look favourable in terms of C abatement (Hammond et al., 2011). According to their sensitivity analysis, if a finding of a suppression of soil CO_2 emissions of 30% were continued into the future within a small-scale biochar-production system, net GHG emissions from the system could be reduced by up to 60%. However, two years is too short a time to say with confidence whether this will be the case in the *Miscanthus* system that we have investigated as a part of this study.

In the field, soil N_2O emissions one month after amendment (June 2010) were high in the un-amended soils, and whilst N_2O emissions from biochar-amended plots were lower, the suppression was not significant. Soil N_2O fluxes were low in all treatments thereafter from September 2010 to May 2012 and in laboratory-incubated soils. Soil N_2O fluxes are highly variable temporally and a large proportion of emissions occur in 'bursts' following wetting or N-fertilisation events, which increase soil denitrifier activity (Dobbie & Smith, 2001; Sänger et al., 2010). High soil N_2O emissions at this field site in June 2010 have been corroborated by other researchers and may be explained by rainfall on the sampling day (Bottoms, 2012). With the exception of the June 2010 sampling, the timing of gas sampling did not

occur shortly following topsoil saturation from a rain event, therefore denitrifier activity was not stimulated.

We found that soil N_2O emissions were highly variable and were a relatively minor component of net soil $CO_{2eq.}$ emissions, which is in agreement with other published data from the same field site (Drewer et al., 2012). Considering only un-amended field plots, soil N_2O emissions contributed only 8% to net soil $CO_{2eq.}$ emissions on an annual basis, compared to 2% from Drewer et al., (2012).We found that N_2O production during the summer season were larger; in the field in 2010, 1.75 ± 0.65 g N_2O m^{-2} $summer^{-1}$ was emitted from un-amended soil and 0.02 ± 0.02 g N_2O m^{-2} $summer^{-1}$ in 2011, while Drewer et al., (2012) found that overall N_2O production to be 0.014 g N_2O m^{-2} $summer^{-1}$. In the laboratory, we found that N_2O fluxes were 0.16 g N_2O m^{-2} $summer^{-1}$ in un-amended soil. In this present study, we used a similar gas sampling technique to that of Drewer et al., (2012). We cannot explain why soil N_2O fluxes in our study were higher than that of Drewer et al., (2012). Nevertheless, we conclude that soil N_2O emissions are a relatively minor component of net soil $CO_{2eq.}$ emissions from *Miscanthus* soil. To support this further, LCAs of biochar/bioenergy production reported that suppression of soil N_2O emissions following biochar amendment was a relatively minor constituent of potential climate forcing, even in arable crop systems (Roberts et al., 2010; Hammond et al., 2011).

3.6 Conclusion

We return to the central question that underlies this study: can biochar reduce net soil $CO_{2eq.}$ emissions from a *Miscanthus* energy crop? Assuming that *Miscanthus* crops are managed with minimal inorganic-N addition and that hardwood-derived biochar produced by slow-pyrolysis is applied to the soil in significant quantities (~ 50 t ha^{-1}), we conclude that biochar amendment may have the potential to reduce net soil $CO_{2eq.}$ emissions from *Miscanthus* soils through the reduction of soil CO_2 emissions. This is particularly relevant when considering the overall GHG balance of bioenergy/biochar production, where reduced soil CO_2 emissions over the long term and the resulting increase in SOM content has been identified as one of the

most significant factor influencing the sustainability of combined bioenergy/biochar production (Hammond et al., 2011). In future studies, soil CO_2 emissions should be analysed regularly from the day that biochar is added to ensure that overall, soil CO_2 emissions are lower with biochar amendment.

Future research should consider that the effect of biochar amendment on climate abatement in *Miscanthus* crop systems may be different to that of biochar in arable systems, particularly when taking into account the low nutrient status of *Miscanthus* crop soil. A key research priority should be to investigate the effects of biochar amendment on the overall GHG balance of bioenergy/biochar production systems on a range of soil types in order to assess the global warming potential of the *Miscanthus* system with and without biochar amendment. We have observed suppression of soil CO_2 emissions with biochar amendment, however, use of eddy covariance techniques would enable the effects of biochar amendment on net ecosystem exchange to be estimated, providing additional information on the effects of biochar on C exchange within the crop/soil and atmosphere. Also, the mechanisms underlying the suppression of soil CO_2 emissions should be further investigated over the long term, such as the effect of biochar on the activity of CO_2-producing soil enzymes, the increased C-use efficiency from the co-location of soil microbes, soil organic matter and nutrients and the precipitation of soil-derived CO_2 onto the biochar surface as carbonates.

3.7 Acknowledgements

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National Centre for Atmospheric Science (NCAS), for providing access to Met Office temperature and rainfall data close to the field site.

Introduction to Chapter 4 - Biochar reduces soil N_2O emissions in incubated arable soil through enhanced reduction of N_2O to N_2

The previous chapters demonstrated that soil N_2O (Chapter 2) and CO_2 (Chapter 3) emissions were reduced by the addition of fresh biochar to *Miscanthus* crop soils. This may have important implications for the sustainability of *Miscanthus* plantations.

The results from Chapter 2 and 3 suggested that biochar addition had significant effects on soil N cycling process, by reducing extractable soil inorganic N concentrations and suppressing soil N_2O emissions. The next chapter expands the scope of this study to arable soils. Arable cropping systems emit significant amounts of N_2O following the addition of N-based fertiliser (Sutton et al., 2011). The next chapter describes our work to investigate the effect of biochar amendment on soil N_2O emissions from an arable soil and the mechanisms underlying this effect. It also investigates the effect of biochar on soil N cycling processes within an arable soil using a ^{15}N-labelling laboratory incubation.

4 Biochar reduces soil N_2O emissions in incubated arable soil through enhanced reduction of N_2O to N_2

Running title: Biochar reduces conversion of N_2O to N_2

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I conducted the experimental work contained within this chapter, including preparation of the ^{15}N-labelled samples. I also performed the analysis and wrote up the paper. Dr Whitaker, Dr McNamara and Dr Reay reviewed and suggested corrections for the manuscript drafts. Dr Andy Stott and Helen Grant ran the $^{15}N_2O$, inorganic ^{15}N and organic ^{15}N samples.

4.1 Abstract

Nitrous oxide (N_2O) emissions from soil are a significant source of greenhouse gas (GHG) emissions. Biochar amendment to soil can contribute to climate change mitigation by suppressing N_2O emissions, although the mechanisms are unclear.

We took soil cores from an arable field in eastern England, incubated them at 16°C and applied a series of wetting/drying cycles. In biochar-amended soils, N_2O emissions were suppressed by 84% under un-wetted conditions and by 88% in wetted soils. Extractable soil ammonium concentrations were lower in soils amended with biochar. We hypothesised that biochar-induced immobilisation of inorganic-N (BII) and increased soil aeration would explain the suppression of soil N_2O emissions.

We conducted an experiment to investigate soil nitrogen (N) transformations in amended soils by separately labelling ^{15}N ammonium and nitrate and saturating the soil so that the effects of BII and increased soil aeration were negligible. Using the FLUAZ model, we quantified nitrification, denitrification and immobilisation with and without biochar amendment over six days.

Nitrous oxide emissions were 95% lower in biochar-amended soil, yet nitrification and denitrification rates were un-affected. We hypothesised that increased soil pH and increased labile carbon mineralisation in saturated soils explained the lower N_2O: N_2 ratio from denitrification which we observed in biochar amended soil following addition of ammonium nitrate. The N_2O: (NO_2^- + nitrate) production ratio from nitrification was reduced by an unknown mechanism. Further studies are needed to investigate the effect of increased soil pH and labile C addition with biochar amendment on the ratio of N_2O + N_2 production during denitrification in soil.

4.2 Introduction

Nitrous oxide (N_2O) is a significant greenhouse gas (GHG) that has a global warming potential 298 times that of carbon dioxide (CO_2) over a 100-year time period and is responsible for approximately 6% of total anthropogenic radiative forcing (Solomon et al., 2007b; Davidson, 2009). Agricultural land contributes approximately 60% to anthropogenic N_2O emissions. New agricultural practices are needed to minimise emissions of N_2O in order to mitigate the effects of climate change (Smith et al., 2007; Reay et al., 2012).

Biochar amendment to soil has been proposed as a method to increase soil carbon (C) storage on a global scale and thus contribute to climate change mitigation (Woolf et al., 2010; Sohi, 2012). It consists of biomass material combusted in an oxygen-free environment, typically heated to between 350 and 600°C and subsequently applied as a soil amendment (Sohi et al., 2010). Short-term laboratory experiments and one short term field study (lasting no more than a few months) have shown that biochar amendment can also suppress soil N_2O emissions (Spokas & Reicosky, 2009; Clough & Condron, 2010; Singh et al., 2010a; Van Zwieten et al., 2010b; Rogovska et al., 2011; Taghizadeh-Toosi et al., 2011a; Case et al., 2012). However, it is not clear whether this suppression will be sustained in the longer term in the field (Scheer et al., 2011; Jones et al., 2012), or the laboratory (Spokas, 2012).

The microbial pathways by which N_2O is produced in soil and the environmental factors that control them are relatively well understood, however the interactions between them are not. "Soil N_2O emissions are produced by two primary processes, nitrification and denitrification (Azam et al., 2002). Denitrifier activity is increased with increasing soil temperature, extractable nitrate (NO_3^-) concentration, availability of labile C, water-filled pore space (WFPS) and pH (up to a pH of ~ 8.3) (Weier et al., 1993; Šimek et al., 2002; Ciarlo et al., 2007; Gillam et al., 2008; Saggar et al., 2012). Dentrification is the primary N_2O-producing process in soil above 70% WFPS, producing N_2O, nitric oxide (NO) and dinitrogen (N_2). Nitric oxide is not a

significant end product of denitrification in saturated soils as the gas does not diffuse fast enough to be converted into N_2O or N_2 during denitrification (Russow et al., 2009). The proportion of N_2O: N_2 produced via denitrification is decreased with increasing pH, labile soil C availability, low soil NO_3^- concentration and greater soil WFPS (Vallejo et al., 2006; Senbayram et al., 2012).

Nitrification is the oxidation of ammonium (NH_4^+) into nitrite (NO_2^-) and NO_3^- and is at a maximum in soils with high NH_4^+ concentration, at a moderate WFPS (~ 60%) and high soil temperature (Norton & Stark, 2011). The proportion of soil N_2O emissions from nitrification (the N_2O: (NO_2^- + NO_3^-) ratio) may be controlled by a number of mechanisms, which are poorly understood (Venterea & Rolston, 2000; Mørkved et al., 2007). A third, less significant N_2O production process is nitrifier denitrification (Wrage et al., 2005).

Addition of nitrogen (N)-based fertiliser to agricultural soil is common practice (Olfs et al., 2005). Increased use of manure or mineral N-based fertiliser can primarily explain the increase in atmospheric N_2O concentrations since 1960 (Davidson, 2009). A significant proportion of annual N_2O emissions occur within a short time following N-fertiliser addition under conditions of high soil temperature, high soil inorganic-N (NH_4^+ and NO_3^-) concentrations and when rainfall events occur close to the time of addition (Clayton et al., 1997; Dobbie et al., 1999; Hénault et al., 2012).

It is currently unclear how biochar amendment affects soil N cycling and suppresses soil N_2O emissions (Spokas et al., 2012b). The structure of biochar is known to affect soil physical properties increasing soil aeration and soil water holding capacity (WHC) and decreasing bulk density (BD) (Karhu et al., 2011; Basso et al., 2012). Amended soils at the same gravimetric water content (GMC) would, therefore, be more aerobic than un-amended soils and soil N_2O production would be decreased due to lower denitrifier activity (Van Zwieten et al., 2010b). However, in a previous study, we demonstrated that the effect of biochar on increased soil aeration did not

solely explain the suppression of soil N_2O emissions following wetting events (Case et al., 2012).

The availability of inorganic-N substrate for nitrification and denitrification may be reduced by biochar amendment, constraining process rates (Norton & Stark, 2011; Saggar et al., 2012). This may occur by one of two processes: abiotic-N adsorption to the biochar surface or indirect immobilisation of soil N into microbial biomass (both processes combined henceforth collectively referred to as biochar-induced immobilisation, BII) (Spokas & Reicosky, 2009; Singh et al., 2010a; Van Zwieten et al., 2010b). Both NH_4^+ and NO_3^- are known to adsorb abiotically to biochar surface, which is often covered in negatively-charged carboxylic groups, although the mechanisms behind this effect are unclear (Mizuta et al., 2004; Kastner et al., 2009; Taghizadeh-Toosi et al., 2011b; Spokas et al., 2012b). This effect may reduce over time as biochar pores clog with organic material (Van Zwieten et al., 2010b). Microbial-N immobilisation is generally the predominant form of N immobilisation in soil, and typically cycles more rapidly than abiotic-N immobilisation (Barrett & Burke, 2000). Microbial-N immobilisation may be increased shortly following biochar amendment, as the labile C fraction of fresh biochar may be mineralised quickly following amendment to soil (Deenik et al., 2010; Lehmann et al., 2011; Ippolito et al., 2012).

In a previous study, we observed lower extractable inorganic-N concentrations in amended compared to un-amended soils, concurrent with lower soil N_2O emissions (Case et al., 2012). Therefore, we hypothesised that BII could primarily explain the suppression of soil N_2O emissions.

Our study had two primary aims. Firstly, we aimed to determine whether biochar addition affected soil N_2O emissions from an arable soil in environmental conditions similar to the field. Secondly, we aimed to investigate whether BII and soil aeration combined would explain the suppression of soil N_2O emissions. By quantifying N transformations in un-amended and amended soil under controlled conditions we

aimed to derive insights into the effects of biochar amendment on the activity of soil nitrifiers and denitrifiers.

In order to investigate these aims, we formed two hypotheses. Our first hypothesis was that suppression of soil N_2O emissions with biochar amendment is due to a combination of altered soil aeration due to biochar and N immobilisation (microbial and abiotic) (hypothesis 1). Our second hypothesis was that transformations of extractable soil NH_4^+ and NO_3^- via nitrification and denitrification under significant emission conditions (i.e. conditions where significant denitrifier activity is expected) are unaffected by biochar amendment (hypothesis 2).

To test hypothesis 1, we incubated arable soil cores under field conditions, undergoing wetting/drying cycles and in a second incubation, we incubated soil samples under significant emission conditions to determine the effect of biochar amendment on soil N_2O emissions. We expected soil N_2O emissions to be suppressed with biochar addition.

To address hypothesis 2 we used a ^{15}N pool dilution technique using paired $^{15}NH_4^+NO_3^-$ and $NH_4^+{}^{15}NO_3^-$ additions to quantify nitrification, denitrification and immobilisation with a numerical analysis model (FLUAZ, Mary et al., (1998)). Additionally, we analysed $^{15}N_2O$ and $^{15}N_2$ emissions from ^{15}N-labelled treatments to identify the sources of N_2O emissions and derive the product ratios of N_2O emissions from nitrification (N_2O: NO_2^- + NO_3^-) and denitrification (N_2O: N_2). We expected that soil N_2O emissions, nitrification and denitrification rates would not be significantly different with biochar amendment. Finally, we expected that N immobilisation rates with biochar would be greater.

4.3 Materials and methods

4.3.1 Biochar and field site description

The field site near Lincoln, Lincolnshire, was cultivated with an arable rotation with three years of wheat (*Triticum aestivum*) followed by one year of oilseed rape at the time of soil sampling (*Brassica Napus*). The soil was a sandy loam with 57% sand, 32% silt and 10% clay, and BD of 1.39 g cm^{-3}. The field site received a total of 140 kg N ha^{-1} as $NH_4^+NO_3^-$ each year, divided into three applications, one just before crop planting (35 kg N ha^{-1}) in February and two more after planting (70 and 35 kg N ha^{-1}).

The biochar, the same feedstock used in Case et al., (2012), was derived from a slow-pyrolysis batch process, heated first to 180°C to release volatile gas, then to 400°C for the next 24 hours. The biochar came from the thinnings of hardwood trees, chipped to a maximum size of 15 mm (ash, oak and cherry, Bodfari Charcoal, UK). It had a total C content of 72.3%, a total N content of 0.71%, low extractable inorganic-N concentrations (< 1.0 and 1.3 mg kg^{-1} of NH_4^+-N and NO_3^--N respectively), soil pH of 9.25 and a GMC of 3.1% at the time of use. More information regarding biochar properties is provided in the Appendix (Section 7.1).

4.3.2 Effects of biochar on N_2O emissions from soil undergoing wetting/drying cycles

We assessed the effect of biochar amendment on arable soil N_2O emissions under representative field conditions (undergoing controlled wetting/drying cycles). We collected 20 soil cores from the field site in March 2011, three weeks after planting and fertiliser N addition to the field. Soil cores of 150 – 180 mm depth were extracted in PVC pipes (H 215 mm D 102 mm) using hand tools and stored at 4°C for 1 month prior to the experiment. Each soil core contained approximately 1.6 kg soil d. wt. We collected additional soil samples to 7 cm depth to analyse for soil physical and chemical properties.

We designed a four-treatment factorial incubation of soil cores un-amended and amended with biochar, un-wetted or wetted with deionised water (n = 5). All soil cores were mixed to 7 cm depth. To half, biochar (ground to < 2 mm) was mixed into the soil cores at a rate of 3% dry soil weight (~ 22 t ha^{-1}). The cores were then placed at 16°C (mean soil temperature of the field site June - September 2009) in the dark for ten days before gas sampling to allow any initial flush of soil CO_2 emissions induced by warming or by newly-mixed soil to pass (Reicosky, 1997; Reichstein et al., 2000). The design of the soil core apparatus to enable air-tight gas sampling and draining of excess water is described in Case et al., (2012).

Un-wetted soil cores were maintained at 23% GMC, (mean monthly soil GMC analysed in the field Feb 2009 to Feb 2010, unpublished data). Wetted soil cores were wetted to 28% GMC at t_0 of the four wetting events on day 17, 46, 67 and 116 (maximum soil GMC observed in the field Feb 2009 to Feb 2010, unpublished data).

The soil core headspace was left open to the atmosphere apart from times of gas sampling. Headspace gas samples were taken from un-wetted cores on day 4, 17, 31, 46, 67, 116 and 120. Samples were taken from wetted cores at 12, 24 and 48 hours after wetting. For each soil core 10 ml (1% of the 0.9 l headspace) of chamber headspace volume was sampled using an air-tight syringe and injected into a 3 ml Labco sampling vial (Labco, USA). At each gas sampling time point, samples were taken at 0, 20, 40 and 60 minutes following enclosure.

On day 120, the soil cores were stored at 4°C and soil samples collected from them within four days to 7 cm depth. The soil was homogenised while wet and analysed for a range of soil chemical properties following the methods in Section 4.3.3. The homogenised soil samples were frozen at – 20°C for up to four weeks before analysis.

4.3.3 Soil physical and chemical properties

The same procedures for analysing soil pH, extractable NH_4^+ and NO_3^-, total C and N, CN ratio, BD, GMC, WHC, WFPS and particle density were used throughout this

study. Soil pH was determined with a Kent-Taylor combination pH electrode (Asea Brown Boveri, Switzerland) by using a 1: 2.5 ratio of soil: deionised water (w: v) (Emmett et al., 2008). For extractable inorganic-N analysis, 50 ml of 0.8 M potassium chloride (KCl, 6%) was used to extract NH_4^+ and NO_3^- from 5 g of soil. The extracts were then analysed on a Seal AQ2 discrete analyser (Bran and Luebbe, UK) using discrete colorimetric procedures (Maynard & Kalra, 1993). We ground 5 g of soil to < 2 mm, oven-dried it and analysed for total C and N content using 0.1 g of sample on a Truspec total CN analyser (LECO, USA) (Sollins et al., 1999). Gravimetric moisture content, BD, WFPS, WHC and particle density analyses were conducted according to standard methods (Blake, 1965; Ohlinger, 1995a; b; Emmett et al., 2008).

4.3.4 Headspace gas analysis

Headspace gas samples were analysed for N_2O concentrations by a Gas Chromatograph (GC). The GC (PerkinElmer Autosystem XL, PerkinElmer, USA) contained a stainless steel Porapak Q 50 – 80 mesh column (L 2 m, outer D 3.17 mm) maintained at 60°C. The GC was fitted with an electron capture detector (ECD) maintained at 360°C and a flame ionisation detector (FID) with methaniser operating at 300°C.

The equations in Holland et al., (1999) were used to calculate GHG fluxes linearly. All results were calibrated with certified standards (Air Products, UK). with the minimum detection limit calculated to be 8.6 μg N_2O-N m^{-2} h^{-1} (Parkin & Venterea, 2010). All gas fluxes were used in statistical analyses whether or not they were below the minimum detection limit (Sjögersten & Wookey, 2002; McNamara et al., 2008).

4.3.5 Biochar effects on soil N transformations using ^{15}N pool dilution

We conducted a ^{15}N pool dilution experiment in order to address both hypotheses. We created a 'significant emission' scenario for soil N_2O emissions by adding AN fertiliser and saturating the soil in order to test hypothesis 1 – that increased soil

aeration and BII are responsible for the suppression of soil N_2O emissions with biochar amendment. We analysed soil inorganic ^{15}N dynamics and headspace $^{15}N_2O$ emissions in order to address hypothesis 2 – that transformations of soil NH_4^+ and NO_3^- via nitrification and denitrification under significant emissions conditions are unaffected by biochar amendment.

We analysed soil extractable NH_4^+ and NO_3^-, organic-N and the ^{15}N % abundance of all analytes to estimate the effect of biochar amendment on nitrification and denitrification using a numerical analysis model (FLUAZ, Mary et al., (1998)). Finally, we analysed soil N_2O, $^{15}N_2O$ and $^{15}N_2$ emissions to determine the proportion of N_2O that came from nitrification (N_2O: NO_2^- + NO_3^-) and denitrification (N_2O: N_2).

Soil was collected from the field in January 2012 (prior to N fertiliser addition for that year), sieved to < 4 mm then stored, covered, at 4°C for forty days. We conducted a factorial laboratory incubation. There were four separate treatments: $^{15}NH_4^+NO_3^-$ un-amended, $^{15}NH_4^+NO_3^-$ amended, $NH_4^{+15}NO_3^-$ un-amended and $NH_4^{+15}NO_3^-$ amended (n = 20). Biochar was mixed with half of the soil with hand tools at a rate of 2% dry soil weight and stored again at 4°C, covered. Seven days later, 100 g d. wt. soil was put into eighty soil containers, divided equally between the four treatments (H 17.4 cm, D 11.6 cm). The soil cores were stored at 16°C (mean soil temperature of the field site June - September 2009) and seven days later (to allow for any initial flush of soil CO_2 emissions induced by warming or by newly-mixed soil to pass (Reicosky, 1997; Reichstein et al., 2000) solutions of 100.0 ± 0.1 mg N kg^{-1} soil $^{15}NH_4^+NO_3^-$ and $NH_4^{+15}NO_3^-$ (10% ^{15}N enrichment, equivalent to 110 kg N ha^{-1}) were applied in a de-ionised water solution to achieve a WFPS of 90% (91.0 ± 0.7% achieved). The solution was added by surface application with a syringe, pre-tested to ensure even surface application.

At five time points after solution addition (30 minutes, 1, 2, 4 and 6 days), sixteen soil cores (four soil cores of each treatment) were destructively sampled for total C and N content, soil pH, GMC and BD (methods in Section 4.3.3), extractable soil

NH_4^+, $^{15}NH_4^+$, NO_3^- and $^{15}NO_3^-$ concentration, organic N and organic ^{15}N concentration (methods in Section 4.3.6).

4.3.6 Inorganic ^{15}N analysis

Extractable inorganic ^{15}N concentrations ($^{15}NH_4^+$ and $^{15}NO_3^-$) were analysed by first extracting inorganic-N from soil using 2 M KCl. Then, 20 ml of the extract was placed in air-tight Kilner jars (Kilner, USA). For $^{15}NH_4^+$, 0.2 g of Magnesium Oxide (MgO) was added. For $^{15}NH_4^+$ + $^{15}NO_3^-$, 1 ml of 0.2 M sulfamic acid was added to decompose NO_2^-, then 0.2 g of MgO and 0.2 g Devarda's Alloy. Whatman no. 41 filter paper disks (Whatman, USA) were suspended above the solution with 5 µl 2.5 M potassium hydrogen sulphate solution added. The jars were sealed and placed in a 30°C environment for at least 72 hours to enable close to 100% adsorption of the extractant N (Khan et al., 1998). The filter disks were then dried at 40°C for 24 hours. This method allowed us to directly analyse $^{15}NH_4^+$ and ($^{15}NH_4^+$ + $^{15}NO_3^-$) concentrations. Three-quarters of each of the two filter papers were weighed together and sealed in a single tin capsule (Elemental Microanalysis Ltd, UK). The samples were combusted using an automated NA1500 elemental analyser (Carlo Erba, Italy) coupled to an Isotope Ratio Mass-Spectrometer (Dennis Leigh Technology, UK).

We calculated $^{15}NO_3^-$ atom % abundance from ($^{15}NH_4^+$ + $^{15}NO_3^-$) atom % abundance and extractable inorganic-N analyses using Eq. 1:

$$A_n = \frac{\left((A_{a+n}) * (Q_{a+n})\right) - \left((A_a) * (Q_a)\right)}{Q_n} \qquad \text{Eq. 1}$$

Where:

A_n = $^{15}NO_3^-$ atom % abundance

A_{a+n} = ($^{15}NH_4^+$ + $^{15}NO_3^-$) atom % abundance

Q_{a+n} = Extractable NH_4^+ + NO_3^- concentration

A_a = $^{15}NH_4^+$ atom % abundance

Q_a = Extractable NH_4^+ concentration

Q_n = Extractable NO_3^- concentration

Organic ^{15}N content was used as an analogue for microbial biomass (Mary et al., 1998). This was determined by oven drying 3 g of soil at 80°C for 24 hours, then mixing the dried soil with 10 ml of 1 M KCl in a 12 ml polystyrene test tube and mechanically shaking for 15 minutes. The tube was then centrifuged for 15 minutes at 3,000 rpm (Recous et al., 1998). The KCl was removed and replaced. This process was repeated four times, after which the KCl was drained. The soil was dried at 80°C for 24 hours. Then, 50 mg of dry soil was sealed in a tin capsule and analysed in the same way as the acidified disks described above. For both inorganic and organic ^{15}N, the standard deviation of control samples was not more than 6 ‰.

4.3.7 Biochar effects on soil N transformations using ^{15}N pool dilution: Modelling

In order to address hypothesis 2 (that transformations of extractable soil NH_4^+ and NO_3^- via nitrification and denitrification under significant emissions conditions are unaffected by biochar amendment), we analysed inorganic and organic-N and respective ^{15}N concentrations, then quantified nitrification and denitrification rates within soil using a numerical analysis model (FLUAZ, Mary et al., (1998)).

The FLUAZ model uses a numerical method using a Runge-Kutta algorithm. Partial differentiation equations using a non-linear fitting method (Haus-Marquardt algorithm) describe the changes in N and ^{15}N concentrations for inorganic, organic and biomass N. Using this method, the model minimises the difference between analysed and modelled data.

Inorganic N, organic N and respective ^{15}N concentrations were analysed according to a paired treatment design and were input into the FLUAZ model to calculate N transformations (Fig. 4.1, Mary et al., (1998)). For each N transformation, 90% confidence intervals (CIs) were reported. The final model fitted mineralisation (m + s, mineralisation of soil humus-derived and biochar-derived N to NH_4^+), nitrification (n, the conversion of NH_4^+ to NO_3^-), immobilisation (We, the sum of NH_4^+ and NO_3^- taken up by the organic N pool) and denitrification rate (kd, the sum of conversion

of NO_3^- to N_2O, NO or N_2) over four time periods (0.02 – 1 day, 1 – 2 days, 2 – 4 days, 4 – 6 days). Fig. 4.1 provides an overview of the rates and pools that we quantified using the FLUAZ model.

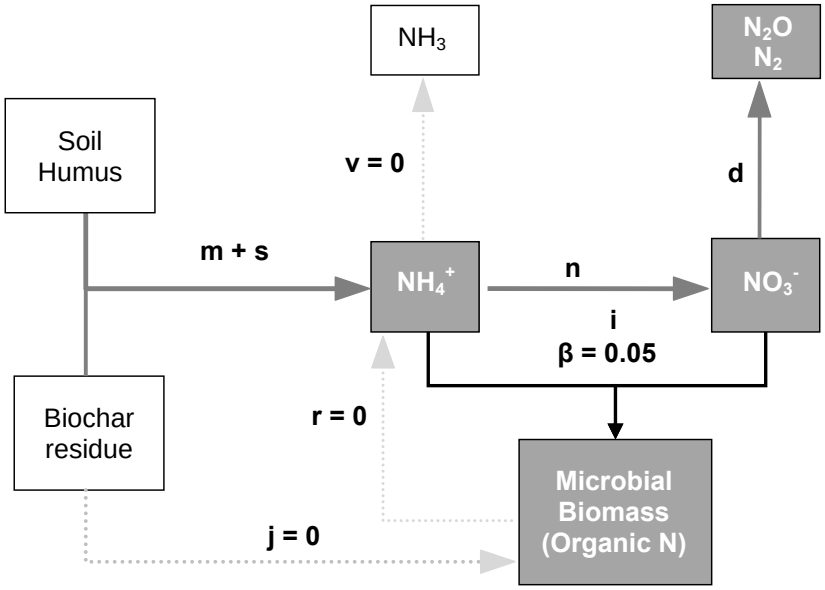

Fig. 4.1. The N-cycling rates modelled as a part of FLUAZ model design using a "paired" ^{15}N labelling experiment, adapted from (Mary et al., 1998). Dark boxes indicate pools that are directly measured; dark, solid lines indicate rates that are estimated by the model. White boxes indicate pools that are not measured by our experimental design, and dotted grey lines indicate N cycling rates that are considered to be negligible in our experimental design. "m + s" is the combined mineralisation of biochar residue and humified organic N. "v" is the volatilisation of NH_4^+ to NH_3. "j" is the direct microbial assimilation of biochar residue N. "r" is the remineralisation of microbially-immobilised N. "n" is the nitrification of NH_4^+ to NO_3^-. "d" is the denitrification of NO_3^- to N_2O or N_2. "i" is the microbial immobilisation of NH_4^+ and NO_3^- combined. "β" is the ratio of NO_3^- over NH_4^+ microbial immobilisation.

We made assumptions about the remaining parameters of the model based on evidence from the literature. We assumed that the ratio between microbial immobilisation of NO_3^- and NH_4^+ (β) was 0.05, as suggested in Mary et al., (1998). We assumed that remineralisation (r) was 0, as the incubation only lasted for six days (Barraclough, 1995). We assumed that the conversion of plant residue N into microbial biomass (j) was 0 and that ammonia volatilisation was negligible (Hayashi et al., 2011). We assumed that the addition of 2% fresh biochar to soil with 0.71% N content added 142 mg N kg^{-1} to the soil as total N, which was included in the model as 'residue N'.

The FLUAZ model was run separately for un-amended and amended soil. The overall match between the observed and modelled data in the FLUAZ model is estimated by the mean weighted error of the model. Using the assumptions described above and the input values described in Section 4.4.2, the overall mean weighted error of the model was 1.0 overall for un-amended soils and 1.7 for amended soils. These relatively low values indicated that the FLUAZ provided a good fit to the data (Mary et al., 1998).

4.3.8 Headspace N_2O, $^{15}N_2O$ and $^{15}N_2$ analysis

We analysed soil N_2O emissions in order to test whether the suppression of soil N_2O emissions with biochar amendment is due to a combination of altered soil aeration due to biochar and N immobilisation (addressing hypothesis 1). We analysed soil $^{15}N_2O$ and $^{15}N_2$ emissions in order to address hypothesis 2, that transformations of extractable soil NH_4^+ and NO_3^- via nitrification and denitrification under significant emissions conditions are unaffected by biochar amendment. At five different time points following ^{15}N addition (0, 1, 2, 4 and 6 days), 10 ml gas samples were taken from sixteen soil cores (1.7 l headspace) using a gas-tight syringe and injected into evacuated 3 ml vials. Headspace N_2O concentrations were analysed using the same method as described in Section 4.3.4. For $^{15}N_2O$ analysis, 80 ml headspace samples were taken and injected into evacuated 60 ml glass serum bottles (Wheaton Science Products, US2). $\delta^{15}N$ values were then derived using a trace gas precursor (20 µl in a 20 ml headspace) coupled to an Isoprime Isotope Ratio Mass Spectrometer (IRMS, GV instruments Ltd, UK). The SD of $^{15}N_2O$ standards was 0.5 ‰ (per mil) N_2O.

We directly analysed the ^{15}N content of N_2 emissions (Khalil et al., 2004; Morley & Baggs, 2010). For $^{15}N_2$ analysis, 20 ml headspace samples were taken and injected into evacuated 10 ml Labco sampling vials (Labco, USA). Gas samples from these vials (4 – 6 µl) were injected into an N_2 prep unit using a gas-tight syringe. Water was removed from the sample by a perchlorate chemical trap and the CO_2 removed cryogenically. The N_2 was passed through reduced copper maintained at 600°C and the N_2 passed into an Isoprime IRMS (Micromass, UK) via an open split. The SD of

$^{15}N_2$ samples was 0.08 ‰ N_2. After gas samples were extracted, lab air of equivalent volume and known concentration was injected into the enclosed sample headspace.

The proportion of soil N_2O emissions attributed to nitrification and denitrification was calculated from $^{15}NO_3^-$ labelled soil cores (Stevens et al., 1997), using equation 1. from Mathieu et al., (2006):

$$d = \frac{(a_m - a_n)}{(a_d - a_n)} (with\ a_d \neq a_n) \quad \text{Eq. 2}$$

Where:

d = the proportion of N_2O emissions from denitrification in a time period

a_m = the average ^{15}N atom enrichment of the N_2O mixture during time period

a_n = the average ^{15}N enrichment of the nitrification pool (NH_4^+) during time period

a_d = the average ^{15}N enrichment of the denitrification pool (NO_3^-) during time period

The proportion of N_2O: N_2 emissions from denitrification was calculated from the change in $^{15}N_2$ concentration from the atmospheric background standards, the N_2O emissions derived from denitrification calculated using Eq. 2 and cumulative denitrification (estimated from the FLUAZ model, Section 4.3.7). The proportion of N_2O emissions from nitrification – or the N_2O: (NO_2^- + NO_3^-) ratio – was calculated by dividing soil N_2O emissions from nitrification using Eq. 2 by cumulative nitrification (estimated from the FLUAZ model, Section 4.3.7).

4.3.9 Statistical analysis

In order to test for significant differences between un-amended and biochar-amended soil and address both hypotheses, we compared soil physico-chemical properties with and without biochar amendment using t-tests. T-tests were also used to test for significant differences between soil N_2O production over six days with and without biochar amendment in the incubation to analyse soil N transformations. For all statistical analyses, data exploration was first conducted using R version 2.15.2 (The R Project, 2013) following the procedure presented in Zuur et al., (2010a). For t-test data, Levene's test was used to resolve whether the

variance in un-amended and amended soil was significantly different ($p < 0.05$). Welch's t-test was used if this was the case; otherwise a two sample t-test was used.

To test for significant differences between un-amended and amended soil N_2O emissions and address hypothesis 1, linear mixed-effects models were used for soil cores undergoing wetting/drying cycles. For all of the models, 'soil core' was used as the random factor and 'biochar amendment' and 'day from start of the experiment' as independent variables. For un-wetted soil cores, soil N_2O emissions were used as the dependent variable. For the wetted soil cores, 'soil N_2O emissions within 48 hours of a wetting event' was used as the dependent variable and 'time from wetting event' as an additional independent variable. The models were run using NLME package version 3.1-108 and refined following the guidance provided in (Zuur et al., 2010b).

4.4 Results

4.4.1 Soil incubation undergoing wetting/drying cycles

We added biochar to soil cores from an arable field and analysed soil N_2O emissions over a 120-day period in order to address hypothesis 1, that the suppression of soil N_2O emissions with biochar amendment is due to a combination of altered soil aeration due to biochar and N immobilisation. Soil N_2O emissions were suppressed with biochar amendment in un-wetted soil, from 103.9 ± 17.1 in un-amended soil to 16.3 ± 2.8 $\mu g\ N_2O\text{-}N\ m^{-2}\ h^{-1}$ in amended soil, a suppression of 84% ($p < 0.001$, Table 4.1, Fig. 4.2).

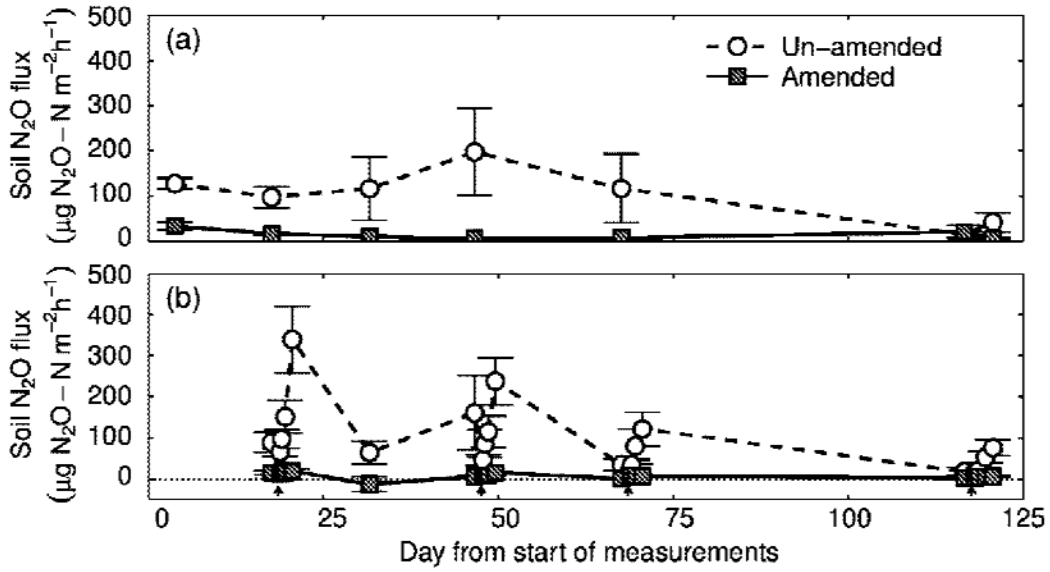

Fig. 4.2. The effect of biochar amendment on soil N_2O emissions from soil cores undergoing wetting/drying cycles either (a) un-wetted or (b) wetted. Arrows on the graph indicate the time of soil wetting. Data points represent mean ± standard error (n = 5). The horizontal dotted line in graph (b) indicates the 0 line. Statistical model outputs underlying these results are presented in Table 4.1.

Soil N_2O emissions significantly increased with wetting in un-amended soil, but not in amended soil (Fig. 4.2, Table 4.2). Soil N_2O emissions within 48 hours of wetting (emissions following wetting) were 88% lower with biochar amendment ($p < 0.001$, Table 4.1, Table 4.2). There was a temporal pattern of the soil N_2O emission pulse getting smaller following each successive wetting event. Following the fourth and final wetting event, soil N_2O emissions following wetting were 83% and 69% lower than for the first wetting event in un-amended and amended soil respectively ($p < 0.001$, Fig. 4.2, Table 4.2). We concluded that hypothesis 1 was supported by our data.

Table 4.1: Variables affecting N_2O emissions within soils undergoing wetting/drying cycles. "N_2O un-wetted" indicates soil cores maintained field moist, while "N_2O wetted" signifies soil N_2O emissions within 48 hours of a wetting event. Data outputs presented are those from refined linear mixed-effects models using plot as the random factor, refined following the procedure in Zuur et al., (2010b). n = 5. Symbols indicate p-value significance of the term: ** = $p < 0.01$, *** = $p < 0.001$. Refer to Fig. 4.2 for the data underlying these statistical outputs.

	Independent variable									
Response variable	**Biochar**		**Time from wetting**		**Biochar * Time from wetting**		**Day from start**		**Biochar * day from start**	
	t	**p**	**t**	**p**	**t**	**p**	**t**	**p**	**t**	**p**
N_2O un-wetted	8.07	***	-	-	-	-	- 7.56	***	- 6.56	***
N_2O wetted	4.99	**	9.63	***	8.47	***	- 8.36	***	- 6.21	***

Biochar amendment affected soil physico-chemical properties, assessed after 120 days incubation. Total soil C content, C: N ratio and pH were all increased with biochar amendment (Fig. 4.3). Soil pH increased from 5.55 ± 0.12 to 6.53 ± 0.24 ($p < 0.01$, Fig. 4.3) in un-wetted soil and from 5.13 ± 0.07 to 6.19 ± 0.26 ($p < 0.05$, Fig. 4.3) in wetted soil. Soil extractable NH_4^+ concentration after 120 days was 70% lower in biochar amended, wetted soil cores ($p < 0.05$, Fig. 4.3).

Table 4.2: The effect of biochar amendment on cumulative N_2O emissions within 48 hours of a wetting event from soils undergoing wetting/drying cycles. Data indicate mean (± standard error, n = 5).

Wetting event	**Treatment**	**Cumulative N_2O production (mg N_2O-N m^{-2} 48 hrs^{-1})**
1	Un-amended	8.49 (1.92)
	Amended	0.84 (0.18)
2	Un-amended	6.53 (1.86)
	Amended	0.59 (0.06)
3	Un-amended	3.51 (1.38)
	Amended	0.37 (0.04)
4	Un-amended	1.39 (0.47)
	Amended	0.26 (0.06)

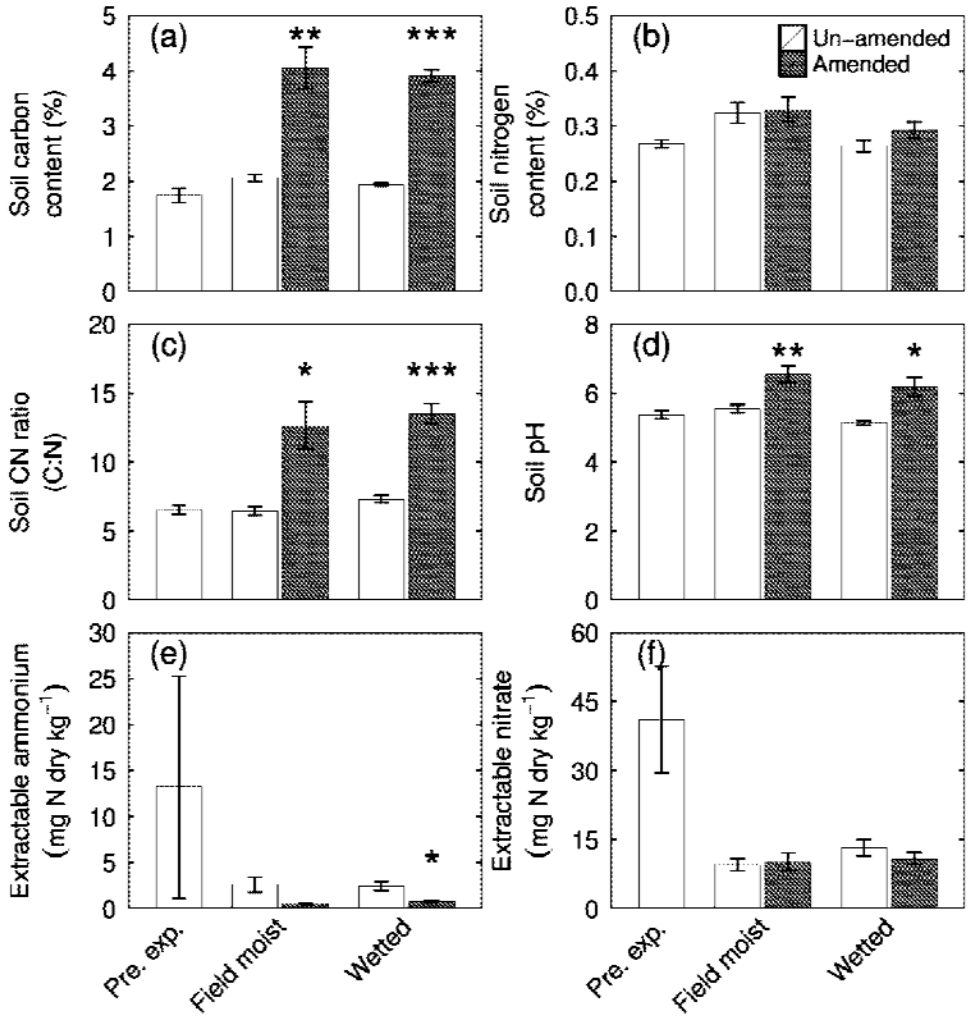

Fig. 4.3. The effect of biochar amendment on physico-chemical properties of soil cores taken from the field prior to biochar amendment (n = 4) and after 125 days incubation from either un-wetted (field moist) or wetted soil cores (n = 5); soil (a) total C content; (b) total N content; (c) CN ratio; (d) pH; (e) extractable NH_4^+ concentration; (f) extractable NO_3^- concentration. Bar plots represent mean ± standard error. Asterisks indicate significant difference between un-amended and amended soils after using t-tests: * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$.

4.4.2 Biochar effects on soil N transformations following fertiliser addition using ^{15}N pool dilution

We wetted arable soil and added AN fertiliser in order to address hypothesis 1, that the suppression of soil N_2O emissions with biochar amendment is due to a combination of altered soil aeration due to biochar and N immobilisation. Despite equalising soil aeration and making BII negligible, soil N_2O emissions were

suppressed with biochar-amendment, disproving hypothesis 1. During the six days following the addition of ^{15}N-labelled substrate, un-amended soil produced 0.80 ± 0.25 compared with 0.05 ± 0.02 mg N_2O-N kg^{-1} from amended soil, a suppression of 95% (two-sample t-test, $p < 0.05$, $t = 2.7$, $df = 13$, Fig. 4.4).

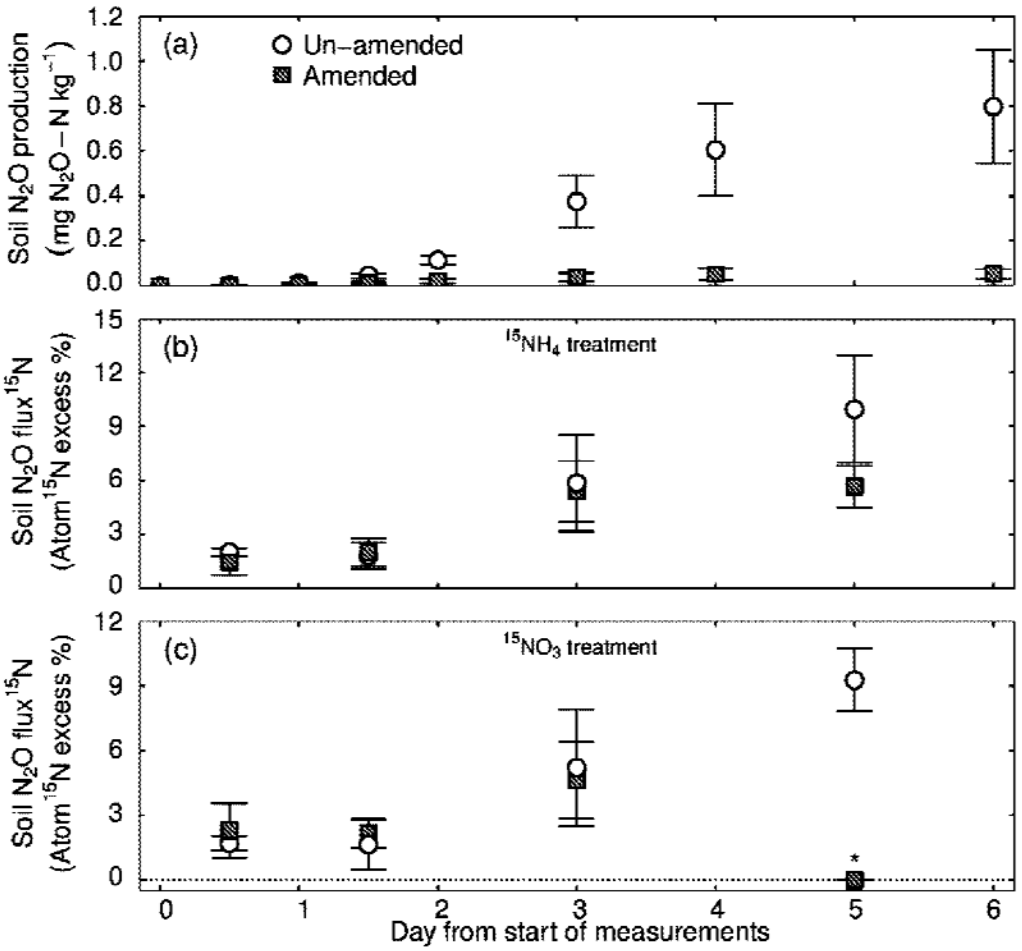

Fig. 4.4. The effect of biochar amendment on (a) cumulative soil N_2O production and the average N_2O atom excess for soil amended with ^{15}N-labelled (b) NH_4^+ or (c) NO_3^- during an incubation to investigate soil N transformations. ^{15}N-labelled NO_3^- or NH_4^+ was added at t = 0 and soil WFPS raised to 90%. Data points represent mean ± standard error (n = 4). The asterisk in graph c) indicates 0, as there were no soil N_2O emissions from biochar-amended soils between day 4 and 6.

In order to address hypothesis 2 – that stated that transformations of extractable soil NH_4^+ and NO_3^- via nitrification and denitrification under significant emissions conditions are unaffected by biochar amendment – we analysed soil inorganic N

and organic N and respective ^{15}N concentrations and input these data into the FLUAZ model. From this we estimated soil cumulative nitrification and denitrification (Fig. 4.7).

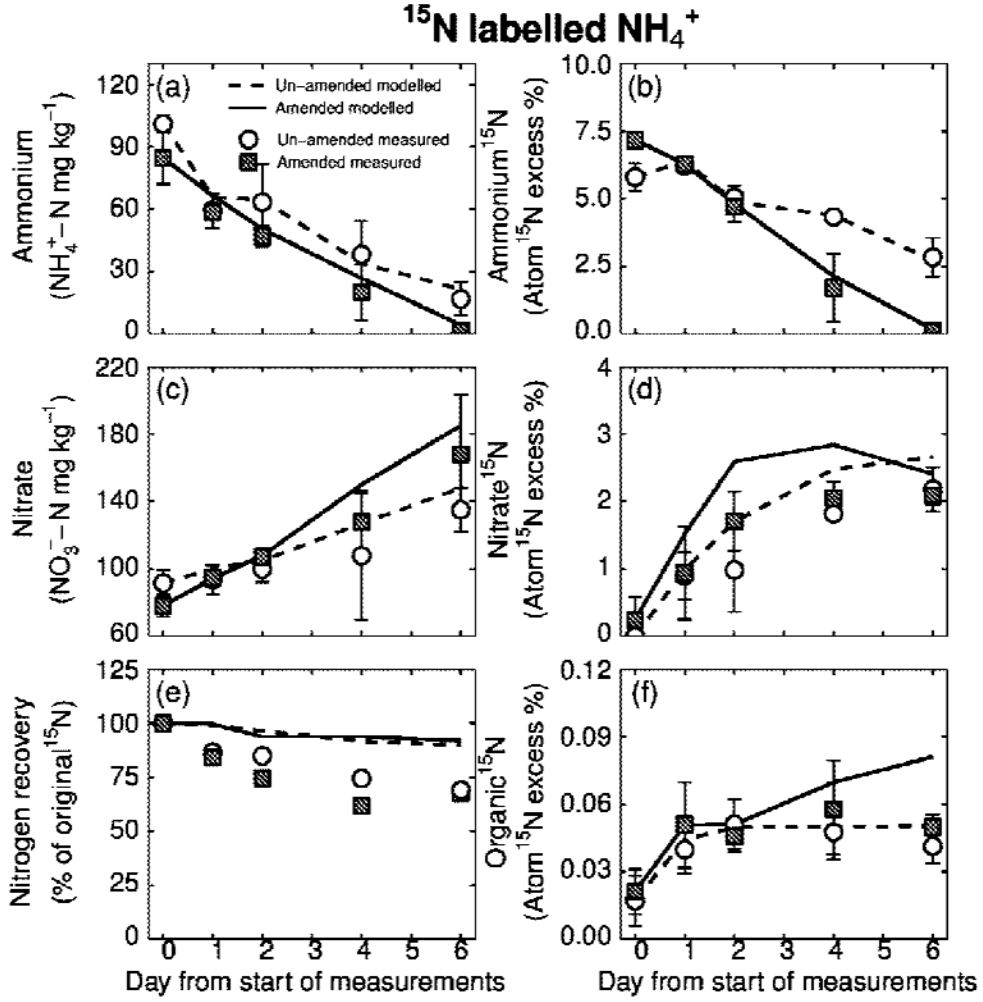

Fig. 4.5. The effect of biochar amendment on: (a) soil extractable NH_4^+ concentration, (b) NH_4^+ atom ^{15}N excess %, (c) soil extractable NO_3^- concentration, (d) soil NO_3^- atom ^{15}N excess %, (e) nitrogen (N) recovery from initial ^{15}N (%, initial ^{15}N = ^{15}N content analysed 30 minutes after addition) and (f) soil organic N atom ^{15}N excess (%), during the incubation to investigate the soil N cycle within $^{15}NH_4^+$-labelled soil. Points indicate the mean of directly measured values ± standard error (n = 4), whereas lines indicate simulated values from subsequent FLUAZ model analysis.

Observed and modelled soil extractable NH_4^+, NO_3^- and ^{15}N concentrations underlying the total N recovery calculations are presented in Fig. 4.5 and Fig. 4.6.

Soil inorganic-N and ^{15}N concentrations in the soil generally fitted well to the modelled data (Fig. 4.5, Fig. 4.6). Extractable soil NH_4^+ concentrations reduced with time while NO_3^- concentrations increased in both un-amended and amended soils (Fig. 4.5, Fig. 4.6). In both $^{15}NH_4^+$ and $^{15}NO_3^-$ labelled soils, extractable soil NH_4^+ concentrations decreased in amended soil at a greater rate and to a lower final concentration after 6 days. In $^{15}NH_4^+$ amended soil, $^{15}NH_4^+$ enrichment decreased more rapidly with time and soil extractable NO_3^- concentrations increased more rapidly.

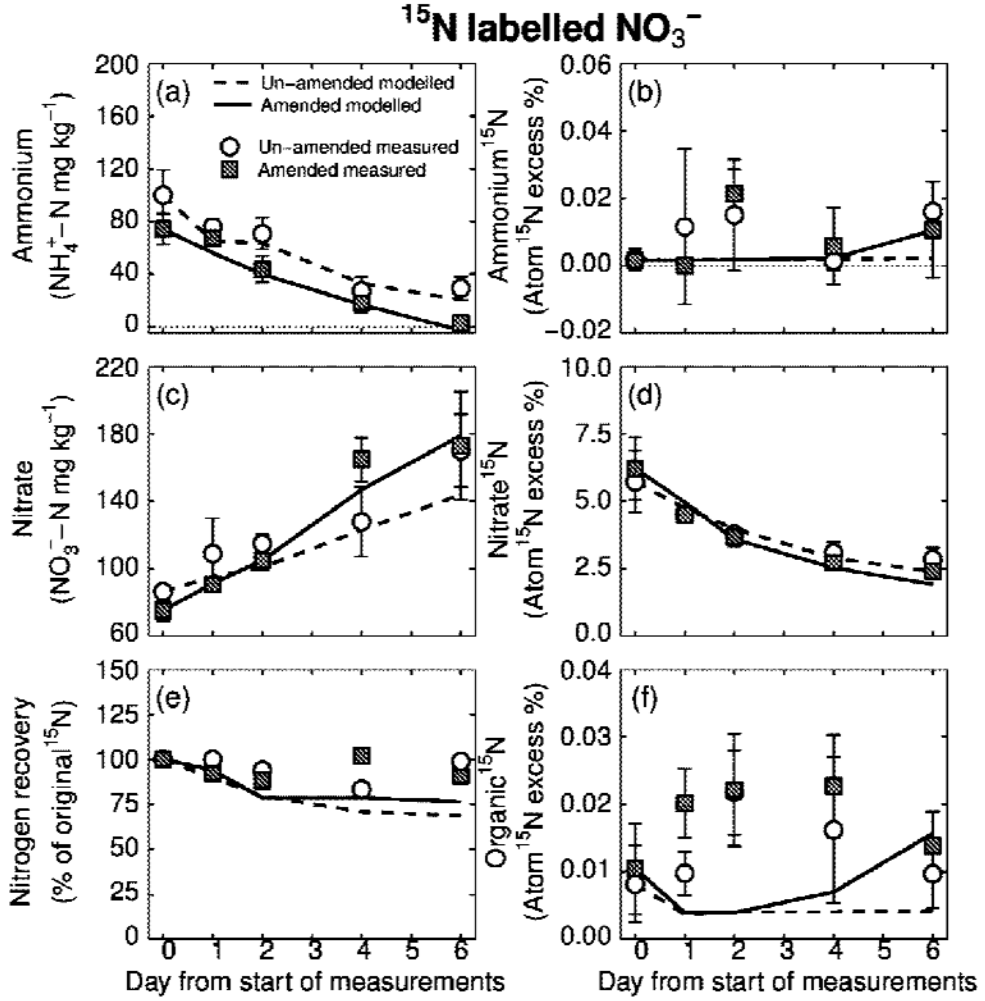

Fig. 4.6. The effect of biochar amendment on: (a) soil extractable NH_4^+ concentration, (b) NH_4^+ atom ^{15}N excess %, (c) soil extractable NO_3^- concentration, (d) soil NO_3^- atom ^{15}N excess %, (e) nitrogen (N) recovery from initial ^{15}N (%, initial ^{15}N = ^{15}N content analysed 30 minutes after addition) and (f) soil organic N atom ^{15}N excess (%),during the incubation to investigate the soil N cycle within $^{15}NO_3^-$-labelled soil. Points indicate the mean of directly measured

values ± standard error (n = 4), whereas lines indicate simulated values from subsequent FLUAZ model analysis. The horizontal dotted lines in graph (a) and (b) indicate 0.

Initial organic-N content was determined to be 2,162 ± 46 mg N kg^{-1} (n = 8) for both un-amended and amended soil and was assumed to have an atom % excess of 0.0025% (Mary et al., 1998). Organic ^{15}N concentrations matched the modelled data well in the $^{15}NH_4^+$ treatments but were lower than the modelled data in the $^{15}NO_3^-$ treatments, a trend that we cannot explain (Fig. 4.5, Fig. 4.6). Organic ^{15}N % excess was not significantly different between un-amended and amended soil. To estimate of the validity of our results we calculated total N recovery from inorganic, organic N and respective ^{15}N concentrations in the soil. Total N recovery for the ^{15}N-labelled NO_3^- treatments remained close to 100% throughout the incubation; whereas total N recovery for the ^{15}N-labelled NH_4^+ treatments were lower (Fig. 4.5, Fig. 4.6). Total N recovery during subsequent time points is shown in Fig. 4.5 e) and Fig. 4.6 e).

Table 4.3: Biochar-induced N immobilisation (BII) via microbial or abiotic processes. Post-^{15}N addition microbial N immobilisation was calculated from the difference between microbial N immobilisation in un-amended and amended soil calculated by the FLUAZ model. Overall BII pre-^{15}N addition from the two processes was calculated from the differences in inorganic-N concentrations pre-^{15}N addition (see text) and dividing this value by the same ratio between microbial N immobilisation and abiotic N immobilisation found post-^{15}N addition (3.95: 1).

	Pre-^{15}N addition immobilisation				**Post-^{15}N addition immobilisation**				**Total**			
	Microbial		**Abiotic**		**Microbial**		**Abiotic**		**Microbial**		**Abiotic**	
	NH_4^+	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**	**NH_4^+**	**NO_3^-**
BII (mg N kg^{-1})	1.27	2.09	0.34	0.57	0	0	0.20	0.01	1.27	2.09	0.54	0.58

Cumulative nitrification, denitrification and N immobilisation (0 – 6 days following ^{15}N addition) estimated from the FLUAZ model is shown in Fig. 4.7. The modelled transformations of N concentrations via each process following ^{15}N addition are henceforth referred to as a cumulative nitrification, immobilisation and denitrification. According to the FLUAZ model output, cumulative nitrification (the conversion of NH_4^+ to NO_3^-) was not different in un-amended and amended soil (98 ± 25 and 139 ± 32 mg N kg^{-1} respectively, $p > 0.1$, Fig. 4.7). Cumulative denitrification

(the sum of conversion of NO_3^- to N_2O, NO or N_2) in both un-amended and amended soil was highly variable and not significantly different (Fig. 4.7). In un-amended soil, cumulative denitrification was 0.35 ± 0.56 mg N kg^{-1} while in amended soil it was 0.23 ± 0.71 mg N kg^{-1} ($p > 0.1$, Fig. 4.7). We therefore concluded that biochar amendment did not affect the concentration of NO_3^- or NH_4^+ transformed by nitrification or denitrification. Cumulative immobilisation (the uptake of NH_4^+ or NO_3^- into organic-N) was not significantly different with biochar amendment (12.7 ± 16.8 mg N kg^{-1} in un-amended soil compared with 34.9 ± 35.1 mg N kg^{-1} in amended soil, $p > 0.1$, Fig. 4.7). The total immobilisation of inorganic-N with biochar amendment was negligibly low compared to total soil inorganic N concentrations. A summary of our estimate for overall BII is shown in is in Table 4.3, with the calculation steps described in the Appendix (Section 7.3.1).

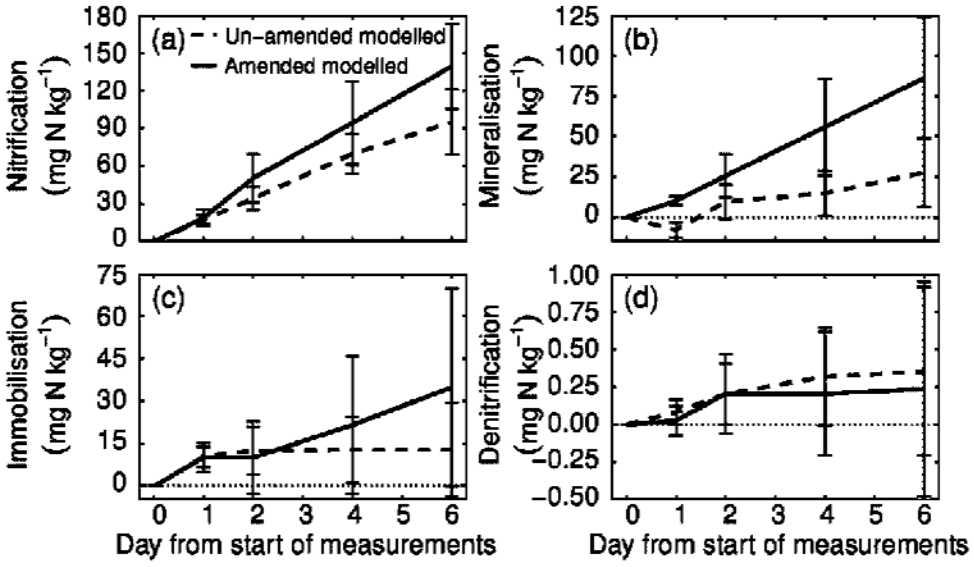

Fig. 4.7. The effect of biochar amendment on cumulative (a) nitrification, (b) mineralisation, (c) immobilisation and (d) denitrification during an incubation to investigate the soil N cycle. The data was derived from outputs of the FLUAZ model and are calculated from the raw data given in Fig. 4.5 and Fig. 4.6 and inline in the Results section (Section 4.4). Error bars represent confidence intervals ± 90%.

To address hypothesis 2, we quantified the proportion of N_2O emissions derived from nitrification and denitrification using Eq. 2. We estimated that soil N_2O

emissions were produced via a mix of both nitrification and denitrification from days 0-2 in amended and un-amended soils (Fig. 4.8). Between day 2 and 4, all soil N_2O emissions came from denitrification in both treatments. Between day 0 and 4, 95% of soil N_2O emissions came from denitrification in un-amended soil, compared to 85% of soil N_2O emissions coming from denitrification in amended soil (Fig. 4.4, Fig. 4.7). After day 4, no further soil N_2O emissions were produced from amended soils (Fig. 4.4, Fig. 4.7). We divided N_2O production from nitrification by cumulative nitrification estimated by the FLUAZ model (using data from Fig. 4.7 and Fig. 4.8). The proportion of N_2O from cumulative nitrification in un-amended soils was 0.080% compared to 0.012% in amended soils (i.e. the ratio of N_2O: (NO_2^- + NO_3^-) production, soil N_2O from nitrification divided by cumulative nitrification after 6 days).

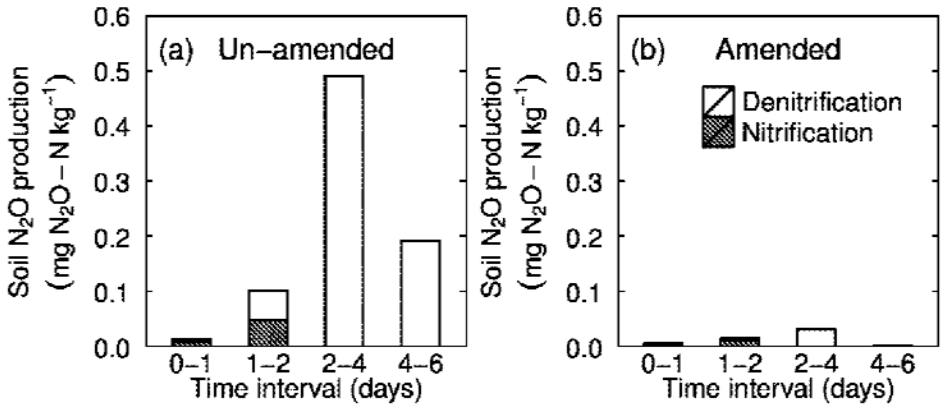

Fig. 4.8. Soil N_2O emissions attributed to denitrification and nitrification in (a) un-amended and (b) amended soils. ^{15}N-labelled NO_3^- or NH_4^+ was added at t = 0 and soil WFPS raised to 91%. The proportion of soil N_2O emissions attributed to nitrification or denitrification during each time interval was derived from the soil cores that had ^{15}N NO_3^- added to them, following equation Eq. 2. n = 4.

Headspace $^{15}N_2$ concentrations could not be accurately measured because the ^{15}N of the soil core headspace was masked by the ^{14}N atmospheric pool. Therefore the ratio of N_2O: N_2 concentrations could not be directly calculated. Cumulative denitrification in un-amended and amended soils was not significantly different (i.e. the total transformation of soil NO_3^- to NO, N_2O or N_2). We assumed that NO

emissions were negligible relative to N_2O and N_2 in saturated soil as it was converted to the final two denitrification products before it diffused to the atmosphere (Russow et al., 2009). The transformation of soil NO_3^- to N_2O was much lower in amended soil (Fig. 4.4). Therefore, the ratio of N_2O: N_2 was also lower, as cumulative denitrification was the same in un-amended and amended soil, yet biochar-amended soil yielded lower soil N_2O emissions.

Table 4.4: The effect of biochar amendment on soil total C content (%); total N content (%), CN ratio and soil pH during an incubation to investigate soil N cycling. ^{15}N-labelled NH_4^+ and NO_3^- was added at t = 0 and soil WFPS raised to 91%. 'Pre. exp.' Refers to analyses conducted before ^{15}N and water addition, while 'Post. exp.' represents analyses from five time points following treatments: 30 minutes, 1 day, 2 days, 4 days, 6 days. Values represent mean (± standard error). Asterisks indicate significant difference between adjacent un-amended and amended soils: *** = $p < 0.001$.

Time	Biochar amendment	Total C (%)	Total N (%)	CN ratio	pH
Pre-^{15}N	Un-amended	2.04 (0.04)	0.25 (0.01)	8.86 (0.25)	6.85 (0.04)
	Amended	3.68 (0.14) ***	0.26 (0.01)	15.69 (0.68) ***	7.14 (0.03) ***
Post-^{15}N	Un-amended	1.98 (0.02)	0.25 (0.01)	8.08 (0.22)	6.29 (0.03)
	Amended	3.7 (0.07) ***	0.26 (0.01)	14.32 (0.42) ***	6.59 (0.03) ***

Soil physico-chemical properties were analysed in order to provide supporting information to explain the effect of biochar amendment on soil N_2O emissions. Biochar amendment significantly increased soil pH from 6.29 ± 0.03 to 6.59 ± 0.03 ($p < 0.001$, Table 4.4). Total soil C content and CN ratios increased in amended soils, while total N contents were not significantly different between un-amended and amended treatments ($p < 0.001$, $p < 0.001$, $p > 0.05$, Table 4.4). Soil WHC was not significantly greater with biochar-amendment (data not shown, $p > 0.05$, $t = -1.5$, $df = 6$). Water holding capacity was 54.6 ± 1.7% and 57.5 ± 0.8% for un-amended and amended soil respectively.

4.5 Discussion

In this study we manipulated soil N status as well as soil aeration to investigate the mechanisms by which biochar amendment suppresses soil N_2O emissions. Our primary aims were to i) investigate whether fresh biochar addition to an arable soil could suppress soil N_2O emissions in typical field conditions and ii) to determine whether BII and increased soil aeration were responsible for this suppression.

We had two main hypotheses to support our aims (Section 4.2). Firstly, we hypothesised that the suppression of soil N_2O emissions with biochar amendment is due to a combination of altered soil aeration due to biochar and N immobilisation (microbial and abiotic) (hypothesis 1). We found that during the soil incubation undergoing wetting/drying cycles, soil N_2O emissions were consistently suppressed with biochar amendment, whether un-wetted or wetted (Fig. 4.2). Under significant emission conditions, soil aeration effects and BII were negligible, nevertheless, soil N_2O emissions were suppressed with biochar amendment, disproving this first hypothesis (Fig. 4.4).

Cumulative soil nitrification and denitrification were the same with biochar amendment under significant emission conditions (Fig. 4.7). However, the ratio of N_2O: (NO_2^- + NO_3^-) production from nitrification and the ratio of N_2O: N_2 production from denitrification was lower with biochar amendment, therefore disproving hypothesis 2. In the following section we consider the mechanisms that may explain the reduction of these two product ratios.

In biochar amended soils exposed to wetting/drying cycles, soil N_2O emissions were suppressed by approximately 84% in un-wetted soil and by 88% in wetted soil within two days of wetting (Fig. 4.2). A variety of mechanisms have been proposed to explain why N_2O emissions are suppressed with biochar amendment under field conditions. We demonstrated previously that WHC was increased and BD reduced with biochar amendment (Case et al., 2012). A combination of these effects may increase soil aeration, therefore reducing the activity of denitrifying enzymes (Yanai et al., 2007; Van Zwieten et al., 2010b). In the soil cores undergoing wetting/drying cycles, soil aeration was consistently greater in amended soil, which may partially explain the observed reduction in soil N_2O emissions following wetting.

Soil extractable NH_4^+ concentrations were 70% lower after a 120-day incubation of biochar-amended soils (Fig. 4.3). Biochar amendment has been shown to immobilise inorganic-N in soil (BII), therefore limiting the availability of N substrate to soil

nitrifiers and denitrifiers. This may occur via abiotic-N adsorption to the biochar surface; or microbial-N immobilisation induced by biochar addition (Clough & Condron, 2010; Van Zwieten et al., 2010b; Bruun et al., 2011a; Case et al., 2012). In this study, BII may have limited the availability of inorganic-N substrate to soil nitrifiers and denitrifiers, resulting in lower soil N_2O emissions. We therefore hypothesised that increased soil aeration with biochar amendment and BII were two of the factors responsible for the suppression of soil N_2O emissions during the first incubation.

To test this hypothesis, we added AN fertiliser and water to saturate the soil and ensure excess available N in the soil solution ('significant emission' conditions for soil N_2O emissions) (Table 4.3). Under these conditions, soil N_2O production was suppressed by 95% (Fig. 4.4); we therefore concluded that BII or increased soil aeration due to biochar addition were not responsible for the suppression of soil N_2O emissions under 'significant emission' conditions. Therefore we needed to consider other mechanisms that may explain the suppression of soil N_2O emissions.

In addition to changes in soil C and N status we observed a significant increase in soil pH. Soil pH can have a significant influence on soil microbial community composition and activity (Fierer & Jackson, 2006). Denitrifier activity in soils is generally greatest close to the 'natural' pH of the soil (Šimek et al., 2002). Biochar often has a high pH, can increase the pH of soil it is added to (Novak et al., 2009; Van Zwieten et al., 2010b; Liu et al., 2012), and may be a key factor in explaining variation in soil N_2O emissions with biochar addition (Stewart et al., 2013). Biochar amendment significantly increased soil pH levels in both experiments reported here, by approximately 1 pH unit in the soil cores and by 0.3 units in the ^{15}N tracer experiment (Fig. 4.3, Table 4.4).

An increase in soil pH may affect denitrification rates. However, during the investigation into soil N transformations, overall denitrification rates (the total of NO_3^- conversion to N_2O, NO or N_2) were not affected by biochar amendment (Fig. 4.7). Since we expected diffusion of the intermediate denitrification product, NO, to

be negligible, we concluded that increased soil pH with biochar amendment contributed to an increased conversion of N_2O to N_2 via denitrification and therefore decreased the N_2O: N_2 ratio from denitrification.

Below pH 6, the conversion of N_2O to N_2 from denitrifiers decreases, as bacterial N_2O reductase (Nos) enzymes are sensitive to low pH (Baggs et al., 2010). As pH increases above this level, the relative production of N_2O compared to N_2 from denitrification may decrease, although it is not known if this is primarily due to the post-transcriptional sensitivity of bacterial Nos enzymes at low pH (Liu et al., 2010), the lower activity of fungi at higher pH that lack Nos enzymes (Saggar et al., 2012), or the soil pH being sufficiently high to remove the interference of low pH on enzyme production, as hypothesised by Bakken et al., (2012). As soil pH only increased by 0.3 units during the ^{15}N tracer experiment, we did not expect it to explain the total 95% decrease in soil N_2O emissions with biochar amendment. To confirm this finding, further experiments are needed using ^{15}N tracers optimised to directly analyse N_2 enrichment in the soil core headspace and also assess the enzyme activity of denitrifying enzymes (e.g. Nos).

Overall, cumulative nitrification was not affected by biochar addition (Fig. 4.7). The proportion of N_2O produced via nitrification (N_2O: (NO_2^- + NO_3^-) was lower in amended soils than in un-amended soils. Increasing soil pH up to 5 has been demonstrated to decrease the ratio of N_2O: (NO_2^- + NO_3^-) production during nitrification, but this has not been shown at higher pH (Mørkved et al., 2007). Therefore, the greater pH with biochar-amendment in this study does not adequately explain lower N_2O emissions from nitrification in amended soil. The reason for these lower emissions from nitrification are unclear, however, nitrification contributed little to overall N_2O emissions compared to denitrification and so only explains a relatively small proportion of the suppression of soil N_2O emissions with biochar amendment.

Some studies have suggested that adding labile C to the soil may affect the production of soil N_2O emissions and the conversion of N_2O to N_2 via

denitrification. The addition of fresh biochar added a significant amount of labile C to soil, which was indicated by greater soil CO_2 emissions following AN fertiliser and water addition in amended compared to un-amended soils (Table 7.3, Appendix 7.3). The effect of increased C availability on soil N_2O emissions from denitrification is poorly understood (Morley & Baggs, 2010). Generally, an increase in the availability of labile C in soil increases the denitrification rate, but may also increase the conversion of N_2O to N_2 via denitrification (and resulting N_2O: N_2 product ratio) as C can be limiting for the final N_2O reduction process (Azam et al., 2002; Morley & Baggs, 2010; Saggar et al., 2012; Senbayram et al., 2012).

Two studies have been published that found similar results to this current study following the addition of both C and N simultaneously to soil and. Vallejo et al., (2006) found that cumulative soil N_2O emissions were decreased following the addition of pig slurry (C + N) compared to urea (N) alone. They proposed that the addition of materials with a high amount of organic C increased soil respiration and therefore provided the anaerobic conditions under which denitrification would occur, therefore increasing the conversion of N_2O to N_2 via denitrification and therefore the N_2O: N_2 product ratio from denitrification. Dittert et al., (2005) found that soil N_2O emissions were reduced with slurry addition compared to mineral-N addition and additionally that the N_2O : N_2 denitrification product ratio was lower following slurry addition. We conclude that the presence of additional labile C in biochar-amended soil resulted in increased short-term C mineralisation following wetting and/or AN fertiliser addition. This decreased soil aeration, decreasing the N_2O: N_2 product ratio from denitrification. We consider that in our study, a combination of the greater soil pH and greater soil labile C content following biochar amendment increased the conversion of N_2O to N_2 during denitrification, and decreased the overall N_2O: N_2 product ratio from denitrification.

Other studies have suggested that inhibitive substances on or within the biochar may explain the suppression of soil N_2O emissions following biochar amendment. Spokas (2012) suggested that soil N_2O suppression may be due to the presence of

nitrification/denitrification inhibitors on the biochar surface. Other authors have posited that other substances, such as α-pinene, PAHs, VOCs and ethylene had significant suppressive effects on microbial activity, therefore reducing soil N_2O emissions (Clough et al., 2010; Spokas et al., 2010, 2011; Taghizadeh-Toosi et al., 2011a; Quilliam et al., 2012). However, in this study, cumulative mineralisation, nitrification, immobilisation, denitrification and soil CO_2 emissions were not inhibited following biochar amendment, so we have no evidence to support the hypothesis that microbial activity was suppressed by any particular inhibitive substance.

We can draw some general conclusions regarding the mechanisms underlying the effect of biochar addition on soil N_2O emissions under differing moisture and N conditions. In un-fertilised arable soils of low moisture content, we expect that soil N_2O emissions will be suppressed by biochar amendment due to increased soil aeration and BII. In un-fertilised arable soils saturated following rainfall events, we expect that BII, labile C mineralisation and increased soil pH will all contribute to lower soil N_2O emissions. In AN fertilised, saturated arable soils we conclude that increased soil pH and labile C mineralisation will lead to suppressed soil N_2O emissions.

If increased soil pH with biochar amendment is partially responsible for the suppression of soil N_2O emissions, this suggests that a similar suppression of soil N_2O emissions following the addition of AN fertiliser could be achieved simply by liming soil. Several studies have observed increased soil N_2 emissions and reduced soil N_2O emissions following liming of the soil under both wetted and un-wetted conditions (Brumme & Beese, 1992; Klemedtsson et al., 1997; Stevens et al., 1998; Clough et al., 2003, 2004; Baggs et al., 2010). Alternatively, the addition of C-based residues in combination with N fertiliser could achieve reduction of soil N_2O emissions compared to addition of N fertiliser alone, especially if high C: N ratio materials are added (Shan & Yan, 2013). However, the decision on whether to lime soil, amend soil with C-based compounds or to amend soil with biochar needs to be

viewed in the context of other potential benefits of biochar amendment, such as increases in crop yield N-use efficiency, or C sequestration, and comparisons of costs such as those of transportation and application (Hammond et al., 2011; Jeffery et al., 2011). Also, the environmental sustainability of liming and agricultural residue addition compared with biochar amendment in terms of other GHG emissions such as CO_2 should be considered (Page et al., 2009). Future studies should be conducted comparing the effect of biochar amendment, the addition of C-based materials and liming treatments on soil N_2O emissions in comparison with the full effects of each option on soil properties and crop productivity.

Between Day 4 and 6, the $^{15}N_2O$ atom % excess was higher than that of the $^{15}NH_4^+$ or $^{15}NO_3^-$ atom excess (Fig. 4.4, Fig. 4.5, Fig. 4.6); for example close to 10% in the un-amended soil between day 4 and 6 compared to inorganic-N atom % excess values of close to 3%. This suggests that N_2O emissions were coming predominantly from the added ^{15}N compounds instead of the resident inorganic-N. We have no explanation for this, which requires further research to establish if it is a frequently-occurring phenomenon.

The days following rainfall or fertiliser addition are the most significant for annual soil N_2O emissions from agricultural soils. We have shown that under field conditions, fresh biochar addition to arable soil can significantly suppress N_2O emissions under un-wetted and wetted conditions within four months of biochar amendment and in the days immediately following AN fertiliser addition. These results are significant as they support the concept that biochar application to soil could significantly contribute to global efforts to mitigate climate change.

4.6 Acknowledgements

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5 Discussion and conclusions

This study investigated the effects of hardwood biochar amendment on soil greenhouse gas (GHG) emissions in two agricultural crops (bioenergy and arable) and the interactive changes in underlying soil physico-chemical properties. The research had two primary aims. The first was to investigate the effects of biochar amendment on soil GHGs (carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4)) under natural environmental conditions from a commercial bioenergy crop (*Miscanthus X Giganteus*) and arable field. The second aim was to investigate the mechanisms underlying any observed effects of biochar on soil GHG emissions.

Following several laboratory incubations and one field incubation to address these aims, the main results and conclusions of this study were: 1. that soil carbon dioxide (CO_2) emissions were suppressed by 33% for two years following biochar amendment in a *Miscanthus* field, 2. soil nitrous oxide (N_2O) emissions were suppressed by 49% following wetting events (Chapter 2, 4), 3. soil N_2O emissions were suppressed by at least 84% in a recently nitrogen (N)-fertilised arable soil, in both field moist (un-wetted) and wetted conditions (Chapter 4) and 4. that four mechanisms:- increased soil aeration; biochar-induced immobilisation of inorganic N (BII); increased soil pH; and increased soil labile C content together explain the suppression of soil N_2O emissions depending on wetting and N-fertilisation conditions (Chapter 4). A summary table for all of the GHG emissions results from the incubations carried out throughout this thesis are presented in the Appendix (Table 7.4, Table 7.5, Table 7.6).

A brief summary of the main results are presented here and discussed in the context of published literature. The implications of the suppression of soil GHG emissions with biochar amendment are discussed in terms of total soil CO_2 equivalent (soil $CO_{2eq.}$) emissions for large-scale applications of biochar worldwide. In addition the limitations of the experimental approaches used are discussed and areas for further research are suggested.

5.1 Biochar amendment and soil carbon dioxide (CO_2) emissions from a bioenergy crop soil: effects and global significance

Soil CO_2 emissions were suppressed by 33% with biochar amendment over two years in a bioenergy crop field and by 53% over several months in un-wetted (field-moist) soils under controlled environment conditions (Chapter 4). The effects of biochar amendment on soil CO_2 emissions from bioenergy soils have not previously been investigated.

The experimental chapters hypothesised that there were several mechanisms underlying the effects of biochar amendment on soil CO_2 emissions, summarised in Table 5.1. A review of published literature suggested that four mechanisms could explain the suppression of soil CO_2 emissions in field-moist *Miscanthus* soils (described below and in Table 5.1). In Chapter 2 and 4, soil CO_2 emissions were shown to increase following biochar addition and wetting; this was attributed to the increased availability of labile carbon (C) from biochar following water addition (Table 5.1).

Table 5.1. The primary mechanisms influencing soil CO_2 emissions following fresh biochar amendment, assuming that the biochar is added at a rate of at least 2% w/w and that the soil is maintained at 23% gravimetric moisture content (GMC) (un-wetted), wetted to 28% GMC or 'Equalised', where un-amended and amended soil was wetted to 90% of water-filled pore space (WFPS). Minus signs (-) indicate that the mechanism reduces soil CO_2 emissions. Plus signs (+) show that the mechanism increases soil CO_2 emissions. 'Direct biochar effect' is a combination of the following mechanisms: Increased C-use efficiency, co-location of soil organic matter (SOM), nutrients and soil microbes on the biochar surface, reductions in the activity of soil C-mineralising enzymes and adsorption of CO_2 to the biochar surface. Data taken from Table 7.4, Table 7.5 and Table 7.6 in the Appendix, Section 7.4).

Land use	Field or laboratory incubation	Inorganic-N concentration (mg N kg^{-1})	Wetting condition	% change with biochar	Direct biochar effect	Increased availability of labile C
Miscanthus	Field	Low (< 20)	Un-wetted	- 33	-	
Miscanthus	Laboratory	Low (< 20)	Wetted	+ 26	-	++
Arable	Laboratory	Med (~ 60)	Un-wetted	0	-	
Arable	Laboratory	Med (~ 60)	Wetted	+ 26	-	++
Arable	Laboratory	High (~ 100)	Wetted, equalised	+ 61	-	+++

Studies have observed both suppression and priming of soil CO_2 emissions following biochar addition to soil (Wardle et al., 1998; Lehmann et al., 2011). Soil CO_2 emissions were hypothesised to increase shortly after biochar due to its labile C content (Zimmerman et al., 2011). However, in this study, soil CO_2 emissions only increased following wetting events in the laboratory, possibly due to increasing labile C availability in more saturated biochar-amended soils.

Extreme soil inorganic-N limitation may also limit soil CO_2 emissions (Henriksen & Breland, 1999). However, as reported in Chapter 3, extractable soil inorganic-N concentrations were not lower with biochar addition in the laboratory or the field. Therefore it was concluded that biochar-induced immobilisation of inorganic-N (BII) did not limit soil CO_2 emissions in soils of low inorganic-N content.

Based on a review of the biochar literature, we hypothesised that there are a number of other mechanisms responsible for the reduction of soil CO_2 emissions with biochar amendment in the field: increased C-use efficiency, the co-location of soil organic matter (SOM), nutrients and soil microbes on the biochar surface, reductions in the activity of soil C-mineralising enzymes and adsorption of CO_2 to the biochar surface (discussed in full in Chapter 3). These mechanisms are

henceforth referred to as the 'direct biochar effect'. All of these mechanisms were not directly observed, but instead hypothesised by a review of biochar studies that observed soil CO_2 emissions (Lehmann et al., 2011), with the exception of reductions in the activity of soil C-mineralising enzymes, which has been directly observed in one unpublished study (Jin, 2010). However, this was not confirmed in a subsequent published study by Bailey et al., (2011), who found highly variable effects of biochar amendment on soil C-mineralising enzymes.

In conclusion, the proposed mechanisms for suppression of CO_2 emissions by biochar (Table 5.1) are not backed up by experimental evidence in this study or in the published literature. Therefore, although we have proven that biochar amendment suppresses soil CO_2 emissions for up to two years, we can only speculate on the mechanisms involved. Despite this lack of evidence to explain the mechanisms, the reduction in soil CO_2 emissions in *Miscanthus* plantations following biochar addition may have significant implications for climate change mitigations (see Section 5.1.1).

5.1.1 Bioenergy, biochar and carbon abatement

There are worldwide efforts to sustainably scale-up bioenergy production in order to substitute for energy derived from fossil fuels (Whitaker et al., 2010). The EU has a target for 20% of all energy to come from renewable sources by 2020 (The European Commission, 2009). There is a second target to produce 10% of all vehicle fuel in Europe from biomass sources by 2020. This fuel must be from sources that release at least 35% less total $CO_{2eq.}$ emissions than fossil fuel sources over the entire production life cycle (The European Commission, 2009). In 2012, this second target was modified to ensure that not more than half the 10% target could come from 'food-crop derived' biofuels (first-generation biofuels) and that by 2018 the life cycle total $CO_{2eq.}$ reduction from biofuels compared to fossil fuel energy production should be 50% (The European Commission, 2012).

Energy from biomass sources currently contributes around two-thirds of the total from renewable energy generation (which itself consisted of 9% of European energy generation in 2010) and is predicted to greatly increase (Don et al., 2012). Generally, second-generation bioenergy crops such as *Miscanthus X Giganteus* are considered to be C neutral or negative if planted on set aside or arable land as opposed to converted forests/grassland. However, there are uncertainties around this point depending on crop type, original land use and land management practice (Mathews, 2009; Rowe et al., 2009, 2011; Cherubini et al., 2009).

The long-term C balance of bioenergy crops is controlled by changes in soil and biomass C (Don et al., 2012). Over the entire life cycle of the crop production, *Miscanthus* has been predicted to sequester 0.68 t C ha^{-1} yr^{-1} as 'additional soil organic C' and up to 0.46 t C ha^{-1} yr^{-1} as 'additional belowground biomass C' if planted on arable land (a total of 1.15 t C ha^{-1} yr^{-1}) (Don et al., 2012). We demonstrated that adding biochar to *Miscanthus* soils could significantly decrease soil CO_2 emissions by 33% (Chapter 3), equivalent to a further 1.25 CO_2-C t ha^{-1} yr^{-1} compared to un-amended soil at the *Miscanthus* field site. Therefore, biochar addition could potentially double the effective increase in soil C stocks in the arable soils planted with *Miscanthus* if the observed reduction of soil CO_2 emissions was to continue into the long term.

If the additional C storage from our one-off application of biochar to soil were to be added to this total (estimated to be 37.6 t C ha^{-1}, based on 49 t ha^{-1} biochar addition of 76.6% C content), then C storage within the soil would be improved further. Note that the magnitude of this biochar-C storage component depends on the long-term stability of the biochar and the frequency of its addition to soil.

Future studies, should take into account that the potential of biochar amendment to reduce the total $CO_{2eq.}$ emissions from the bioenergy life cycle may be further increased by producing biochar concurrently with electricity and biofuel during pyrolysis. However, there is a trade-off between the amount of biochar produced and the amount of biogas and bio-oil products. The re-application of such 'dual

purpose' biochar to bioenergy plantation soil creates a circular production process that limits the need for external energy inputs (Laird et al., 2009; Sohi et al., 2010). The production of biochar concurrently with these other useful products may also improve the economic viability of biochar production as opposed to the production of biochar alone (Roberts et al., 2010; Shackley et al., 2011).

Overall, we suggest that biochar amendment could significantly increase the soil C storage of *Miscanthus* plantations firstly by the direct addition of recalcitrant biochar C to the soil and secondly through the long-term reduction of soil CO_2 emissions and subsequent increase in SOC, provided that the biomass yield and calorific value of the crop are maintained. This benefit could be compounded by producing biochar by advanced production processes that utilises the biogas and bio-oil by-products from pyrolysis.

5.2 The effect of biochar on soil nitrous oxide (N_2O) emissions: underlying mechanisms and global significance

Research presented in this study has demonstrated that soil N_2O emissions are suppressed by biochar amendment under controlled environmental conditions. Several experiments were conducted to identify the underlying mechanisms. Prior to this study, research into the mechanisms underlying the suppression of soil N_2O emissions following biochar amendment was limited (see Clough and Condron, (2010) and Spokas et al., (2012b) for a summary of the work conducted so far). This current work is the first to bring some clarity to the mechanisms, particularly in terms of the soil N cycle. Chapter 4 demonstrated that biochar addition suppressed soil N_2O emissions without suppressing nitrification and denitrification rates in the soil. These findings contradict with some studies in the published literature, which hypothesised that substances on or within the biochar, such as volatile organic compounds (VOCs) or ethylene, may inhibit nitrifier or denitrifier activity (Spokas et al., 2010; Taghizadeh-Toosi et al., 2011a).

Chapter 2 and 4 demonstrated that soil N_2O emissions were suppressed in a *Miscanthus* and arable soil following wetting and in an arable soil when field moist. We hypothesised that four mechanisms were involved in the suppression; increased soil aeration, BII, increased soil pH and increased soil labile C content. All these factors may have an influence on soil N_2O emissions depending on the inorganic-N content and soil wetting status (Table 5.2), discussed in turn below. This study is the first to demonstrate that several mechanisms may be acting simultaneously to suppress soil N_2O emissions.

Table 5.2. The primary mechanisms influencing the suppression of soil N_2O emissions following fresh biochar amendment assuming that the biochar is added at a rate of at least 2% w/w and that the soil is maintained at 23% GMC (un-wetted), wetted to 28% GMC or 'Equalised', where un-amended and amended soil was wetted to 90% of water-filled pore space (WFPS). 'Increased N immob.' represents biochar induced immobilisation of inorganic-N. Minus signs (-) indicate that the mechanism reduces soil N_2O emissions. Data taken from Table 7.4, Table 7.5 and Table 7.6 in the Appendix, Section 7.4).

Land use	Inorganic-N conc. (mg N kg^{-1})	Wetting condition	% change with biochar	Soil aeration increase	Increased N immob.	Soil pH increase	Labile C increase
Miscanthus	Low (< 20)	Un-wetted	- 14	-	-		
Miscanthus	Low (< 20)	Wetted	- 84	-	-	-	-
Arable	Med (~ 60)	Un-wetted	- 83	-			
Arable	Med (~ 60)	Wetted	- 88	-		-	-
Arable	High (~ 100)	Wetted, equalised	- 95			-	-

Increased soil aeration partially explained lower soil N_2O emissions in the soils subjected to wetting/drying cycles, because water was not added to compensate for the increased water holding capacity (WHC) of biochar (Chapter 2, 4). We suggested that under conditions where the soil was un-wetted (field moist), increased soil aeration with biochar suppressed nitrifier activity; when soil was wetted, biochar-related aeration suppressed denitrifier activity.

In low or moderate N-content soils (Table 5.2), BII limited the N substrate available to nitrifiers or denitrifiers (Chapter 2, 4). Immobilisation of soil inorganic-N, especially ammonium (NH_4^+), has been demonstrated in several studies following biochar addition (Ding et al., 2010; Taghizadeh-Toosi et al., 2011b; Bruun et al., 2012;

Hollister et al., 2013). This is consistent with other studies that found lower soil N_2O emissions concurrent with lower soil inorganic-N concentrations with biochar addition (Van Zwieten et al., 2010b; Bruun et al., 2011b).

Soil N_2O emissions in N-fertilised, saturated soil were suppressed by 95% with biochar amendment, which was not explained by increased soil aeration or BII (Table 5.2, Chapter 4). We hypothesised that two other mechanisms could explain the suppression in such soils. Increased production of atmospheric nitrogen (N_2) relative to N_2O from denitrification may have occurred due to increased pH or increased labile C content following biochar addition. The investigation into soil N transformations in Chapter 4 did not prove this directly as the incubation was unable to quantify N_2 emissions from biochar-amended soil, however based on our evidence this appears to be the most likely mechanism to explain the suppression of soil N_2O emissions under these conditions (discussed in Chapter 4). Increases in soil pH (Van Zwieten et al., 2010b) and increases in labile C content with biochar amendment (Bruun et al., 2011b) have both been proposed as potential mechanisms to explain a reduction in soil N_2O emissions. Both these mechanisms only act to suppress soil N_2O emissions in wetted soils, because they both affect the product ratio from denitrification, which only occurs to a significant degree in soils of a high water-filled pore space (WFPS) (Bateman & Baggs, 2005).

Soil GHG emissions were not analysed beyond four months after biochar addition in laboratory incubations. The effects of biochar amendment on soil N_2O emissions after this time can therefore not be confirmed with certainty. With time (within three years), the pH of the biochar reduces due to oxidation reactions on the biochar surface and labile C on the biochar surface is mineralised (Spokas, 2012; Jones et al., 2012). Also, it has been hypothesised that biochar pores become clogged with time, thus limiting its adsorption capacity (Van Zwieten et al., 2010a). However, if fresh biochar is added regularly (e.g. annually) to soil (Schmidt, 2012), we suggest that it is possible that biochar amendment would have a long-term effect on suppressing soil N_2O emissions from arable soils.

5.2.1 Biochar amendment to decrease the N_2O emission factor and increase N-use efficiency of added fertiliser

Since 1960, the growth of N_2O emissions from agriculture has been derived almost exclusively from increased use of organic or inorganic N fertiliser (Davidson, 2009). The International Panel on Climate Change (IPCC) uses the term 'N_2O emission factor', or the % of added N emitted as N_2O to quantify the amount of N_2O derived from the application of fertiliser (De Klein et al., 2007). The IPCC cited a default N_2O emission factor of 1% of applied N fertiliser, agricultural residues or organic amendments applied to croplands, which was used in emissions inventories (EF1) (De Klein et al., 2007). However, recent research has suggested that this emission factor may be an underestimate, with an N_2O emission factor of 3 to 5% predicted for the fixed N application to agro biofuel production (Crutzen et al., 2007), 2.5% following inorganic N-fertiliser application to agricultural soil (Davidson, 2009), or 2.5% for NO_3^--based fertiliser compared to 0.7% for NH_4^+-based fertiliser (including urea) (Lesschen et al., 2011). Despite these differing emission factors, there is still general agreement with the IPCC in terms of overall soil N_2O emissions on a global scale (Reay et al., 2012). Therefore, there is great interest in reducing N_2O emissions from applied N-based fertiliser (as represented by the N_2O emission factor) in order to limit the climate change impacts of agriculture (Erisman et al., 2008).

The direct N_2O emission factor calculations are based on the annual emissions of N_2O from added N fertiliser (De Klein et al., 2007). However, many studies assumed that it was appropriate to extrapolate the results seen in shorter durations to annual emissions, which we have assumed is appropriate when applying this methodology to our study (Table 5.3). Chapter 4 demonstrated that soil N_2O emissions were suppressed by 95% following N-based fertiliser addition. This was equivalent to 0.80% of the added N-fertiliser in un-amended soil compared to 0.05% in amended soil within 6 days of N addition. This laboratory incubation (Chapter 4) only lasted for six days, therefore a complete quantification of the direct N_2O emission factor from the added AN fertiliser was not possible as N_2O emissions following biochar

amendment generally continue for longer than this (Van Zwieten et al., 2010b; Taghizadeh-Toosi et al., 2011a). However, four month-long laboratory incubations were conducted using soil that had been N-fertilised approximately one month prior to the start of the experiment (Chapter 4). Soil N_2O emissions were consistently suppressed by 84% for the duration of these incubations, a similar magnitude to that of our six day-long incubation (Table 5.2). Based on these findings we suggest that a reduction of the direct soil N_2O emission factor continues for at least 120 days. The findings from these two experiments suggest that biochar amendment has the potential to significantly reduce the direct N_2O emission factor from applied ammonium nitrate (AN) fertiliser. However, as the amount of added N fertiliser remaining in the soil at the start of the 120-day laboratory incubation was not analysed, this reduction of the N_2O emission factor could not be quantified precisely.

Our findings of a lower N_2O emission factor with biochar amendment are in general agreement with publications where N-based fertiliser was added to the soil in conjunction with biochar. However, the N_2O emissions factor from added AN fertiliser following biochar addition has not been previously studied; other studies instead added urea to the soil (Clough et al., 2010; Van Zwieten et al., 2010b; Zhang et al., 2010; Taghizadeh-Toosi et al., 2011a). Generally, the N_2O emissions factor following urea application is reduced following biochar addition (Table 5.3).

Table 5.3. Nitrous oxide (N_2O) emission factors derived from this and other studies in the biochar literature. AN = ammonium nitrate. 'Lab' = laboratory.

Study reference	Field or lab	Study length (days)	N form	N addition rate (mg N kg^{-1})	N_2O emission factor un-amended (%)	N_2O emission factor amended (%)	Significantly different?
This study, (Chapter 4)	Lab	6	AN	100	0.8	0.05	Yes
Clough et al., (2010)	Field	55	Urea	760	29	17	No
Tahidizadeh-Toozi et al., (2011a)	Field	86	Urea	960	0.12	0.04	Yes
Zhang et al., (2010)	Field	123	Urea	300	0.4	0.1	Yes
Van Zwieten et al., (2010b)	Lab	47	Urea	165	15.1	2.2	Yes

We conclude that greatly-expanded use of biochar amendment could have an impact on soil N_2O emissions from N fertiliser globally. The N_2O emission factors used within future IPCC reports may need to be reconsidered if biochar amendment is widely adopted as a standard agricultural practice. Studies involving much longer incubation time periods (at least 3-5 years) and at the field scale are needed to verify these findings.

Nitrogen-based fertiliser use efficiency was approximately 30% globally in arable soils in 2000 (Erisman et al., 2008). There is a great need to increase N-use efficiency for agriculture to keep up with the long-term increase in N-based fertiliser use from global agriculture due to population growth and demand for meat (van Beek et al., 2010; Popp et al., 2010). One way to effectively do this is by reducing the combined emissions of NO, N_2O NH_3 or N_2 gas from N-amended soils. Chapter 4 showed that soil N_2O emissions were suppressed with biochar amendment. However, as we did not quantify soil NO, NH_3 or N_2 emissions, conclusions cannot be drawn about biochar amendment and its implications for the N-use efficiency of agriculture.

Another important component of the N-use efficiency of added N-based fertiliser is the movement of N through soil via runoff or leaching (Raun & Johnson, 1999; Cassman et al., 2002; Fageria & Baligar, 2005). Several published studies have

reported immobilisation of inorganic N (Van Zwieten et al., 2010b; Ding et al., 2010; Taghizadeh-Toosi et al., 2011b; Spokas, 2012; Hollister et al., 2013). By increasing the retention of inorganic-N in the soil, biochar amendment could significantly reduce run off and leaching. Two laboratory incubations demonstrated that biochar may increase the retention of inorganic-N within the soil through BII (Chapter 2, 4). However, evidence of inorganic N immobilisation was not consistent throughout the incubations (Chapter 3); therefore this study does not prove that biochar amendment consistently immobilises of inorganic-N. We cannot conclude whether biochar amendment reduces runoff or leaching or inorganic-N.

Based on our findings, we conclude that there is the potential for biochar addition to reduce the direct N_2O emission factor from agricultural soils amended with AN fertiliser; however this needs to be confirmed with longer field applications of inorganic N and biochar. Our findings did not confirm whether biochar amendment affected N-use efficiency. Further research is needed to analyse a wider range of N-based gaseous emissions from soils (NH_3, NO, N_2O and N_2) to examine whether biochar amendment can consistently immobilise inorganic-N compounds (NH_4^+, NO_3^-), while ensuring that they are still available to plants.

5.3 Comparison of field and laboratory results

Several laboratory incubations were conducted as a part of this research. The main objective of the incubations was to investigate specific mechanisms and changes in physico-chemical properties underlying soil GHG emissions with and without biochar. Previous sections extrapolated some of our laboratory results to the field scale to provide some wider context to our findings. This section discusses the validity of this approach.

Extrapolation to the field from laboratory incubations is confounded by the fact that the soil cores in the laboratory were maintained at a constant 16°C, whilst field conditions were naturally variable. Comparing our laboratory and field incubations to the summer only where temperatures were similar, soil CO_2 emissions were

lower in the laboratory than in the field, (Table 7.6, Appendix, Section 7.4). Differences, in assumed order of importance, may occur due to soil mixing during soil core preparation (Reicosky et al., 1997; Reicosky, 1997), the lack of root input or nutrient deposition (Reichstein & Janssens, 2009) or the failure to sample during the times of increased soil GHG emissions following soil wetting (Table 5.4). The lack of root input is likely to be a significant factor as it is responsible for approximately half of soil CO_2 emissions in the field during the summer months (McNamara, pers. comm.).

Table 5.4. The mechanisms that can explain lower net soil $CO_{2eq.}$ emissions between our field and laboratory incubations.

Incubation	Mechanism
Field incubation	Sampling too infrequent to catch 'bursts' of soil GHG emissions following wetting The first gas analyses occurred at a different time following biochar addition in the field (three weeks) compared to the laboratory incubations (generally two weeks)
Laboratory incubation undergoing wetting/drying cycles	1. No root input or nutrient deposition 2. Soil mixing depletes soil of labile C and/or nutrients 3. Soil $CO_{2eq.}$ calculated from unwetted soil – sampling did not include 'bursts' of soil GHG emissions following wetting

Despite soil CO_2 emissions being lower in the laboratory incubation than in the field, the effects of biochar amendment on soil CO_2 emissions were similar in both the field and laboratory incubations (Table 7.6, Appendix, Section 7.4). Therefore, we conclude that the relative effects of biochar amendment on soil GHG emissions can be extrapolated from the laboratory incubations to the field scale.

5.4 Overall climate impact of biochar on agricultural systems

The previous sections highlighted that the suppression of soil CO_2 and N_2O emissions with biochar amendment may reduce the life cycle $CO_{2eq.}$ emissions from bioenergy systems and the N_2O emission factor from arable soils amended with AN fertiliser. They also discussed whether the results from the laboratory incubations could be extrapolated to the field scale. Based on the discussion, this section now

questions the assumptions of the long-term total $CO_{2eq.}$ mitigation potential of biochar amendment globally, the only large-scale estimate of which was made by Woolf et al., (2010).

In considering the potential of biochar amendment to mitigate GHG emissions on a global scale, Woolf et al., (2010) predicted that up to 1.8 Pg CO_2-$C_{eq.}$ yr^{-1}, or 12% of anthropogenic GHG emissions (130 Pg CO_2-$C_{eq.}$ by 2100), could be abated by sustainable bioenergy + biochar production and subsequent biochar amendment globally. The predicted magnitude of this mitigation is based on the 'Maximum Sustainable Technological Potential' of biochar that assumed that biochar feedstock was not derived from the conversion of natural or productive agricultural land to biomass production and that the maximum possible feedstock was collected from a number of sources without endangering habitats, soil conservation or food security (Woolf et al., 2010).

The intention of this discussion is not to question the viability of the sources of large-scale biochar production proposed by Woolf et al., (2010), but instead to examine the assumptions underlying the total $CO_{2eq.}$ mitigation potential calculated by their model. The authors made a number of assumptions concerning the suppression of soil N_2O emissions, increased CO_2 emissions due to the diversion of agricultural residue to biochar production and soil CH_4 uptake, which this section now considers in turn in light of our findings.

The authors assumed that soil N_2O emissions would be reduced by 25% (Woolf et al., 2010). The laboratory incubations in Chapter 4 demonstrated soil N_2O emission reductions of 85 to 95% in arable soil, and 50% following wetting in a *Miscanthus* soil in Chapter 2. The evidence from this study suggests that the suppression of N_2O emissions suggested in Woolf et al., (2010) could be an under-estimate in certain circumstances. However, this was not proven in the long term and other publications within the biochar literature where soil N_2O emissions have not been reduced following biochar amendment. Therefore the effect of biochar amendment on soil N_2O emissions need to be analysed within many more land uses over the

long term in order to confirm that soil N_2O emissions are significantly suppressed by more than 25%.

Woolf et al., (2010) suggested that biochar addition increases soil CO_2 emissions within the bioenergy life cycle, which they attribute to two sources. Biochar decomposition may increase cumulative CO_2-$C_{eq.}$ emissions from biochar-amended soils by up to 17 Pg CO_2-$C_{eq.}$ (by 2100) and also SOC loss from the diversion of agricultural residue biomass to biochar production may increase cumulative CO_2-$C_{eq.}$ emissions by up to 10 Pg in the same time period. We could not determine the proportion of CO_2 emissions from biochar or native soil C sources from the system as we did not use ^{13}C stable isotope studies to quantify either of these processes. However, the possibility of medium-term suppression of soil CO_2 emissions with biochar amendment, as observed in the *Miscanthus* field (Chapter 3), was not taken into account by Woolf et al., (2010). The suppression of soil CO_2 emissions in the field may counter the emissions from biochar decomposition and the loss of C due to the diversion of agricultural residue biomass to biochar production assumed by Woolf et al., (2010). Future life cycle analyses of biochar and bioenergy production need to include the possibility for increased SOC accumulation in biochar-amended bioenergy croplands.

Finally, Woolf et al., (2010) assumed that CH_4 emissions from soil following biochar amendment were reduced overall by 100 mg CH_4 m^{-2} yr^{-1}. However, the contribution of increased soil CH_4 oxidation to overall total soil $CO_{2eq.}$ emissions by 2100 was negligible for all scenarios. In this present study, there was no increase in CH_4 oxidation or emissions with biochar amendment because CH_4 flux was minimal in all of the incubations. Therefore, the assumption that Woolf et al., (2010) made regarding soil CH_4 fluxes may also be incorrect in some soils. Again, studies to analyse the microbial activity underlying methanogenesis and methanotrophy would need to be analysed in-depth within bioenergy soils in order to draw confident conclusions. However, soil CH_4 uptake was a very minor component of

the overall total $CO_{2eq.}$ emissions from bioenergy + biochar systems (Woolf et al., 2010).

In conclusion, assumptions regarding the suppression of soil N_2O emissions and the effect of biochar on increasing SOC stocks need to be reconsidered in future bioenergy + biochar production models similar to the study conducted by Woolf et al., (2010). Changes in these assumptions may increase the climate change mitigation of the bioenergy + biochar life cycle and the long-term potential for climate change mitigation of biochar amendment on a global scale.

5.5 Future research needs

The previous sections concluded that biochar addition may reduce the climate change impact of agriculture in both perennial bioenergy crop soils and arable soils. Our findings suggested that Woolf et al., (2010) may have underestimated the potential of biochar amendment to suppress soil N_2O and CO_2 emissions. Global-scale biochar life cycle analyses such as that used in Woolf et al., (2010) should be reconsidered to take these effects into account. However, further research is required to confirm these results in a variety of soils using a variety of biochar types. Longer-term experiments need to be installed in order to monitor the effect of biochar on soil GHG emissions as it ages (i.e. over 3 to 5 years), with frequent analyses to capture bursts of GHG emissions following rainfall or N-fertilisation events, taking measurements from the day of biochar application onwards. Until the data from these studies are available, laboratory-based biochar ageing experiments could be used to investigate these effects.

More research is needed to investigate the effect of biochar amendment on the soil C and N cycle and other mechanisms underlying the suppression of soil N_2O and CO_2 emissions. Studies on N cycling should focus specifically on testing whether the increase in soil pH or increase in labile C availability in biochar-amended soils leads to an increased reduction of N_2O to N_2. Studies on the soil C cycle should focus on soil C-mineralising enzymatic activity with biochar amendment, the rate of

mineralisation of added biochar and native SOM and the effect of biochar amendment on crop yield and the calorific value of the crop. These studies should be conducted using a combination of ^{13}C or ^{15}N stable isotope studies with molecular techniques.

Future studies should investigate whether biochar amendment can affect the N-use efficiency of agriculture. Research should focus on the whether biochar amendment consistently immobilises inorganic-N and whether it is plant available. Additionally, future studies should analyse all of the N-based gaseous products following N-based fertiliser and biochar addition to soil, such as NH_3, NO, N_2O and N_2 under a range of environmental conditions – e.g. different soil types, N input rates, N application timings and repeated biochar applications.

The choice of feedstock, production method and application method can have a large effect on the sustainability of biochar amendment to soil. Future research should ensure that the biochar production and application methods used are sustainable in a social, environmental and economic context.

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7 Appendix

7.1 Chapter 2 supplementary information – The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil – the role of soil aeration

Table 7.1 Physical and chemical properties of the biochar used during the course of this study. Data represent mean (n), or mean ± standard error (n).

Property	Units	Value
Feedstock		Hardwood charcoal (oak, cherry, ash thinnings)
Production conditions		To 180°C to release volatile gases, then 400°C over 24 hours
Particle size	mm	< 2
Bulk density	g cm^{-3}	0.24 (1)
LOI	%	78.3 (1)
C	g kg^{-1}	723 ± 1.5 (3)
N	g kg^{-1}	7.1 ± 0.01 (3)
H	g kg^{-1}	21.6 (1)
S	g kg^{-1}	undetectable
C/N		85.8
H/C		0.03
WHC	%	146 ± 4 (4)
Extractable NH_4^+	mg kg^{-1}	< 1 (3)
Extractable NO_3^-	mg kg^{-1}	< 1.3 (3)
pH (1: 2.5 H_2O)		9.3 ± 0.1 (3)
CEC	$cmol_+$ kg^{-1}	144.9 (1)
K (exchangeable)	$cmol_+$ kg^{-1}	78.2 (1)
Ca (exchangeable)	$cmol_+$ kg^{-1}	79.4 (1)
Mg (exchangeable)	$cmol_+$ kg^{-1}	35.3 (1)
Na (exchangeable)	$cmol_+$ kg^{-1}	6.0 (1)
P	mg kg^{-1}	1,263 (1)
K	mg kg^{-1}	13,780 (1)
Al	mg kg^{-1}	912 (1)
As	mg kg^{-1}	< 4.1 (1)
Cd	mg kg^{-1}	< 0.8 (1)
Cr	mg kg^{-1}	11 (1)
Cu	mg kg^{-1}	18 (1)
Fe	mg kg^{-1}	3,204 (1)
Pb	mg kg^{-1}	6 (1)
Mn	mg kg^{-1}	521 (1)
Hg	mg kg^{-1}	< 4.1 (1)
Ni	mg kg^{-1}	7 (1)
Si	mg kg^{-1}	158 (1)
Ti	mg kg^{-1}	12 (1)
Zn	mg kg^{-1}	81 (1)

Ba	mg kg^{-1}	125 (1)
Na	mg kg^{-1}	948 (1)
Ca	mg kg^{-1}	27,451 (1)
Mg	mg kg^{-1}	2,409 (1)
Sr	mg kg^{-1}	69 (1)
B	mg kg^{-1}	44 (1)
BETX (HS-GC-MS)	mg kg^{-1}	18 (1)
USEPA 16 PAHs (GC-MS)	mg kg^{-1}	8 (1)

7.2 Chapter 3 supplementary information – Can biochar reduce soil greenhouse gas (GHG) emissions from a Miscanthus bioenergy crop?

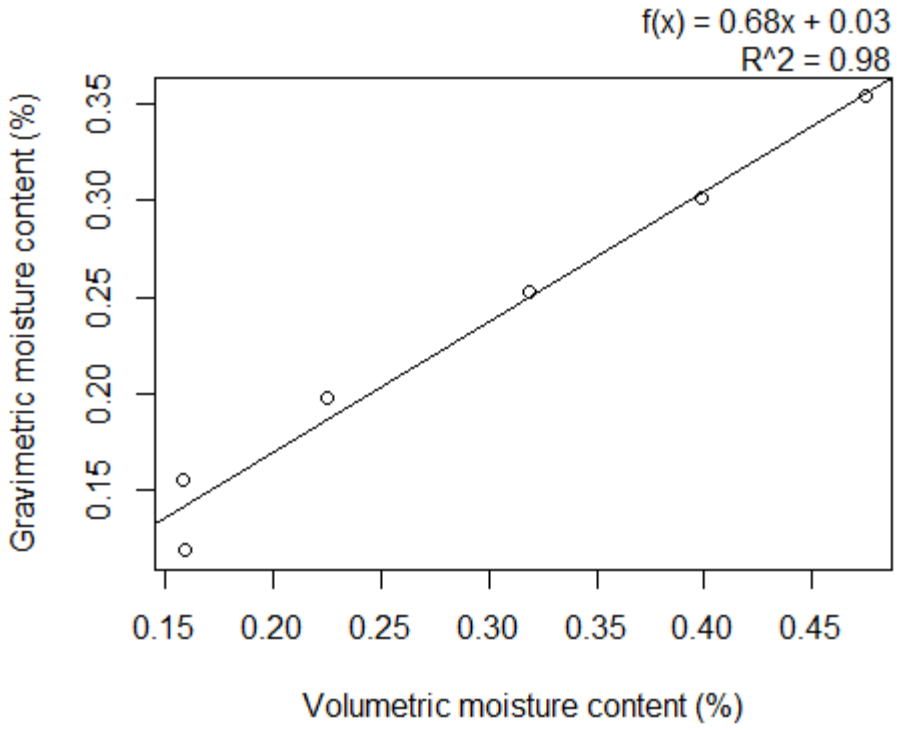

Fig. 7.1 The calibration line used to convert field-experiment soil volumetric moisture content into gravimetric moisture content in un-amended soil

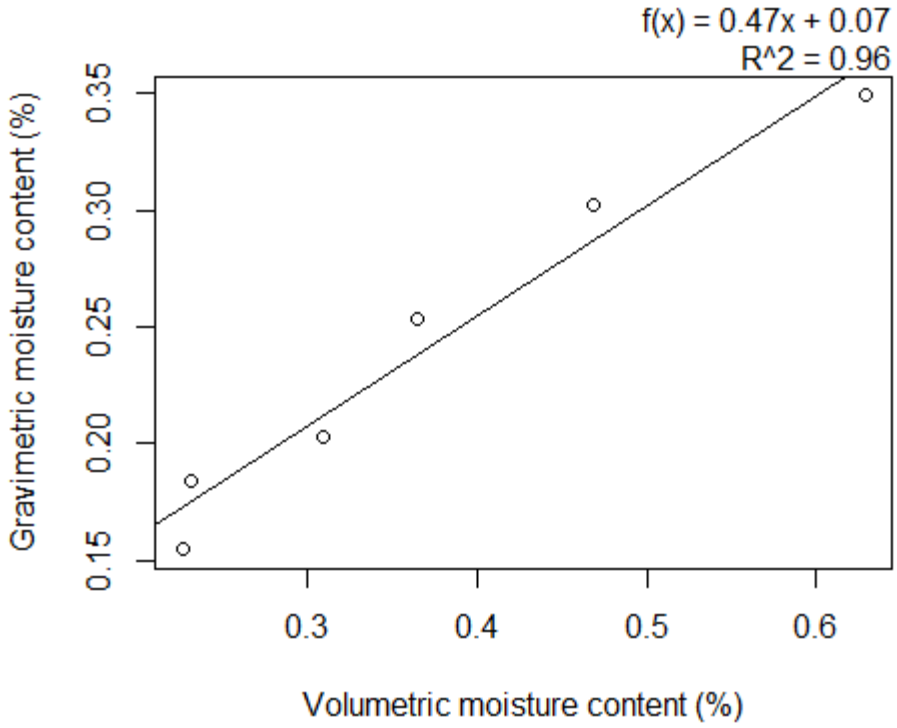

Fig. 7.2. The calibration line used to convert field-experiment soil volumetric moisture content into gravimetric moisture content in biochar-amended soil.

7.3 Chapter 4 supplementary information – Biochar amendment reduces soil nitrous oxide (N_2O) emissions through enhanced reduction of N_2O to N_2

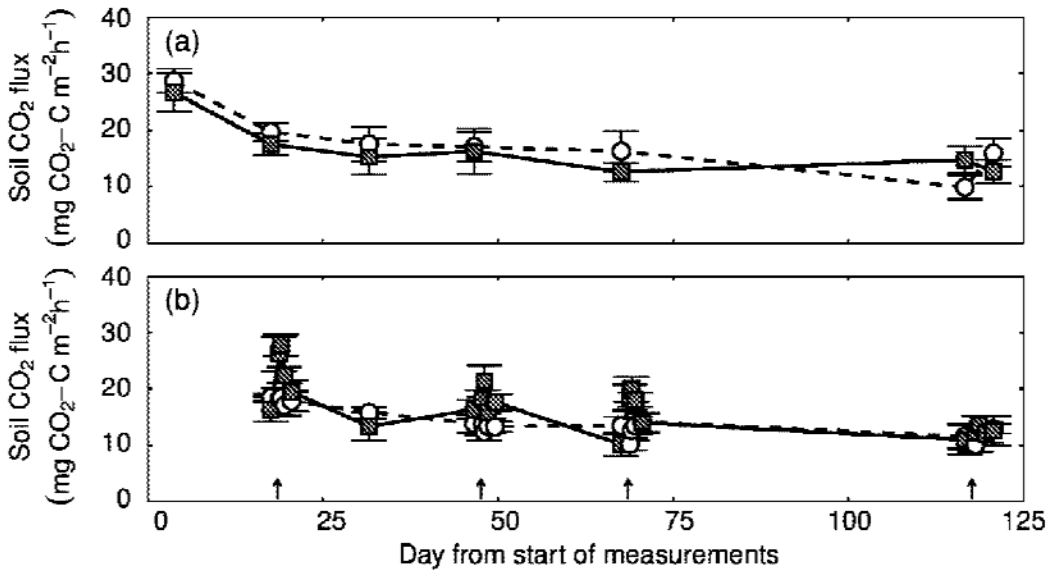

Fig. 7.3. The effect of biochar amendment on soil N_2O emissions from soil cores undergoing wetting/drying cycles either (a) un-wetted or (b) wetted. Arrows on the graph indicate the time of soil wetting. Data points represent mean ± standard error (n = 5). The horizontal dotted line in graph (b) indicates the 0 line. Statistical model outputs underlying these results are presented in Table 7.2.

Table 7.2. Variables affecting N_2O emissions within soils undergoing wetting/drying cycles. "N_2O un-wetted" indicates soil cores maintained field moist, while "N_2O wetted" signifies soil N_2O emissions within 48 hours of a wetting event. Data outputs presented are those from refined linear mixed-effects models using plot as the random factor, refined following the procedure in Zuur et al., (2010b). n = 5. Symbols indicate p-value significance of the term: ns = not significant, * = $p < 0.05$, *** = $p < 0.001$. Refer to Fig. 7.3 for the data underlying these statistical outputs.

	Independent variable					
	Biochar		Day from start		Biochar * Day from start	
Response variable	t	p	t	p	t	p
CO_2 un-wetted	0.82	ns	- 5.38	***	0.75	ns
CO_2 wetted	-2.66	*	-7.93	***	-1.82	ns

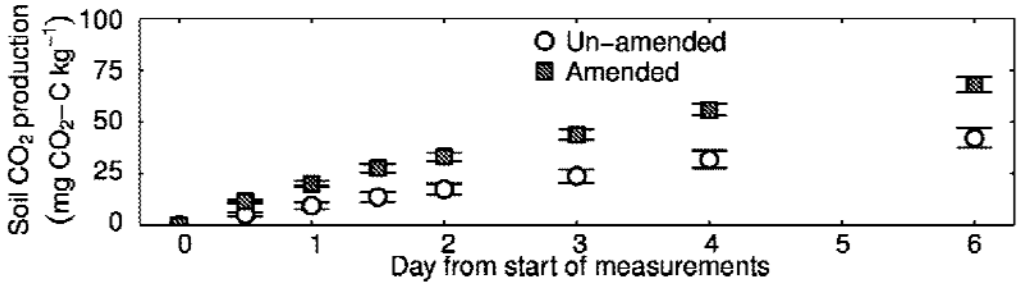

Fig. 7.4. The effect of biochar amendment on cumulative soil CO_2 production during an incubation to investigate soil N transformations. ^{15}N-labelled NO_3^- or NH_4^+ was added at t = 0 and soil WFPS raised to 91%. Data points represent mean ± standard error (n = 4). The star in graph c) indicates 0, as there were no soil N_2O emissions from biochar-amended soils between day 4 and 6.

Table 7.3. The effect of biochar amendment on carbon dioxide (CO_2), production from soil un-amended or amended with biochar during an incubation to investigate soil N transformations. Carbon dioxide production was compared 6 days following water and nitrogen addition. The outputs presented in the table are those from two-sample t-tests. ** = $p < 0.01$.

	Biochar		
Response variable	t	df	p
CO_2 production	-4.1	13	**

7.3.1 Calculation steps for biochar-induced immobilisation

There was no difference in microbial-N immobilisation over the 6 days between un-amended (13.0 ± 17.0 mg N kg^{-1}) and amended (32.0 ± 30.0 mg N kg^{-1}) soils therefore we consider microbial-N immobilisation to be the same in both treatments for the following calculations ($p > 0.1$, Fig. 4.7). Cumulative microbial-N immobilisation for

both un-amended and amended soil was 22.5 mg N kg^{-1} over the six days (21.4 mg NH_4^+-N kg^{-1}, 1.1 mg NO_3^--N kg^{-1}, assuming $\beta = 0.05$, Fig. 4.7). Abiotic-N adsorption within 30 minutes of ^{15}N addition was 5.84 and 5.63 mg N kg^{-1} respectively in amended and un-amended soils (Data not shown). Therefore, only 0.21 mg N kg^{-1} soil can be attributed to abiotic-N adsorption to biochar, which consisted of 0.20 mg NH_4^+-N kg^{-1} soil and 0.01 mg NO_3^--N kg^{-1} soil (assuming $\beta = 0.05$). The ratio between biological-N immobilisation and abiotic-N adsorption was 3.95: 1 from day 0 to 6 for both un-amended and amended soil.

Before ^{15}N nitrogen addition (pre-experiment), the soil NH_4^+ concentration was not significantly different between un-amended and amended soil. Un-amended soil had a mean NH_4^+ concentration of 7.7 ± 0.7 compared to 6.0 ± 0.7 mg NH_4^+-N kg^{-1} in amended soil (data not shown, $p > 0.05$, $t = 1.9$, $df = 12$). Soil NO_3^- content was significantly lower with biochar content 11.0 ± 0.8 and 8.2 ± 0.3 mg NO_3^--N kg^{-1} respectively (data not shown, $p < 0.01$, $t = 3.3$, $df = 14$). We assumed that the ratio of 3.95: 1 microbial-N immobilisation: N adsorption also applied to the pre- ^{15}N addition results. We estimated that the biochar fixed 0.34 mg NH_4^+-N kg^{-1} soil and 0.57 mg NO_3^--N kg^{-1} soil pre- ^{15}N addition.

Adding the pre- and post- ^{15}N addition abiotic-N adsorption together, we estimate the total N adsorption to the biochar surface to be 0.54 mg NH_4^+-N kg^{-1} soil and 0.58 mg NO_3^--N kg^{-1} soil, equivalent to 27 mg NH_4^+-N kg^{-1} biochar and 29 mg NO_3^--N kg^{-1} biochar. We estimated total potential biochar-induced microbial-N immobilisation to be 1.4 mg NH_4^+-N kg^{-1} soil and 2.5 mg NO_3^--N kg^{-1} soil. The combined BII during the incubation to investigate the soil N cycle was therefore 1.9 mg NH_4^+-N kg^{-1} and 3.0 mg NO_3^--N kg^{-1} soil.

7.4 Chapter 5 supplementary information – Discussion and conclusions

Table 7.4. Net soil cumulative $CO_{2eq.}$ emissions from a low inorganic-N concentration *Miscanthus* soil within 48 hours of wetting at 16°C. Data indicates mean (standard error). Data adapted from Chapter 2.

Wetting event	Treatment	Total $CO_{2eq.}$ production (g $CO_{2eq.}$ m^{-2})	CO_2 production (g $CO_{2eq.}$ m^{-2})	N_2O production (g $CO_{2eq.}$ m^{-2})	CH_4 production (g $CO_{2eq.}$ m^{-2})
1	Un-amended	2.67 (0.43)	2.42 (0.37)	0.24 (0.60)	0.002 (0.001)
	Amended	2.93 (0.42)	2.89 (0.43)	0.05 (0.13)	0.001 (0.0004)
2	Un-amended	1.69 (0.37)	1.31 (0.28)	0.38 (0.94)	0.0001 (0.0002)
	Amended	1.58 (0.33)	1.53 (0.31)	0.05 (0.015)	- 0.0002 (0.002)
3	Un-amended	0.66 (0.14)	0.57 (0.12)	0.08 (0.016)	0.002 (0.001)
	Amended	0.90 (0.15)	0.86 (0.15)	0.04 (0.005)	- 0.002 (0.001)
Average	**Un-amended**	**1.10 (0.22)**	**1.01 (0.20)**	**0.09 (0.02)**	**- 0.0008 (0.002)**
	Amended	**1.21 (0.23)**	**1.15 (0.21)**	**0.07 (0.02)**	**- 0.0007 (0.002)**

Table 7.5. Net soil cumulative $CO_{2eq.}$ emissions from a moderate inorganic-N concentration arable soil within 48 hours of wetting at 16°C. Data indicates mean (standard error). Data adapted from Chapter 4.

Wetting event	Treatment	Total $CO_{2eq.}$ production (g $CO_{2eq.}$ m^{-2})	CO_2 production (g $CO_{2eq.}$ m^{-2})	N_2O production (g $CO_{2eq.}$ m^{-2})	CH_4 production (g $CO_{2eq.}$ m^{-2})
1	Un-amended	7.10 (0.86)	3.13 (0.28)	3.98 (0.90)	-0.002 (0.004)
	Amended	4.41 (0.19)	4.02 (0.15)	0.39 (0.09)	-0.005 (0.003)
2	Un-amended	5.40 (0.97)	2.34 (0.22)	3.06 (0.87)	-0.002 (0.001)
	Amended	3.45 (0.26)	3.18 (0.24)	0.28 (0.03)	-0.0003 (0.002)
3	Un-amended	3.96 (0.82)	2.31 (0.25)	1.64 (0.64)	0.003 (0.002)
	Amended	3.21 (0.04)	3.18 (0.24)	0.17 (0.02)	-0.002 (0.005)
4	Un-amended	2.74 (0.24)	3.04 (0.05)	0.65 (0.22)	0.0005 (0.002)
	Amended	2.42 (0.27)	2.30 (0.25)	0.12 (0.03)	-0.007 (0.003)
Average	**Un-amended**	**4.80 (0.72)**	**2.47 (0.22)**	**2.33 (0.66)**	**- 0.003 (0.02)**
	Amended	**3.37 (0.20)**	**3.13 (0.17)**	**0.24 (0.40)**	**- 0.001 (0.009)**

Table 7.6. The effect of biochar amendment on net soil $CO_{2eq.}$ emissions from field plots or soil cores placed under controlled environmental conditions. Mean $CO_{2eq.}$ emissions were calculated from the mean soil GHG emissions sampled during the period specified by the 'Sample dates included' column, and mean $CO_{2eq.}$ production was calculated by multiplying this value by the number of days specified by the column 'Time Period'. The time period 'Year' indicates 365 days, while 'Summer' indicates 92 days (the number of days in June, July and August). The sample date 'Lab incubation' indicates that gas sampling data was used from the whole 120-day laboratory incubation. Data indicate mean, SE indicates ± standard error, n = 5.

Incubation	Time period (tp)	Sample dates included	Biochar	Soil $CO_{2\ eq.}$ emissions (net soil $CO_{2eq.}$ μg m^{-2} h^{-1})	Soil $CO_{2\ eq.}$ emissions (net soil $CO_{2eq.}$ t ha^{-1} tp^{-1})	Soil CO_2 emissions (net soil $CO_{2eq.}$ t ha^{-1} tp^{-1})	Soil N_2O emissions (net soil $CO_{2eq.}$ t ha^{-1} tp^{-1})	Soil CH_4 emissions (net soil $CO_{2eq.}$ t ha^{-1} tp^{-1})
(Chapter 3) Field	Year	2010-2012	Un-amended	172.2 (23.5)	15.05 (2.42)	13.87 (1.77)	1.18 (0.64)	- 0.01 (0.01)
	Year	2010-2012	Amended	108.9 (13.0)	9.54 (1.26)	9.27 (1.11)	0.25 (0.15)	0.02 (0.01)
(Chapter 3) Field	Year (-1st)	2010-2012	Un-amended	137.3 (20.0)	12.03 (1.83)	12.02 (1.74)	0.02 (0.08)	-0.01 (0.01)
	Year (-1st)	2010-2012	Amended	100.8 (13.8)	8.83 (1.28)	8.73 (1.19)	0.07 (0.08)	0.03 (0.01)
(Chapter 3) Field	Summer	2010/2011	Un-amended	289.4 (43.1)	6.39 (1.20)	5.25 (0.66)	1.13 (0.54)	0.003 (0.004)
	Summer	2010/2011	Amended	138.3 (16.1)	3.05 (0.46)	2.83 (0.33)	0.23 (0.12)	- 0.004 (0.009)
(Chapter 3) Field	Summer	2010	Un-amended	395.1 (51.5)	8.72 (1.92)	6.49 (1.07)	2.24 (0.84)	0.001 (0.01)
	Summer	2010	Amended	175.9 (16.3)	3.88 (0.72)	3.47 (0.45)	0.43 (0.25)	- 0.02 (0.02)
(Chapter 3) Field	Summer	2011	Un-amended	183.6 (11.2)	4.06 (0.26)	4.02 (0.24)	0.03 (0.02)	0.004 (0.001)
	Summer	2011	Amended	108.2 (16.2)	2.39 (0.37)	2.32 (0.35)	0.06 (0.02)	0.006 (0.003)
(Chapter 2) Laboratory	Summer	Lab incubation	Un-amended	35.1 (3.1)	0.77 (0.07)	0.76 (0.07)	0.016 (0.005)	- 0.001 (0.001)
	Summer	Lab incubation	Amended	24.7 (4.1)	0.55 (0.09)	0.54 (0.09)	0.013 (0.002)	- 0.002 (0.001)
(Chapter 3) Laboratory (field incubated soil)	Summer	Lab incubation	Un-amended	120.2 (9.7)	2.65 (0.24)	2.44 (0.17)	0.05 (0.01)	-0.01 (0.01)
	Summer	Lab incubation	Amended	54.6 (6.0)	1.20 (0.14)	1.14 (0.12)	0.21 (0.06)	0.002 (0.01)
(Chapter 4) Laboratory	Summer	Lab incubation	Un-amended	119.4 (10.9)	2.61 (0.27)	1.54 (0.97)	1.06 (0.17)	0.01 (0.01)
	Summer	Lab incubation	Amended	73.0 (5.7)	1.59 (0.13)	1.43 (0.10)	0.17 (0.03)	- 0.02 (0.01)