Article (refereed) - postprint

Case, Sean D.C.; McNamara, Niall P.; Reay, David S.; Stott, Andy W.; Grant, Helen K.; Whitaker, Jeanette. 2015. Biochar suppresses N2O emissions while maintaining N availability in a sandy loam soil.

Copyright © 2014 Elsevier B.V.

This version available http://nora.nerc.ac.uk/509004/

NERC has developed NORA to enable users to access research outputs wholly or partially funded by NERC. Copyright and other rights for material on this site are retained by the rights owners. Users should read the terms and conditions of use of this material at http://nora.nerc.ac.uk/policies.html#access

NOTICE: this is the author’s version of a work that was accepted for publication in Soil Biology and Biochemistry. Changes resulting from the publishing process, such as peer review, editing, corrections, structural formatting, and other quality control mechanisms may not be reflected in this document. Changes may have been made to this work since it was submitted for publication. A definitive version was subsequently published in Soil Biology and Biochemistry, 81. 178-185. 10.1016/j.soilbio.2014.11.012

www.elsevier.com/

Contact CEH NORA team at noraceh@ceh.ac.uk

The NERC and CEH trademarks and logos (‘the Trademarks’) are registered trademarks of NERC in the UK and other countries, and may not be used without the prior written consent of the Trademark owner.
Biochar suppresses N$_2$O emissions while maintaining N availability in a sandy loam soil

Running title: Biochar, soil N$_2$O suppression and N availability

Sean D. C. Case$^{1,2}$, Niall P. McNamara$^1$, David S. Reay$^2$, Andy W. Stott$^1$, Helen K. Grant$^1$, Jeanette Whitaker$^*$

$^1$Centre for Ecology & Hydrology, Lancaster Environment Centre, Library Avenue, Bailrigg, LA1 4AP, UK

$^2$School of Geosciences, University of Edinburgh, High School Yards, Edinburgh, EH8 9XP, UK

*corresponding author: jhart@ceh.ac.uk; telephone +44 (0) 1524 595888

Keywords

Biochar, Nitrous oxide, Immobilisation, Denitrification, Mineralisation, Nitrification, Ammonium, Nitrate, FLUAZ
Abstract

Nitrous oxide (N₂O) from agricultural soil is a significant source of greenhouse gas emissions. Biochar amendment can contribute to climate change mitigation by suppressing emissions of N₂O from soil, although the mechanisms underlying this effect are poorly understood. We investigated the effect of biochar on soil N₂O emissions and N cycling processes by quantifying soil N immobilisation, denitrification, nitrification and mineralisation rates using ¹⁵N pool dilution techniques and the FLUAZ numerical calculation model. We then examined whether biochar amendment affected N₂O emissions and the availability and transformations of N in soils.

Our results show that biochar suppressed cumulative soil N₂O production by 91 % in near-saturated, fertilised soils. Cumulative denitrification was reduced by 37 %, which accounted for 85 - 95 % of soil N₂O emissions. We also found that physical/chemical and biological ammonium (NH₄⁺) immobilisation increased with biochar amendment but that nitrate (NO₃⁻) immobilisation decreased. We concluded that this immobilisation was insignificant compared to total soil inorganic N content. In contrast, soil N mineralisation significantly increased by 269 % and nitrification by 34 % in biochar-amended soil.

These findings demonstrate that biochar amendment did not limit inorganic N availability to nitrifiers and denitrifiers, therefore limitations in soil NH₄⁺ and NO₃⁻ supply cannot explain the suppression of N₂O emissions. These results support the concept that biochar application to soil could significantly mitigate agricultural N₂O emissions through altering N transformations, and underpin efforts to develop climate-friendly agricultural management techniques.
1 Introduction

Nitrous oxide 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 (Davidson, 2009). Agricultural land contributes approximately 60% to global anthropogenic N$_2$O emissions; new agricultural practices are therefore needed to minimise soil N$_2$O emissions and mitigate the effects of climate change (Reay et al., 2012; Smith et al., 2007).

Biochar amendment to soil has been proposed as a method to increase soil C storage and suppress soil N$_2$O emissions on a global scale (Woolf et al., 2010). Biochar consists of biomass heated in an O$_2$-limited environment (typically to between 350 and 600 °C) that can be subsequently applied as a soil amendment (Sohi et al., 2010). Laboratory incubations and several short-term field studies have shown that biochar amendment can suppress soil N$_2$O emissions (Clough et al., 2013; Taghizadeh-Toosi et al., 2011; Zhang et al., 2012). However, more extensive studies are needed to conclude with certainty whether biochar addition has a consistent and long-term effect on soil N$_2$O emissions (Jones et al., 2012; Spokas, 2012).

Denitrification, nitrification and nitrifier-denitrification are the three main processes that produce N$_2$O in agricultural soils (Butterbach-Bahl et al., 2013; Kool et al., 2011). Denitrification is the primary source, which also produces nitric oxide (NO) and dinitrogen (N$_2$) from nitrite (NO$_2$) and nitrate (NO$_3^-$), whilst nitrification comprises the oxidation of ammonium (NH$_4^+$) to NO$_2^-$ and NO$_3^-$. The rates of denitrification and the relative proportions of N$_2$O, NO and N$_2$ produced by this process depend on complex interactions between soil physico-chemical properties and climatic factors such as soil temperature, pH, moisture status, and the availability of oxygen (O$_2$), nitrogen (N) and labile carbon (C) (Gillam et al., 2008; Saggar et al., 2013; Šimek et al., 2002). The ratio of N$_2$O: N$_2$ produced via denitrification decreases with increasing soil pH, labile C availability, soil water-filled pore space (WFPS) and decreasing soil NO$_3^-$ concentrations (Senbayram et al., 2012). Conditions that favour
nitrification include high soil NH$_4^+$ concentrations, high soil temperature and aerobic conditions (greatest at a moderate WFPS, ~ 60 %) (Norton and Stark, 2011).

The mechanisms to explain how biochar amendment influences soil N$_2$O emissions are uncertain (Spokas et al., 2012). Biochar affects soil aeration by increasing soil water holding capacity (WHC) and decreasing soil bulk density (BD), conditions under which denitrifier activity is typically lower (Basso et al., 2012; Karhu et al., 2011). However, we recently demonstrated that biochar-induced suppression of soil N$_2$O emissions in soil subjected to wetting/drying cycles was not due to increased soil aeration (Case et al., 2012).

One alternative mechanism for biochar N$_2$O suppression is a restriction in the availability of inorganic N to soil nitrifiers and denitrifiers via immobilisation in biochar-amended soil (Bruun et al., 2012; Case et al., 2012; Nelissen et al., 2014). Inorganic N availability may be affected by changes in the rates of N mineralisation or nitrification. Increased gross mineralisation rates following biochar addition have been attributed to stimulated mineralisation of native soil organic matter (Nelissen et al., 2012), whilst increased nitrification rates have been attributed to greater soil pH in a biochar-amended arable soil (Nelissen et al., 2012) and the uptake of inhibitive phenolic compounds by biochar in a forest soil (DeLuca et al., 2006). However, research in this area is limited; the effect of biochar amendment on the net availability of inorganic N to soil nitrifiers and denitrifiers and the subsequent effect on soil N$_2$O emissions is poorly understood (Clough et al., 2013). This represents a significant knowledge gap in determining the potential for biochar to contribute to climate change mitigation. To address this knowledge gap, we analysed those soil N cycling processes that control substrate availability for N$_2$O production (i.e. denitrification, nitrification, immobilisation and mineralisation) in fertilised, near-saturated soil amended with biochar. Our aim was to identify whether biochar affects the availability and transformations of N in arable soils underlying soil N$_2$O emissions.
2 Materials and methods

2.1 Biochar and field site description

The field site near Lincoln, Lincolnshire, UK was cultivated with an arable rotation of three years of wheat (*Triticum aestivum*) followed by one year of oilseed rape (*Brassica Napus*). The field received a total of 140 kg N ha\(^{-1}\) yr\(^{-1}\) as ammonium nitrate (NH\(_4\)NO\(_3\)) divided into three separate applications. The soil association of the field the samples were taken from was Beccles 1, which was a fine loam over clay. The bedrock was a Charnmouth mudstone formation. The soil was a sandy loam (57 % sand, 32 % silt and 10 % clay) with a bulk density (BD) of 1.39 g cm\(^{-3}\). The biochar (also used in a previous study, 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, using the thinnings of hardwood trees as feedstock (ash, oak and cherry, Bodfari Charcoal, UK). The biochar 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 N kg\(^{-1}\) of NH\(_4\)\(^+\) and NO\(_3\)\(^-\) respectively), and a pH of 9.25. For more biochar properties refer to the supplementary information of Case et al. (2012).

A four-treatment factorial experiment using \(^{15}\)N pool dilution was designed to investigate the effects of biochar amendment on N transformations in arable soil. Soil was collected from the field in January 2012 (during which time winter wheat was growing), sieved to < 4 mm then covered and stored at 4 °C. Biochar (< 2 mm) was mixed with soil at a rate of 2 % d. wt. soil (equivalent to 28 t ha\(^{-1}\)). One week later, 100 g d. wt. soil was put into plastic containers (H 17.4 cm, D 11.6 cm, V = 1.7 l) to 10 mm depth (bulk density, BD = 0.91 ± 0.02 g cm\(^{-3}\)) and pre-incubated in the dark at 16 °C for seven days to allow for any initial flush of soil CO\(_2\) emissions (Reichstein et al., 2000; Reicosky, 1997). Mineral fertiliser in de-ionised water solution was added to the soil at a rate of 100 mg N kg\(^{-1}\) (d. wt. soil, equivalent to 110 kg N ha\(^{-1}\)) in the form of \(^{15}\)NH\(_4\)NO\(_3\) or NH\(_4\)\(^{15}\)NO\(_3\) (10 atom % \(^{15}\)N enrichment, Sigma’Aldrich, USA), adjusting the soil to 90 % WFPS to create favourable conditions for denitrification, and also N\(_2\)O production (Weier et al., 1993). Pre-tests had demonstrated that soil
CO2 emissions were linear, and O2 concentrations adequate over at least four days of enclosure, so the containers were sealed for the duration of the incubation to enable a mass balance to be calculated.

At four time points after 15N addition (30 mins, 1, 2 and 4 days), four replicates of each treatment were destructively sampled for total C and N content, soil pH, gravimetric moisture content (GMC), extractable soil NH4+ and 15NH4+, NO3- and 15NO3-, and organic N and 15N concentrations (methods in Section 3.3). The first sampling time point was chosen as 30 minutes after 15N addition, when it was assumed that the chemical or physical immobilisation of N was completed, and any further N immobilisation came exclusively from biological processes (Mary et al., 1998).

At seven time points following 15N addition (0, 0.5, 1, 1.5, 2, 3 and 4 days), 10 ml gas samples were taken from the soil container headspace for N2O and CO2 analysis using a gas-tight syringe and injected into evacuated 3 ml vials (Labco, USA). For 15N2O analysis, 80 ml headspace samples were injected into evacuated 60 ml glass serum bottles (Wheaton Science Products, USA). After gas samples were removed, laboratory air of equivalent volume (N2O and CO2 concentration analysed) was injected into the enclosed sample headspace. This dilution of laboratory air was taken into account in the final calculations of GHG emissions.

2.2 Gas sampling and N2O source separation

Headspace gas samples were analysed for N2O and CO2 concentrations using the same Gas Chromatograph system (PerkinElmer Autosystem XL, PerkinElmer, USA) described in Case et al. (2014) and calibrated against certified standards (Air Products, UK).

For 15N2O analysis, ~ 4 ml of the 80 ml sample was injected into a TraceGas Preconcentrator coupled to an isotope ratio mass spectrometer (IRMS, Isoprime Ltd, UK) whereupon the sample was directed through a series of chemical traps to remove H2O and CO2. The N2O was cryogenically trapped under liquid N. The waste was flushed out, and then the N2O was further cryofocused in a second liquid N trap prior to being introduced onto a 25 m x 0.32 mm Poraplot Q column (Chrompack column, Varian, UK). The column separated N2O from any residual CO2, and both entered the IRMS via an open split. The retention time between the first eluting CO2 (< 2E-10 amplitude) and second eluting
N₂O peak typically fell in the range between 60 - 70 seconds to avoid isobaric interference of the CO₂ with the calculated ¹⁵N. The N₂O was directed towards the triple collectors of the IRMS where m/z 44, m/z 45 and m/z 46 mass ions were measured. Mass/charge ratios for the m/z 44, m/z 45 and m/z 46 NO were then recorded for each sample and delta values for both ¹⁵N were calculated with respect to N₂O reference gas (BOC Industrial Gases, UK).

The experimental design allowed us to differentiate the source of N₂O emissions from nitrification + nitrifier-denitrification and denitrification. The proportions of soil N₂O emissions attributed to the two processes were calculated using Equation 1, based on data from the analysis of the ¹⁵NO₃⁻ labelled soil treatment (Mathieu et al., 2006). Outputs greater than 100% and lower than 0% were rounded to the nearest boundary.

\[ d = \frac{(a_m - a_n)}{(a_d - a_n)} \]  
where \(a_d\) ≠ \(a_n\)  

Where ‘d’ is the proportion of N₂O emissions from denitrification in a time period, ‘\(a_m\)’ is the average % ¹⁵N atom enrichment of the N₂O mixture during the time period, ‘\(a_n\)’ is the average % ¹⁵N enrichment of the nitrification pool (NH₄⁺) during the time period and ‘\(a_d\)’ is the average % ¹⁵N enrichment of the denitrification pool (NO₃⁻) during the time period.

### 2.3 Analysis of soil properties and soil N isotopic composition

Extractable inorganic NH₄⁺ and NO₃⁻ concentrations were determined using 5 g d. wt. equivalent of wet soil and 50 ml of 0.8 M potassium chloride (KCl, 6 %). The samples were shaken for 1 hour, and then filtered through Whatman no. 44 filter paper disks (Whatman, USA). Extracts were analysed on a Seal AQ2 analyser (Bran and Luebbe, UK) using discrete colorimetric procedures (Maynard and Kalra, 1993).

Extractable inorganic ¹⁵N concentrations (¹⁵NH₄⁺ and ¹⁵NO₃⁻) were analysed following the acidified disk method (Khan et al., 1998). First, inorganic N was extracted from soil, using 2 M KCl and the same method as that described for inorganic N extraction above. Then, 20 ml of the extract was placed in air-tight 500 ml glass jars (Kilner, USA). For ¹⁵NH₄⁺ concentrations, 0.2 g of magnesium
oxide (MgO) was added. For $^{15}$NH$_4^+$ + $^{15}$NO$_3^-$ concentrations, 1 ml of 0.2 M sulfamic acid was added to decompose NO$_2^-$, followed by 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 added 5 μl of 2.5 M potassium hydrogen sulphate solution. The jars were sealed and placed in a 30 °C environment for at least 72 hours to enable near 100 % adsorption of the extractant N. The filter disks were then dried at 40 °C for 24 hours.

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 IRMS (Dennis Leigh Technology, UK). Atom % abundances of $^{15}$NO$_3^-$ were calculated from the ($^{15}$NH$_4^+$ + $^{15}$NO$_3^-$) atom % abundance and respective inorganic N concentrations using the method described in Khan et al., (1998).

Organic $^{15}$N contents were used as an analogue for microbial biomass and were assumed to have an atom % $^{15}$N excess of 0.0025 % (Mary et al., 1998). First, 3 g of soil was oven dried at 80 °C for 24 hours, and then the dried soil was mixed with 10 ml of 1 M KCl in a 12 ml polystyrene test tube and mechanically shaken for 15 minutes. The tube was then centrifuged for 15 minutes at 3,000 rpm and subsequently the KCl was removed and replaced (Recous et al., 1998). This process was repeated four times. Afterwards, the soil was dried at 80 °C for 24 hours. 50 mg of dried soil was sealed in a tin capsule and analysed in the same fashion as described for the analysis of the acidified disks above.

The total C and N contents of dried, ground soil samples (0.1 g, < 1 mm) were analysed using a Tru-spec total CN analyser (Leco Corp., USA) (Sollins et al., 1999). Gravimetric moisture content, soil pH (soil: H$_2$O, 1: 2.5), particle density, BD and WFPS analyses were conducted according to standard methods (Blake, 1965; Emmett et al., 2008; Ohlinger, 1995a, 1995b).

2.4 Estimating soil N transformations with and without biochar

To assess whether biochar amendment affected the availability and transformations of soil N underlying N$_2$O production, we quantified mineralisation, immobilisation, nitrification and denitrification rates using the FLUAZ numerical N-cycling model (Mary et al., 1998). The model
consists of two parts (Mary et al., 1998). First, a numerical model that solves differential equations
from the N and $^{15}$N mass equations based on a 4th order Runge-Kutta algorithm with a variable time
step. Second, a non-linear fitting program to calculate N rates based on Marquardt’s algorithm
(Marquardt, 1963).

Inorganic N, organic N and respective $^{15}$N concentrations were input into the FLUAZ model and
analysed using a paired treatment design. The final model fitted mineralisation (‘m + s’,
mineralisation of soil organic N and biochar-derived N to $\text{NH}_4^+$), nitrification (‘n’, the conversion of
$\text{NH}_4^+$ to $\text{NO}_3^-$), immobilisation of $\text{NH}_4^+$ and $\text{NO}_3^-$ (‘ia’ and ‘in’, the sum of $\text{NH}_4^+$ and $\text{NO}_3^-$ taken up by
the organic N pool) and denitrification rates (‘kd’, the sum of conversion of $\text{NO}_3^-$ to $\text{N}_2\text{O}$, $\text{NO}$ or $\text{N}_2$),
over three time periods following $^{15}$N addition (30 minutes - 1 day, 1 - 2 days, 2 - 4 days).

For the FLUAZ model analysis we made several assumptions. As the incubation only lasted for four
days, and the temperature was maintained at 16 °C it was assumed that remineralisation of
immobilised N (‘r’) was negligible (Murphy et al., 2003). It was also assumed that the conversion of
plant residue N directly into microbial biomass (‘j’, N humification) and ammonia volatilisation were
negligible (Mary et al., 1998; Whitehead and Raistrick, 1990).

2.5 Statistical analysis

Student’s t tests were used to test for significant differences in soil $\text{N}_2\text{O}$ and $\text{CO}_2$ emissions, inorganic
N contents, total C, N and pH between un-amended and amended soil. For all statistical analyses the
software package R was used (version 3.0.2, The R Project, 2013).
Cumulative soil N\(_2\)O emissions after four days were suppressed by 91% with biochar amendment, from 0.61 ± 0.20 to 0.05 ± 0.02 mg N\(_2\)O·N kg\(^{-1}\) for un-amended and amended soils respectively (two-sample t-test, \(p < 0.05\), \(t = 2.5\), df = 13, Fig. 1a). Soil CO\(_2\) production was 56 compared to 32 mg CO\(_2·C\) kg\(^{-1}\) in amended and un-amended soil respectively over the same time period, equivalent to a 75% increase (two-sample t-test, \(p < 0.001\), \(t = 4.7\), df = 13, Fig. 1b).

Using \(^{15}\)N analysis of N\(_2\)O emissions (Fig. 1c, d), and soil NH\(_4^+\) and NO\(_3^-\) concentrations (Fig. 1e, f), soil N\(_2\)O emissions were source partitioned over the four-day incubation period. Nitrification + nitrifier denitrification produced 40% and 33% of N\(_2\)O emissions in amended and un-amended soils respectively from day 0 to 2 (Fig. 1e, f). Between day 2 and 4, all soil N\(_2\)O emissions were produced via denitrification in both treatments. Considering the entire four-day incubation, 95% of un-amended soil N\(_2\)O emissions came from denitrification, compared to 85% in amended soil (Fig. 1e, f).

To test whether transformations of soil N were affected by biochar amendment we analysed the concentrations and isotope ratios of inorganic and organic N and input these data into the FLUAZ model. Soil NH\(_4^+\) concentrations decreased over time whilst soil NO\(_3^-\) concentrations increased over time in both un-amended and amended soils (Fig. 2a, c, Fig. 3a, c). Soil NH\(_4^+\) concentrations decreased at a similar rate in all treatments (Fig. 2a, c, Fig. 3a, c). Soil NO\(_3^-\) concentrations were initially lower in biochar-amended soil (88.7 ± 2.1 vs 77.2 ± 2.6 NO\(_3^-\)·N mg kg\(^{-1}\), \(p < 0.01\) for un-amended and amended soil respectively), but during the four-day period increased more rapidly (28.9 ± 13.8 vs 69.1 ± 8.9 NO\(_3^-\)·N mg kg\(^{-1}\), \(p < 0.05\) for un-amended and amended soil respectively, Fig. 2a, c, Fig. 3a, c). Soil \(^{15}\)NH\(_4^+\) enrichment decreased more rapidly in amended soils (Fig. 2b), but there was no difference in \(^{15}\)NO\(_3^-\) enrichment between the treatments (Fig. 2d, 3d). Initial soil organic N content was 2,162 ± 46 mg N kg\(^{-1}\); organic \(^{15}\)N enrichment did not vary significantly between un-amended and amended soil over the course of the incubation (Fig. 2f, 3f).

The FLUAZ model outputs generally fitted well to analysed soil inorganic N and \(^{15}\)N concentrations, resulting in a mean-weighted error of 0.8 for the un-amended and 1.3 for the amended soil models.
Total N recovery was calculated from inorganic, organic N and respective $^{15}\text{N}$ concentrations in the soil. Total N recovery for the $^{15}\text{N}$-labelled $\text{NO}_3^{-}$ treatments remained close to 100 % throughout the incubation, whereas it was lower for the $^{15}\text{N}$-labelled $\text{NH}_4^{+}$ treatments (typically above 80 %, but attained a minimum of 62 % on day 4 in the amended treatment, Fig. 2e, 3e).

Cumulative mineralisation, nitrification, denitrification and immobilisation of N over 4 days were estimated by using the FLUAZ model. Cumulative denitrification after four days was 37 % lower in amended than in un-amended soil (0.17 and 0.27 mg N kg$^{-1}$ respectively, Table 1). Mineralisation of N and nitrification were greater in amended compared to un-amended soil. Cumulative mineralisation was 55.0 in amended soil compared to 14.9 mg N kg$^{-1}$ in un-amended soil (269 % greater), and cumulative nitrification was increased by 34%, from 75.6 to 101.1 mg N kg$^{-1}$ (Table 1).

The magnitude of initially immobilised N (within 30 minutes of $^{15}\text{N}$ addition) was similar in biochar-amended (5.7 mg N kg$^{-1}$) and un-amended (5.5 mg N kg$^{-1}$) soils (Table 1). Biological $\text{NH}_4^{+}$ immobilisation over the subsequent four days was 50 % greater in amended compared with un-amended soil according to the FLUAZ outputs (17.6 and 11.9 mg N kg$^{-1}$, respectively, Table 1). Soil $\text{NO}_3^{-}$ immobilisation only increased between day 0 and 2 (Table 1). After two days, $\text{NO}_3^{-}$ immobilisation was 17 % lower in amended than un-amended soil (7.8 compared to 9.4 mg N kg$^{-1}$, Table 1).

Biochar amendment significantly altered soil physico-chemical properties. Soil pH increased from 6.31 ± 0.03 to 6.62 ± 0.03 in amended soil (p < 0.001, Table 2). Total soil C content was also greater in amended treatments (3.71 ± 0.19 compared to 1.99 ± 0.01 mg C kg$^{-1}$, p < 0.001, Table 2), while total N contents were similar. The soil C: N ratio increased with biochar amendment (p < 0.001, Table 2).
4 Discussion

Suppression of soil N₂O emissions following biochar application has been demonstrated in a number of short-term studies. Here, we optimised experimental conditions to favour for denitrification and also high soil N₂O emissions and observed a significant (91%) suppression of those emissions with biochar amendment, consistent with suppressions of 50 - 80% reported in studies using other soil and biochar combinations (Ameloot et al., 2013; Cayuela et al., 2013; Nelissen et al., 2012). The proportions of N₂O emissions derived from nitrification + nitrifier denitrification and denitrification (calculated by source partitioning) were similar in un-amended and amended soils; 95% of emissions came from denitrification in un-amended soil compared with 85% in amended soil over four days (Fig. 1b). This is consistent with results from a near-saturated, agricultural soil (not amended with biochar), where 85% of N₂O emissions were attributed to denitrification (Mathieu et al., 2006).

Our findings indicated that denitrification was the dominant source of N₂O emissions and that N₂O emissions from both denitrification and nitrification were suppressed by biochar addition. The suppression of soil N₂O emissions from denitrification may have been due to reduced denitrifier activity or increased complete denitrification (i.e. increased conversion of N₂O to N₂). To examine this, we estimated denitrifier activity with the FLUAZ model and found that denitrification was 37% lower with biochar amendment (Table 1). Lower overall denitrifier activity could feasibly be due to a lower supply of substrate (i.e. NO₃⁻) for denitrifying organisms. We observed that initial concentrations of NO₃⁻ in soil were lower than in un-amended soil, but they increased at a more rapid rate than in un-amended soil, and were not significantly different on day 4 (Fig. 2a, c, Fig. 3a, c). Therefore it was unlikely that NO₃⁻ substrate limitation could explain the suppression of denitrification activity in this study. To confirm this, we considered the processes that controlled N transformations of inorganic N in the soil, including N mineralisation, nitrification and immobilisation.

Biochar addition increased gross N mineralisation by 269%, and an additional 40 mg N kg⁻¹ soil was mineralised in biochar-amended soil over four days (FLUAZ, Table 1). Mineralised N could be derived from the biochar itself; recent studies have suggested that organic N derived from biochar
may be mineralised in a matter of weeks (de la Rosa and Knicker, 2011; Hilscher and Knicker, 2011).

The biochar addition rate used in this study added 142 mg N kg\(^{-1}\) soil in organic form, some of which may have been mineralised during the incubation. Alternatively, the addition of labile C as fresh biochar to this relatively low C agricultural soil may have stimulated soil microbial activity, priming the mineralisation of native soil C and the release of bound N (Luo et al., 2011; Nelissen et al., 2012). We could not discern the source of mineralised N (biochar or native soil organic matter) using this experimental design. This could be investigated using \(^{15}\)N-labelled biochar to differentiate between biochar and SOM-derived mineralised N.

Cumulative nitrification was also increased with biochar (34 %), with nitrification rates greater than 20 mg N kg\(^{-1}\) per day in biochar-amended soil (Table 1). An increase in nitrification with biochar addition is consistent with previous biochar studies, although the magnitude of effect has been observed to vary with N addition rate (Nelissen et al., 2012; Prommer et al., 2014). For example, nitrification rates between 1 and 9 mg N kg\(^{-1}\) were observed following the addition of < 5 mg N kg\(^{-1}\) of inorganic N (Nelissen et al., 2012; Prommer et al., 2014). Possible explanations for this increase in nitrification include increased soil pH or increased soil NH\(_4^+\) concentrations as a result of biochar amendment (Mørkved et al., 2007; Norton and Stark, 2011). Soil pH, which was greater than 6.3 in this study, has been found to have little effect on nitrification rates above pH 5 (Mørkved et al., 2007), and so does not explain the increased nitrification observed here. Furthermore, we did not directly observe an increase in NH\(_4^+\) concentrations in soil with biochar, although there was a more rapid increase in NO\(_3^-\) concentrations (Fig. 2c, 3c). Assuming that the NH\(_4^+\) provided by freshly mineralised organic N was rapidly nitrified, we suggest that biochar amendment did increase soil NH\(_4^+\) availability (Fig. 2c, 3c).

Examining N immobilisation more closely, we found that initial chemical or physical N immobilisation was minimal following biochar addition (Table 1). Furthermore, biological N immobilisation in un-amended and amended soil was also small relative to the magnitude of mineralisation and nitrification; equivalent to less than 8 % of the initial soil NH\(_4^+\)-N content (Table 1, Fig. 1a, 2a). This magnitude of N immobilisation was insignificant compared to total inorganic N.
availability in soil and therefore was not sufficient to explain the 91 % suppression of soil N\textsubscript{2}O emissions. This corroborates findings from a similar study in which hardwood biochar suppressed soil \textsubscript{N}2O emissions in excess N conditions (Cayuela et al., 2013). The rates of biological NH\textsubscript{4}+ immobilisation reported here (2 - 9 mg N kg\textsuperscript{-1} d\textsuperscript{-1}) were greater than those from a comparative study of maize biochar (~ 2 mg N kg\textsuperscript{-1} d\textsuperscript{-1}), potentially due to greater N addition rates in this study (< 3 mg N kg\textsuperscript{-1} compared to 100 mg N kg\textsuperscript{-1}) (Nelissen et al., 2012). We also observed a small decrease in NO\textsubscript{3}– immobilisation in biochar–amended soil (~ 1.6 mg N kg\textsuperscript{-1}), possibly as a result of decreased anion exchange capacity and increased soil pH (Nelissen et al., 2012).

Increased mineralisation, nitrification, insignificant increases in N immobilisation, and similar final NO\textsubscript{3}– concentrations are indicative of similar N substrate availability to soil nitrifiers and denitrifiers in biochar-amended compared to un-amended soil. Despite similar N availability, soil N\textsubscript{2}O emissions were significantly decreased. We therefore concluded that the processes underlying N supply (from mineralisation, nitrification and immobilisation) did not explain the suppression of soil N\textsubscript{2}O emissions in biochar-amended soil, or reduced denitrification rates. Alternative hypotheses to explain reduced denitrification rates include: pH increase (Šimek et al., 2002); the capacity of biochar to act as an electron sink for NO\textsubscript{3}–, therefore competing with soil denitrifiers (Cayuela et al., 2013); or the presence of inhibitory compounds in biochar (Quilliam et al., 2012; Spokas et al., 2011, 2010; Taghizadeh-Toosi et al., 2011). The first two hypotheses were not supported by evidence from this study: the increase in soil pH was relatively small (0.3), and the biochar did not contain significant amounts of magnesium (0.24 %) or iron (0.32 %) compared to other biochars that could act as electron acceptors (from supplementary information of Case et al. (2012). We would therefore suggest that the presence of inhibitory compounds in biochar and their effects on denitrification should be the focus of further research.

As discussed above, suppression of N\textsubscript{2}O emissions could result from reduced rates of denitrification, however it could alternatively result from a difference in the proportions of N\textsubscript{2}O, N\textsubscript{2} and NO produced through denitrification, (e.g. a reduction in the N\textsubscript{2}O: N\textsubscript{2} ratio) (Baggs, 2011). This was demonstrated in a recent study which showed that biochar consistently reduced the N\textsubscript{2}O: N\textsubscript{2} ratio promoting the last
step of denitrification (Cayuela et al., 2013). We did not analyse N$_2$ emissions as a part of this study and so could not confirm this finding. The observed increase in soil pH may, however, have either directly decreased the proportion of N$_2$O: N$_2$ emitted from soil, or enabled the biochar to act as an ‘electron shuttle’ increasing the transfer of electrons to denitrifying bacteria (Cayuela et al., 2013). On addition, the incorporation of biochar into the soil introduces fresh labile C which may have increased the conversion of N$_2$O to N$_2$, by increasing the availability of C electron acceptors for denitrifying organisms (Azam et al., 2002; Morley and Baggs, 2010; Saggar et al., 2013; Senbayram et al., 2012).

In this study we observed a 75 % increase in soil CO$_2$ emissions with biochar amendment (Fig. 1b), equivalent to 0.35 % of the biochar C added to the soil (assuming that biochar emission did not prime the mineralisation of soil C), indicating that a significant proportion of labile C was present in the biochar. This provided evidence in support of this mechanism but was not conclusive.

Taken together, the evidence presented in this study indicates that the supply of inorganic N, and particularly NO$_3^-$, to N$_2$O-producing organisms was not a limiting factor constraining soil N$_2$O emissions in biochar-amended soil. Future research should focus on the potential of inhibitive substances and labile C in biochar to alter the N$_2$O: N$_2$ ratio from denitrification.
5 Conclusions

Biochar amendment has been observed to suppress soil N₂O emissions; this characteristic could be of great value in efforts to reduce agricultural greenhouse gas emissions and therefore mitigate anthropogenic climate change. However, it is not known how and under which environmental conditions biochar consistently suppresses soil N₂O emissions.

In this study, several soil N transformation processes were affected following the addition of biochar to a sandy loam soil, including increased mineralisation and nitrification, slightly increased immobilisation and decreased denitrification. Nitrate-supplying transformation rates were increased or un-affected by biochar amendment, so we concluded that the suppression of soil N₂O emissions was not due to limitations of inorganic N availability in the soil caused by biochar-induced inorganic N immobilisation.

This investigation into N transformations in soil following addition of biochar adds to the body of knowledge regarding the efficient utilisation of biochar in agriculture with minimal environmental impact. The findings suggest that adding biochar to agricultural soil with mineral fertilisers can suppress N₂O emissions without suppressing the activity of soil biota involved in N transformation processes such as mineralisation or nitrification. Finally, they support the concept that biochar application to agriculture could significantly mitigate agricultural N₂O emissions.
6 Acknowledgements

We thank the Natural Environment Research Council for providing a PhD studentship award to Sean Case (NE/H525346/1) and additional support from CEH project number NEC03487. We thank Jonathan Wright for access to the field site. Thanks to Dr Bruno Mary (INRA, Laon, France) for access to the FLUAZ model. Thanks to Emily Bottoms and Andy Robertson for assistance during sample collection and analysis. Thanks to Clive Woods, Alan Lawlor, Gloria dos Santos Pereira, Anne Petit and Kathryn Lehto for assistance with chemical analyses.
Table 1. The effect of biochar amendment on soil N cycling processes in soils treated with $^{15}$N-labelled NH$_4$NO$_3$ and wetted to 90% WFPS for 4 days from N addition. Nitrogen transformations were estimated from the FLUAZ model, described in Section 3.4.

<table>
<thead>
<tr>
<th>Nitrogen cycling process</th>
<th>Day 0-1</th>
<th>Day 0-2</th>
<th>Day 0-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mineralisation Un-amended</td>
<td>-8.2</td>
<td>9.3</td>
<td>14.9</td>
</tr>
<tr>
<td>Mineralisation Amended</td>
<td>9.8</td>
<td>25.2</td>
<td>55</td>
</tr>
<tr>
<td>Nitrification Un-amended</td>
<td>17.2</td>
<td>34.8</td>
<td>75.6</td>
</tr>
<tr>
<td>Nitrification Amended</td>
<td>23.4</td>
<td>54.9</td>
<td>101.1</td>
</tr>
<tr>
<td>NH$_4^+$ immobilisation Un-amended</td>
<td>9.2</td>
<td>9.7</td>
<td>11.9</td>
</tr>
<tr>
<td>NH$_4^+$ immobilisation Amended</td>
<td>6.7</td>
<td>6.9</td>
<td>17.6</td>
</tr>
<tr>
<td>NO$_3^-$ immobilisation Un-amended</td>
<td>3.1</td>
<td>9.4</td>
<td>9.4</td>
</tr>
<tr>
<td>NO$_3^-$ immobilisation Amended</td>
<td>6.8</td>
<td>7.8</td>
<td>7.8</td>
</tr>
<tr>
<td>Denitrification Un-amended</td>
<td>0.06</td>
<td>0.12</td>
<td>0.27</td>
</tr>
<tr>
<td>Denitrification Amended</td>
<td>0.00</td>
<td>0.15</td>
<td>0.17</td>
</tr>
</tbody>
</table>

Table 2. The effect of biochar amendment on physico-chemical properties of soil, treated with $^{15}$N-labelled NH$_4$NO$_3$ and wetted to 90% WFPS. Values represent mean ($\pm$ standard error) of analyses from four time points following addition: 30 minutes, 1 day, 2 days and 4 days. Asterisks indicate significant difference between adjacent un-amended and amended soils: *** = $p < 0.001$.

<table>
<thead>
<tr>
<th>Biochar amendment</th>
<th>Total C (%) $\pm$ SE</th>
<th>Total N (%) $\pm$ SE</th>
<th>CN ratio $\pm$ SE</th>
<th>pH $\pm$ SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Un-amended</td>
<td>1.99 (0.03)</td>
<td>0.26 (0.001)</td>
<td>7.94 (0.41)</td>
<td>6.31 (0.03)</td>
</tr>
<tr>
<td>Amended</td>
<td>3.71 (0.19) ***</td>
<td>0.27 (0.001)</td>
<td>13.90 (1.29) ***</td>
<td>6.62 (0.03) ***</td>
</tr>
</tbody>
</table>
Fig. 1. The effect of biochar amendment on (a) cumulative soil N₂O production, (b) cumulative soil CO₂ production, mean soil N₂O ¹⁵N flux in un-amended and biochar-amended soils treated with (c) ¹⁵N-labelled NH₄⁺ or (d) ¹⁵N-labelled NO₃⁻, and the source partitioning of soil N₂O emissions attributed to denitrification and nitrification + nitrifier denitrification in (e) un-amended and (f) biochar-amended soils treated with ¹⁵N-labelled NO₃⁻. Data points for graphs a) – d) represent mean ± standard error.
Fig. 2. The effect of biochar amendment on soil inorganic-N concentrations and $^{15}$N atom abundance. In soils labelled with $^{15}$NH$_4^+$. Soil properties presented are: (a) soil extractable NH$_4^+$ concentration; (b) soil NH$_4^+$ atom $^{15}$N % excess; (c) soil extractable NO$_3^-$ concentration; (d) soil NO$_3^-$ atom $^{15}$N % excess; (e) % N recovery of $^{15}$N measured at $t_0$; and (f) soil organic N atom $^{15}$N % excess. Points indicate the mean of directly measured values ± standard error (n = 4), whereas lines indicate simulated values from FLUAZ model analysis.
Fig. 3. The effect of biochar amendment on soil inorganic-N concentrations and $^{15}$N atom abundance. In soils labelled with $^{15}$NO$_3^{-}$. Soil properties presented are: (a) soil extractable NH$_4^{+}$ concentration; (b) soil NH$_4^{+}$ atom $^{15}$N % excess; (c) soil extractable NO$_3^{-}$ concentration; (d) soil NO$_3^{-}$ atom $^{15}$N % excess; (e) % N recovery of $^{15}$N measured at $t_0$; and (f) soil organic N atom $^{15}$N % excess. Points indicate the mean of directly measured values ± standard error (n = 4), whereas lines indicate simulated values from FLUAZ model analysis.
References


Senbayram, M., Chen, R., Budai, A., Bakken, L., Dittert, K., 2012. N$_2$O emission and the N$_2$O/(N$_2$O + N$_2$) product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. Agriculture, Ecosystems & Environment 147, 4–12. doi:10.1016/j.agee.2011.06.022


