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Biochar suppresses N$_2$O emissions while maintaining N availability in a sandy loam soil

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Abstract

Nitrous oxide (N_2O) from agricultural soil is a significant source of greenhouse gas emissions. Biochar amendment can contribute to climate change mitigation by suppressing emissions of N_2O from soil, although the mechanisms underlying this effect are poorly understood. We investigated the effect of biochar on soil N_2O emissions and N cycling processes by quantifying soil N immobilisation, denitrification, nitrification and mineralisation rates using ^{15}N pool dilution techniques and the FLUAZ numerical calculation model. We then examined whether biochar amendment affected N_2O emissions and the availability and transformations of N in soils.

Our results show that biochar suppressed cumulative soil N_2O production by 91 % in near-saturated, fertilised soils. Cumulative denitrification was reduced by 37 %, which accounted for 85 - 95 % of soil N_2O emissions. We also found that physical/chemical and biological ammonium (NH_4^+) immobilisation increased with biochar amendment but that nitrate (NO_3^-) 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 NH_4^+ and NO_3^- supply cannot explain the suppression of N_2O emissions. These results support the concept that biochar application to soil could significantly mitigate agricultural N_2O 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_2O emissions; new agricultural practices are therefore needed to minimise soil N_2O 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_2O 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_2O 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_2O emissions (Jones et al., 2012; Spokas, 2012).

Denitrification, nitrification and nitrifier-denitrification are the three main processes that produce N_2O 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_2O, 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_2O: 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

60   nitrification include high soil NH_4^+ concentrations, high soil temperature and aerobic conditions

61   (greatest at a moderate WFPS, ~ 60 %) (Norton and Stark, 2011).

62   The mechanisms to explain how biochar amendment influences soil N_2O emissions are uncertain

63   (Spokas et al., 2012). Biochar affects soil aeration by increasing soil water holding capacity (WHC)

64   and decreasing soil bulk density (BD), conditions under which denitrifier activity is typically lower

65   (Basso et al., 2012; Karhu et al., 2011). However, we recently demonstrated that biochar-induced

66   suppression of soil N_2O emissions in soil subjected to wetting/drying cycles was not due to increased

67   soil aeration (Case et al., 2012).

68   One alternative mechanism for biochar N_2O suppression is a restriction in the availability of inorganic

69   N to soil nitrifiers and denitrifiers via immobilisation in biochar-amended soil (Bruun et al., 2012;

70   Case et al., 2012; Nelissen et al., 2014). Inorganic N availability may be affected by changes in the

71   rates of N mineralisation or nitrification. Increased gross mineralisation rates following biochar

72   addition have been attributed to stimulated mineralisation of native soil organic matter (Nelissen et

73   al., 2012), whilst increased nitrification rates have been attributed to greater soil pH in a biochar-

74   amended arable soil (Nelissen et al., 2012) and the uptake of inhibitive phenolic compounds by

75   biochar in a forest soil (DeLuca et al., 2006). However, research in this area is limited; the effect of

76   biochar amendment on the net availability of inorganic N to soil nitrifiers and denitrifiers and the

77   subsequent effect on soil N_2O emissions is poorly understood (Clough et al., 2013). This represents a

78   significant knowledge gap in determining the potential for biochar to contribute to climate change

79   mitigation. To address this knowledge gap, we analysed those soil N cycling processes that control

80   substrate availability for N_2O production (i.e. denitrification, nitrification, immobilisation and

81   mineralisation) in fertilised, near-saturated soil amended with biochar. Our aim was to identify

82   whether biochar affects the availability and transformations of N in arable soils underlying soil N_2O

83   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_4NO_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 $^{15}NH_4NO_3$ 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_2O production (Weier et al., 1993). Pre-tests had demonstrated that soil

109   CO_2 emissions were linear, and O_2 concentrations adequate over at least four days of enclosure, so the

110   containers were sealed for the duration of the incubation to enable a mass balance to be calculated.

111   At four time points after ^{15}N addition (30 mins, 1, 2 and 4 days), four replicates of each treatment

112   were destructively sampled for total C and N content, soil pH, gravimetric moisture content (GMC),

113   extractable soil NH_4^+ and $^{15}NH_4^+$, NO_3^- and $^{15}NO_3^-$, and organic N and ^{15}N concentrations (methods in

114   Section 3.3). The first sampling time point was chosen as 30 minutes after ^{15}N addition, when it was

115   assumed that the chemical or physical immobilisation of N was completed, and any further N

116   immobilisation came exclusively from biological processes (Mary et al., 1998).

117   At seven time points following ^{15}N addition (0, 0.5, 1, 1.5, 2, 3 and 4 days), 10 ml gas samples were

118   taken from the soil container headspace for N_2O and CO_2 analysis using a gas-tight syringe and

119   injected into evacuated 3 ml vials (Labco, USA). For $^{15}N_2O$ analysis, 80 ml headspace samples were

120   injected into evacuated 60 ml glass serum bottles (Wheaton Science Products, USA). After gas

121   samples were removed, laboratory air of equivalent volume (N_2O and CO_2 concentration analysed)

122   was injected into the enclosed sample headspace. This dilution of laboratory air was taken into

123   account in the final calculations of GHG emissions.

124   2.2   Gas sampling and N_2O source separation

125   Headspace gas samples were analysed for N_2O and CO_2 concentrations using the same Gas

126   Chromatograph system (PerkinElmer Autosystem XL, PerkinElmer, USA) described in Case et al.

127   (2014) and calibrated against certified standards (Air Products, UK).

128   For $^{15}N_2O$ analysis, ~ 4 ml of the 80 ml sample was injected into a TraceGas Preconcentrator coupled

129   to an isotope ratio mass spectrometer (IRMS, Isoprime Ltd, UK) whereupon the sample was directed

130   through a series of chemical traps to remove H_2O and CO_2. The N_2O was cryogenically trapped under

131   liquid N. The waste was flushed out, and then the N_2O was further cryofocused in a second liquid N

132   trap prior to being introduced onto a 25 m x 0.32 mm Poraplot Q column (Chrompack column,

133   Varian, UK). The column separated N_2O from any residual CO_2, and both entered the IRMS via an

134   open split. The retention time between the first eluting CO_2 ($< 2^{E-10}$ amplitude) and second eluting

135 N_2O peak typically fell in the range between 60 - 70 seconds to avoid isobaric interference of the CO_2

136 with the calculated ^{15}N. The N_2O was directed towards the triple collectors of the IRMS where m/z 44,

137 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

138 were then recorded for each sample and delta values for both ^{15}N were calculated with respect to N_2O

139 reference gas (BOC Industrial Gases, UK).

140 The experimental design allowed us to differentiate the source of N_2O emissions from nitrification +

141 nitrifier-denitrification and denitrification. The proportions of soil N_2O emissions attributed to the two

142 processes were calculated using Equation 1, based on data from the analysis of the $^{15}NO_3^-$ labelled soil

143 treatment (Mathieu et al., 2006). Outputs greater than 100% and lower than 0% were rounded to the

144 nearest boundary.

$$d = \frac{(a_m - a_n)}{(a_d - a_n)} \; with \; a_d \neq a_n \tag{1}$$

145 Where 'd' is the proportion of N_2O emissions from denitrification in a time period, 'a_m' is the average

146 % ^{15}N atom enrichment of the N_2O mixture during the time period, 'a_n' is the average % ^{15}N

147 enrichment of the nitrification pool (NH_4^+) during the time period and 'a_d' is the average % ^{15}N

148 enrichment of the denitrification pool (NO_3^-) during the time period.

2.3   Analysis of soil properties and soil N isotopic composition

150 Extractable inorganic NH_4^+ and NO_3^- concentrations were determined using 5 g d. wt. equivalent of

151 wet soil and 50 ml of 0.8 M potassium chloride (KCl, 6 %). The samples were shaken for 1 hour, and

152 then filtered through Whatman no. 44 filter paper disks (Whatman, USA). Extracts were analysed on

153 a Seal AQ2 analyser (Bran and Luebbe, UK) using discrete colorimetric procedures (Maynard and

154 Kalra, 1993).

155 Extractable inorganic ^{15}N concentrations ($^{15}NH_4^+$ and $^{15}NO_3^-$) were analysed following the acidified

156 disk method (Khan et al., 1998). First, inorganic N was extracted from soil, using 2 M KCl and the

157 same method as that described for inorganic N extraction above. Then, 20 ml of the extract was

158 placed in air-tight 500 ml glass jars (Kilner, USA). For $^{15}NH_4^+$ concentrations, 0.2 g of magnesium

159   oxide (MgO) was added. For $^{15}NH_4^+ + {}^{15}NO_3^-$ concentrations, 1 ml of 0.2 M sulfamic acid was added

160   to decompose NO_2^-, followed by 0.2 g of MgO and 0.2 g Devarda's alloy. Whatman no. 41 filter

161   paper disks (Whatman, USA) were suspended above the solution with added 5 μl of 2.5 M potassium

162   hydrogen sulphate solution. The jars were sealed and placed in a 30 °C environment for at least 72

163   hours to enable near 100 % adsorption of the extractant N. The filter disks were then dried at 40 °C

164   for 24 hours.

165   Three-quarters of each of the two filter papers were weighed together and sealed in a single tin

166   capsule (Elemental Microanalysis Ltd, UK). The samples were combusted using an automated

167   NA1500 elemental analyser (Carlo Erba, Italy) coupled to an IRMS (Dennis Leigh Technology, UK).

168   Atom % abundances of $^{15}NO_3^-$ were calculated from the ($^{15}NH_4^+ + {}^{15}NO_3^-$) atom % abundance and

169   respective inorganic N concentrations using the method described in Khan et al., (1998).

170   Organic ^{15}N contents were used as an analogue for microbial biomass and were assumed to have an

171   atom % ^{15}N excess of 0.0025 % (Mary et al., 1998). First, 3 g of soil was oven dried at 80 °C for 24

172   hours, and then the dried soil was mixed with 10 ml of 1 M KCl in a 12 ml polystyrene test tube and

173   mechanically shaken for 15 minutes. The tube was then centrifuged for 15 minutes at 3,000 rpm and

174   subsequently the KCl was removed and replaced (Recous et al., 1998). This process was repeated four

175   times. Afterwards, the soil was dried at 80 °C for 24 hours. 50 mg of dried soil was sealed in a tin

176   capsule and analysed in the same fashion as described for the analysis of the acidified disks above.

177   The total C and N contents of dried, ground soil samples (0.1 g, < 1 mm) were analysed using a Tru-

178   spec total CN analyser (Leco Corp., USA) (Sollins et al., 1999). Gravimetric moisture content, soil pH

179   (soil: H_2O, 1: 2.5), particle density, BD and WFPS analyses were conducted according to standard

180   methods (Blake, 1965; Emmett et al., 2008; Ohlinger, 1995a, 1995b).

2.4   Estimating soil N transformations with and without biochar

182   To assess whether biochar amendment affected the availability and transformations of soil N

183   underlying N_2O production, we quantified mineralisation, immobilisation, nitrification and

184   denitrification rates using the FLUAZ numerical N-cycling model (Mary et al., 1998). The model

185   consists of two parts (Mary et al., 1998). First, a numerical model that solves differential equations

186   from the N and ^{15}N mass equations based on a 4th order Runge-Kutta algorithm with a variable time

187   step. Second, a non-linear fitting program to calculate N rates based on Marquardt's algorithm

188   (Marquardt, 1963).

189   Inorganic N, organic N and respective ^{15}N concentrations were input into the FLUAZ model and

190   analysed using a paired treatment design. The final model fitted mineralisation ('m + s',

191   mineralisation of soil organic N and biochar-derived N to NH_4^+), nitrification ('n', the conversion of

192   NH_4^+ to NO_3^-), immobilisation of NH_4^+ and NO_3^- ('ia' and 'in', the sum of NH_4^+ and NO_3^- taken up by

193   the organic N pool) and denitrification rates ('kd', the sum of conversion of NO_3^- to N_2O, NO or N_2),

194   over three time periods following ^{15}N addition (30 minutes - 1 day, 1 - 2 days, 2 - 4 days).

195   For the FLUAZ model analysis we made several assumptions. As the incubation only lasted for four

196   days, and the temperature was maintained at 16 $^{\circ}$C it was assumed that remineralisation of

197   immobilised N ('r') was negligible (Murphy et al., 2003). It was also assumed that the conversion of

198   plant residue N directly into microbial biomass ('j', N humification) and ammonia volatilisation were

199   negligible (Mary et al., 1998; Whitehead and Raistrick, 1990).

2.5 Statistical analysis

201   Student's t tests were used to test for significant differences in soil N_2O and CO_2 emissions, inorganic

202   N contents, total C, N and pH between un-amended and amended soil. For all statistical analyses the

203   software package R was used (version 3.0.2, The R Project, 2013).

3 Results

Cumulative soil N_2O emissions after four days were suppressed by 91 % with biochar amendment, from 0.61 ± 0.20 to 0.05 ± 0.02 mg $N_2O\text{-}N$ 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 $CO_2\text{-}C$ 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_2O emissions (Fig. 1c, d), and soil NH_4^+ and NO_3^- concentrations (Fig. 1e, f), soil N_2O emissions were source partitioned over the four-day incubation period. Nitrification + nitrifier denitrification produced 40% and 33% of N_2O emissions in amended and un-amended soils respectively from day 0 to 2 (Fig. 1e, f). Between day 2 and 4, all soil N_2O emissions were produced via denitrification in both treatments. Considering the entire four-day incubation, 95% of un-amended soil N_2O 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 $2,162 \pm 46$ 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.

230   Total N recovery was calculated from inorganic, organic N and respective ^{15}N concentrations in the

231   soil. Total N recovery for the ^{15}N-labelled NO_3^- treatments remained close to 100 % throughout the

232   incubation, whereas it was lower for the ^{15}N-labelled NH_4^+ treatments (typically above 80 %, but

233   attained a minimum of 62 % on day 4 in the amended treatment, Fig. 2e, 3e).

234   Cumulative mineralisation, nitrification, denitrification and immobilisation of N over 4 days were

235   estimated by using the FLUAZ model. Cumulative denitrification after four days was 37 % lower in

236   amended than in un-amended soil (0.17 and 0.27 mg N kg^{-1} respectively, Table 1). Mineralisation of

237   N and nitrification were greater in amended compared to un-amended soil. Cumulative mineralisation

238   was 55.0 in amended soil compared to 14.9 mg N kg^{-1} in un-amended soil (269 % greater), and

239   cumulative nitrification was increased by 34%, from 75.6 to 101.1 mg N kg^{-1} (Table 1).

240   The magnitude of initially immobilised N (within 30 minutes of ^{15}N addition) was similar in biochar-

241   amended (5.7 mg N kg^{-1}) and un-amended (5.5 mg N kg^{-1}) soils (Table 1). Biological NH_4^+

242   immobilisation over the subsequent four days was 50 % greater in amended compared with un-

243   amended soil according to the FLUAZ outputs (17.6 and 11.9 mg N kg^{-1}, respectively, Table 1). Soil

244   NO_3^- immobilisation only increased between day 0 and 2 (Table 1). After two days, NO_3^-

245   immobilisation was 17 % lower in amended than un-amended soil (7.8 compared to 9.4 mg N kg^{-1},

246   Table 1).

247   Biochar amendment significantly altered soil physico-chemical properties. Soil pH increased from

248   6.31 ± 0.03 to 6.62 ± 0.03 in amended soil ($p < 0.001$, Table 2). Total soil C content was also greater

249   in amended treatments (3.71 ± 0.19 compared to 1.99 ± 0.01 mg C kg^{-1}, $p < 0.001$, Table 2), while

250   total N contents were similar. The soil C: N ratio increased with biochar amendment ($p < 0.001$, Table

251   2).

4 Discussion

Suppression of soil N_2O 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 N_2O 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 N_2O 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 N_2O emissions were attributed to denitrification (Mathieu et al., 2006).

Our findings indicated that denitrification was the dominant source of N_2O emissions and that N_2O emissions from both denitrification and nitrification were suppressed by biochar addition. The suppression of soil N_2O emissions from denitrification may have been due to reduced denitrifier activity or increased complete denitrification (i.e. increased conversion of N_2O to N_2). 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. NO_3^-) for denitrifying organisms. We observed that initial concentrations of NO_3^- 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 NO_3^- 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

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279   may be mineralised in a matter of weeks (de la Rosa and Knicker, 2011; Hilscher and Knicker, 2011).

280   The biochar addition rate used in this study added 142 mg N kg^{-1} soil in organic form, some of which

281   may have been mineralised during the incubation. Alternatively, the addition of labile C as fresh

282   biochar to this relatively low C agricultural soil may have stimulated soil microbial activity, priming

283   the mineralisation of native soil C and the release of bound N (Luo et al., 2011; Nelissen et al., 2012).

284   We could not discern the source of mineralised N (biochar or native soil organic matter) using this

285   experimental design. This could be investigated using ^{15}N-labelled biochar to differentiate between

286   biochar and SOM-derived mineralised N.

287   Cumulative nitrification was also increased with biochar (34 %), with nitrification rates greater than

288   20 mg N kg^{-1} per day in biochar-amended soil (Table 1). An increase in nitrification with biochar

289   addition is consistent with previous biochar studies, although the magnitude of effect has been

290   observed to vary with N addition rate (Nelissen et al., 2012; Prommer et al., 2014). For example,

291   nitrification rates between 1 and 9 mg N kg^{-1} were observed following the addition of < 5 mg N kg^{-1}

292   of inorganic N (Nelissen et al., 2012; Prommer et al., 2014). Possible explanations for this increase in

293   nitrification include increased soil pH or increased soil NH_4^+ concentrations as a result of biochar

294   amendment (Mørkved et al., 2007; Norton and Stark, 2011). Soil pH, which was greater than 6.3 in

295   this study, has been found to have little effect on nitrification rates above pH 5 (Mørkved et al., 2007),

296   and so does not explain the increased nitrification observed here. Furthermore, we did not directly

297   observe an increase in NH_4^+ concentrations in soil with biochar, although there was a more rapid

298   increase in NO_3^- concentrations (Fig. 2c, 3c). Assuming that the NH_4^+ provided by freshly mineralised

299   organic N was rapidly nitrified, we suggest that biochar amendment did increase soil NH_4^+ availability

300   (Fig. 2c, 3c).

301   Examining N immobilisation more closely, we found that initial chemical or physical N

302   immobilisation was minimal following biochar addition (Table 1). Furthermore, biological N

303   immobilisation in un-amended and amended soil was also small relative to the magnitude of

304   mineralisation and nitrification; equivalent to less than 8 % of the initial soil NH_4^+-N content (Table 1,

305   Fig. 1a, 2a). This magnitude of N immobilisation was insignificant compared to total inorganic N

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306 availability in soil and therefore was not sufficient to explain the 91 % suppression of soil N_2O

307 emissions. This corroborates findings from a similar study in which hardwood biochar suppressed soil

308 N_2O emissions in excess N conditions (Cayuela et al., 2013). The rates of biological NH_4^+

309 immobilisation reported here (2 - 9 mg N kg^{-1} d^{-1}) were greater than those from a comparative study

310 of maize biochar (~ 2 mg N kg^{-1} d^{-1}), potentially due to greater N addition rates in this study (< 3 mg

311 N kg^{-1} compared to 100 mg N kg^{-1}) (Nelissen et al., 2012). We also observed a small decrease in NO_3^-

312 immobilisation in biochar–amended soil (- 1.6 mg N kg^{-1}), possibly as a result of decreased anion

313 exchange capacity and increased soil pH (Nelissen et al., 2012).

314 Increased mineralisation, nitrification, insignificant increases in N immobilisation, and similar final

315 NO_3^- concentrations are indicative of similar N substrate availability to soil nitrifiers and denitrifiers

316 in biochar-amended compared to un-amended soil. Despite similar N availability, soil N_2O emissions

317 were significantly decreased. We therefore concluded that the processes underlying N supply (from

318 mineralisation, nitrification and immobilisation) did not explain the suppression of soil N_2O emissions

319 in biochar-amended soil, or reduced denitrification rates. Alternative hypotheses to explain reduced

320 denitrification rates include: pH increase (Šimek et al., 2002); the capacity of biochar to act as an

321 electron sink for NO_3^-, therefore competing with soil denitrifiers (Cayuela et al., 2013); or the

322 presence of inhibitory compounds in biochar (Quilliam et al., 2012; Spokas et al., 2011, 2010;

323 Taghizadeh-Toosi et al., 2011). The first two hypotheses were not supported by evidence from this

324 study: the increase in soil pH was relatively small (0.3), and the biochar did not contain significant

325 amounts of magnesium (0.24 %) or iron (0.32 %) compared to other biochars that could act as

326 electron acceptors (from supplementary information of Case et al. (2012). We would therefore suggest

327 that the presence of inhibitory compounds in biochar and their effects on denitrification should be the

328 focus of further research.

329 As discussed above, suppression of N_2O emissions could result from reduced rates of denitrification,

330 however it could alternatively result from a difference in the proportions of N_2O, N_2 and NO produced

331 through denitrification, (e.g. a reduction in the N_2O: N_2 ratio) (Baggs, 2011). This was demonstrated

332 in a recent study which showed that biochar consistently reduced the N_2O: N_2 ratio promoting the last

333   step of denitrification (Cayuela et al., 2013).We did not analyse N_2 emissions as a part of this study

334   and so could not confirm this finding. The observed increase in soil pH may, however, have either

335   directly decreased the proportion of $N_2O: N_2$ emitted from soil, or enabled the biochar to act as an

336   'electron shuttle' increasing the transfer of electrons to denitrifying bacteria (Cayuela et al., 2013). On

337   addition, the incorporation of biochar into the soil introduces fresh labile C which may have increased

338   the conversion of N_2O to N_2, by increasing the availability of C electron acceptors for denitrifying

339   organisms (Azam et al., 2002; Morley and Baggs, 2010; Saggar et al., 2013; Senbayram et al., 2012).

340   In this study we observed a 75 % increase in soil CO_2 emissions with biochar amendment (Fig. 1b),

341   equivalent to 0.35 % of the biochar C added to the soil (assuming that biochar emission did not prime

342   the mineralisation of soil C), indicating that a significant proportion of labile C was present in the

343   biochar. This provided evidence in support of this mechanism but was not conclusive.

344   Taken together, the evidence presented in this study indicates that the supply of inorganic N, and

345   particularly NO_3^-, to N_2O-producing organisms was not a limiting factor constraining soil N_2O

346   emissions in biochar-amended soil. Future research should focus on the potential of inhibitive

347   substances and labile C in biochar to alter the $N_2O: N_2$ ratio from denitrification.

5 Conclusions

Biochar amendment has been observed to suppress soil N_2O 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 N_2O 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 N_2O 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 N_2O 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 N_2O emissions.

6 Acknowledgements

373 **Table 1.** The effect of biochar amendment on soil N cycling processes in soils treated with ^{15}N-

374 labelled NH_4NO_3 and wetted to 90% WFPS for 4 days from N addition. Nitrogen transformations

375 were estimated from the FLUAZ model, described in Section 3.4.

|		Cumulative production (mg N kg^{-1})		
Nitrogen cycling process	Day	0-1	0-2	0-4
Mineralisation	Un-amended	-8.2	9.3	14.9
	Amended	9.8	25.2	55
Nitrification	Un-amended	17.2	34.8	75.6
	Amended	23.4	54.9	101.1
NH_4^+ immobilisation	Un-amended	9.2	9.7	11.9
	Amended	6.7	6.9	17.6
NO_3^- immobilisation	Un-amended	3.1	9.4	9.4
	Amended	6.8	7.8	7.8
Denitrification	Un-amended	0.06	0.12	0.27
	Amended	0.00	0.15	0.17

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377

378 **Table 2.** The effect of biochar amendment on physico-chemical properties of soil, treated with ^{15}N-

379 labelled NH_4NO_3 and wetted to 90% WFPS. Values represent mean ($\pm$ standard error) of analyses

380 from four time points following addition: 30 minutes, 1 day, 2 days and 4 days. Asterisks indicate

381 significant difference between adjacent un-amended and amended soils: *** = $p < 0.001$.

Biochar amendment	Total C (%)	Total N (%)	CN ratio	pH
Un-amended	1.99 (0.03)	0.26 (0.001)	7.94 (0.41)	6.31 (0.03)
Amended	3.71 (0.19) ***	0.27 (0.001)	13.90 (1.29) ***	6.62 (0.03) ***

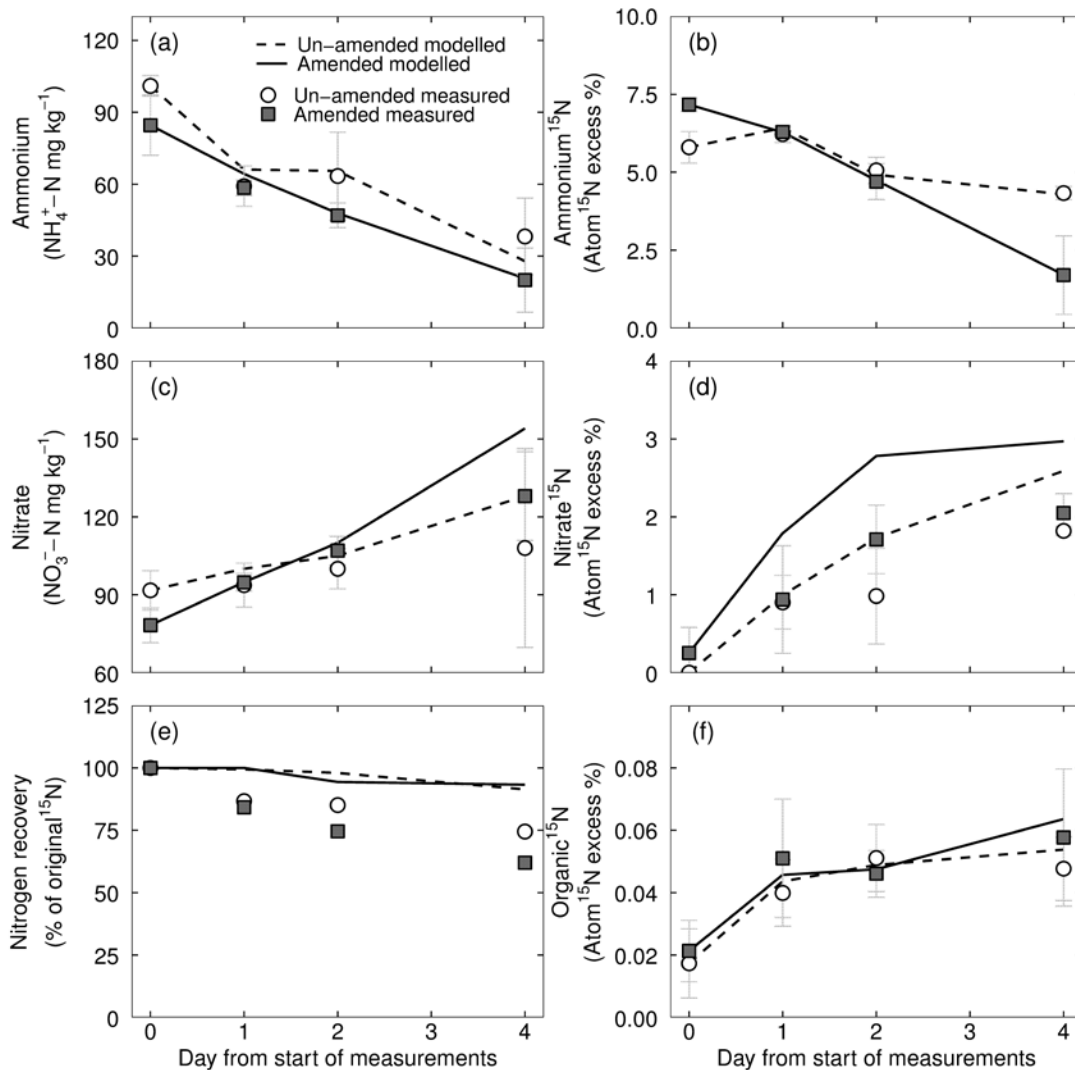382

383

Fig. 1. The effect of biochar amendment on (a) cumulative soil N_2O production, (b) cumulative soil
385 CO_2 production, mean soil N_2O ^{15}N flux in un-amended and biochar-amended soils treated with (c) ^{15}N-
386 labelled NH_4^+ or (d) ^{15}N-labelled NO_3^-, and the source partitioning of soil N_2O emissions attributed to
387 denitrification and nitrification + nitrifier denitrification in (e) un-amended and (f) biochar-amended
388 soils treated with ^{15}N-labelled NO_3^-. Data points for graphs a) – d) represent mean ± standard error.
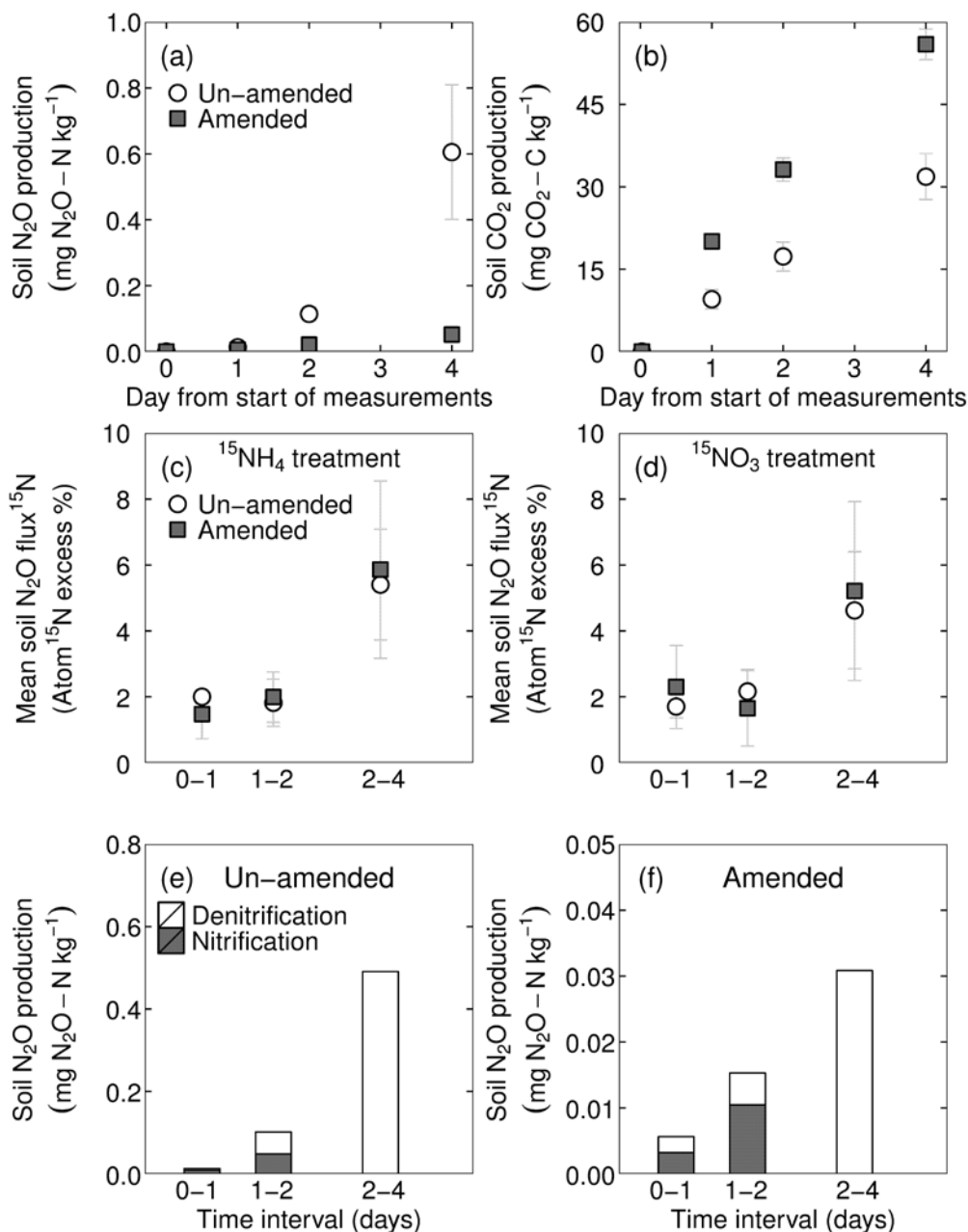

389

390 **Fig. 2.** The effect of biochar amendment on soil inorganic-N concentrations and ^{15}N atom abundance.
391 in soils labelled with $^{15}NH_4^+$. Soil properties presented are: (a) soil extractable NH_4^+ concentration; (b)
392 soil NH_4^+ atom ^{15}N % excess; (c) soil extractable NO_3^- concentration; (d) soil NO_3^- atom ^{15}N %
393 excess; (e) % N recovery of ^{15}N measured at t_0; and (f) soil organic N atom ^{15}N % excess. Points
394 indicate the mean of directly measured values ± standard error (n = 4), whereas lines indicate
395 simulated values from FLUAZ model analysis.

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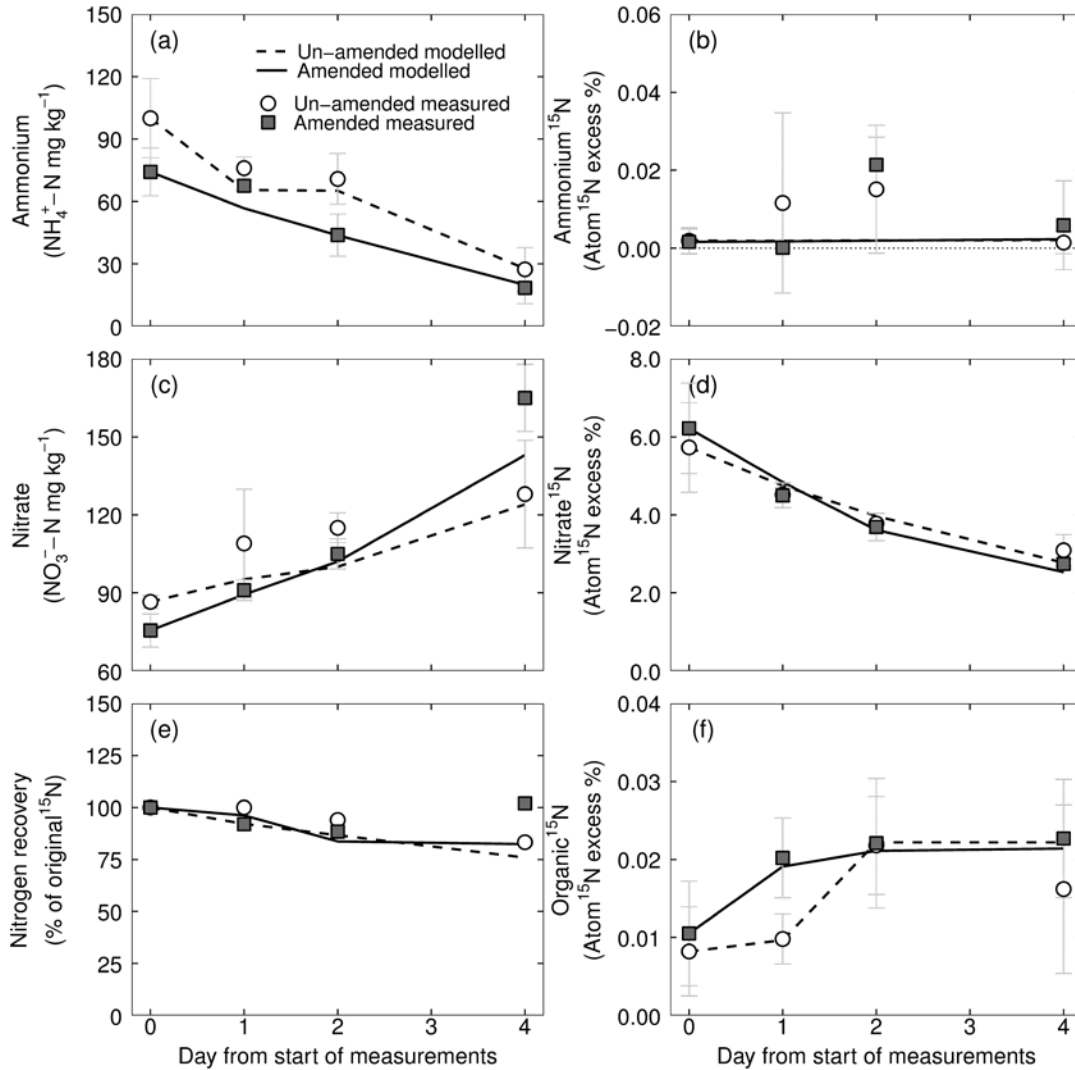
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