Greenhouse gas emissions and soil properties following amendment with manure-derived biochars: Influence of pyrolysis temperature and feedstock type

HIGHLIGHTS

- Manure-derived biochars are richer in soil available nutrients than standard biochar.
- Nitrates and sulphates availability in soils is higher in biochar from manure than in standard biochar.
- Low temperature manure-derived biochars increase N_2O emissions when compared with standard biochar (from gasification of wood chips).
- N_2O emissions, as well as CO_2 emissions, were positively correlated with volatile matter and N content of biochars.
- Manure-derived biochars are less effective for C sequestration with respect to standard biochar, which does not show other important effects on nutrient.

Greenhouse gas emissions and soil properties following amendment with manure-derived biochars: influence of pyrolysis temperature and feedstock type

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Abstract

Manure-derived biochars can offer a potential option for the stabilization of manure, while mitigating changing climate through carbon sequestration and the attenuation of nitrous oxide emission. A laboratory incubation study was conducted to assess the effects of four different manure-derived biochars produced from different feedstocks (poultry litter and swine manure) at different temperatures (400 or 600 °C). A commonly available standard wood chip biochar, produced at a greater temperature (1000 °C), and non-amended treatments were used as references. Two different soils (sandy and silt-loam) were amended with 2% (w/w) biochar on a dry soil weight basis (corresponding to 20 Mg ha^{-1}), with the soil moisture being adjusted to 75% saturation level. After a pre-incubation period (21 days), 170 kg N ha^{-1} of NH_4NO_3 fertilizer was added. Measurements of CO_2, N_2O, CH_4 emissions and soil N mineralisation were carried out on different days during the 85 days of incubation. The net C mineralization and N_2O emissions from both soils amended with poultry litter biochar at 400°C were significantly greater than the other biochar treatments. Nitrate availability was greater in both soils in which the manure-derived biochar was used instead of the standard biochar. All of the biochars increased the pH of the silt-loam, sub-acid soil, but failed to improve the cation exchange capacities (CEC) in either soil. Total C and N, P, K and Mg (except Ca) were significantly increased in the manure-derived biochar amended soils, compared to the Control, and were positively correlated to the biochar nutrient contents. This study indicates that the soil application of biochar engenders effects that can vary considerably according to the biochar properties, as determined on the basis of the feedstock types and process conditions. Low-temperature biochar production from manure represents a possible way of producing a soil amendment that can stabilize C while supplying a significant quantity of nutrients.

1. Introduction

Biochar obtained from the thermo-chemical conversion of biomass is being promoted as a potential solution to reduce greenhouse gases (GHGs) (Gaunt and Lehmann, 2008), and as a beneficial soil amendment (Forbes et al., 2006). It has been claimed that biochar has the potential to reduce the impact of agricultural systems on global warming through carbon (C) sequestration and nitrous oxide (N_2O) suppression (Sohi et al., 2010; Taghizadeh-Toosi et al., 2011; Case et al., 2014; Van Zwieten et al., 2014; Gwenzi et al., 2015). The enhanced physico-chemical properties of biochar amended soils are believed to be the result of increased nutrient retention and availability, a greater water holding capacity, CEC and surface area, and a rise in pH in acidic soils (Liard et al., 2010; Lehmann et al., 2011; Anderson et al., 2011; Jeong et al., 2015). The improved biological properties are linked with the increased microbial diversity along with the provision of a suitable aerated habitat for soil flora and fauna due to the porous nature of biochar (O'Neill et al., 2009; Lehmann et al., 2011). However, all of the aforementioned effects vary considerably according to the specific properties of the biochar. Jeffery et al. (2015) and Gwenzi et al. (2015) have reviewed the possible trade-offs between the expected biochar benefits, potential risks and associated uncertainties, as this debate continues to grow among biochar researchers.

The stability of biochar in soils and its interaction with the soil micro-organisms that are responsible for its decomposition depend on several factors, such as the nature of the biochar feedstock, the type of process used for the biochar production (dry/wet pyrolysis, slow/fast pyrolysis, gasification), and the operating conditions (temperature as well as residence time) (Lehmann et al., 2011; Ameloot et al., 2013; Jeong et al., 2015). The microbial degradation of biochar C in soil is often associated with labile organic compounds, such as alkanoic and

benzoic acids, and phenols, whose concentration falls with increasing pyrolysis temperature (Liang et al., 2010; Novak et al., 2010; Graber et al., 2010; Troy et al., 2014). Biochars produced from plant residues contain stable aromatic structures and are more resistant to microbial attack (Foereid et al., 2011; Ippolito et al., 2012; Case et al., 2014; Jeong et al., 2015).

The results of N_2O emissions from biochar amended soils are not consistent. Some authors reported decreased N_2O emissions (Rondon et al., 2005; Van Zwieten et al., 2010; Taghizadeh-Toosi et al., 2011; Jia et al., 2012; Nelissen et al., 2014; Martin et al., 2015), some reported no-effects (Clough et al., 2010; Nelissen et al., 2014), and yet others reported increased N_2O emissions (Yanai et al., 2007, Scheer et al., 2011; Troy et al., 2013). Increased soil porosity, associated with soil biochar amendment, results in improved soil aeration, which ultimately suppresses N_2O emissions (Richardson et al., 2009; Clough et al., 2013), whereas the anaerobic conditions, a more biochar labile C content, and conditions that favour a greater abundance of denitrifiers in the soil, due to biochar additions, can lead to increased soil N_2O emissions (Cayuela et al., 2014; Kammann et al., 2012; Dalal et al., 2003). Therefore, the extent of N_2O emissions from biochar-amended soils is greatly affected by the fertilizer types (Nelissen et al., 2014), biochar C:N ratio and the nature of C present (Troy et al., 2014; Zhu et al., 2014), biochar porosity, surface area and particle size (Jeong et al., 2015; Martin et al., 2015) and the response of denitrifiers (Van Zwieten et al., 2014). Despite many studies being conducted (both incubation and in field), there still remain substantial scientific doubts on the mechanisms by which biochar affects soil N_2O emissions, thus seeking further investigation studies (Troy et al., 2013).

The modification of N dynamics on biochar amended soil has been suggested to have shift (either increase or decrease) on GHG emissions and the availability of other nutrients after fertiliser application (Clough and Condron, 2010; Clough et al., 2013). Enhanced crop growth, associated with increased efficiency in N use and reduced leaching in biochar-amended soils, has also been reported by some authors (Major et al., 2012; Schomberg et al., 2012). The immobilization of N can occur when a biochar with a large C:N ratio is amended in a soil with a smaller C:N ratio (Kuzyakov et al., 2009).

Pyrolysis may play a role in the manure processing chain, especially in EU regions where intensive livestock production exist, via energy production, nutrients recovery, and reducing both the manure volume and transportation costs (Cantrell et al., 2007; Hossain et al., 2011). Additionally, the deployment of biochars derived from the pyrolysis of locally available resources as a soil conditioner can be a strategically important option for soil fertility improvement and mitigation of GHG emissions (Steiner et al., 2007; Kimetu et al., 2008). While biochars derived from woody biomass and their effects on soil have been reported extensively, there have been fewer reports on the use of biochar derived from animal manures in which amendment effects in different soil types are compared.

A laboratory incubation study was undertaken, with two different soil types, to test the following hypothesis: (i) manure-derived biochars play a different role in amending the soil than wood-derived biochars, and could combine the agronomic advantages of manure and the environmental benefits of biochar (ii) the feedstock type and pyrolysis temperature can be used as a means to modulate the expected effects of manure-derived biochars on GHG emissions and soil properties; (iii) the effects of such biochars also vary according to the soil type. Therefore, the N_2O and CH_4 emissions, the potential for C sequestration, nutrient

availability and the final soil quality (chemical) were measured, after amending two soils (sandy and silt-loam) with biochars from different feedstocks (poultry and swine manures) at two temperatures (400 or 600 °C), and were compared with a readily available standard wood-derived biochar.

2. Materials and Methods

2.1. Soil collection and characterization

Two types of soil with contrasting characteristics were used in this experiment (Table 1): i) a less C content sandy soil with sub-alkaline pH, designated "Soil A", and ii) a more C content silt-loam soil with sub-acidic pH, called "Soil B" (USDA, 2013). They were both collected from the top 20 cm of arable fields (NW Italy) in order to mimic the ploughed layer. They were subsequently air-dried and mechanically sieved to below 2 mm, using an electric auto-rotating sieving device (Neotron s.r.l., Autopack NTR 83). Both soils were less in N, P and K contents, but rich in Ca content (Table 1).

2.2. Biochar production and characterization

Four different manure-derived biochars, produced from two different feedstocks (poultry litter and swine manure) at two different temperatures (400 or 600 °C), were used for this experimental study. The poultry litter biochars ("PL400" and "PL600") were produced at the University of Limerick in Ireland, using a laboratory pyrolysis plant. The swine manure biochars ("SM400" and "SM600") were supplied by ECN, (the Netherlands) (www.ecn.nl). These manure-derived biochars were compared with the most widely available biochar i.e.

wood chip biochar produced at a greater temperature (1000 °C) ("WC"), and with another control treatment i.e. unamended soil ("Control"). The wood chip biochar was produced from kiwi fruit pruning residue via industrial gasification (1000 °C) at Agrindustria, Italy (www.agrind.it).

The biochars were analysed for their total C and N contents by means of elemental analysis (Vario El Cube CNS analyser, Elementar, Germany). The pH was measured in deionized water at a 1:10 biochar-water ratio using a pH electrode (Cyberscan 510 pH meter) after shaking the mixture for 18 hours. The moisture, ash and volatile matter (VM) contents were analysed according to the NSAI standard testing method (NSAI, 2009). Available P was extracted in a 2% formic acid solution and measured spectrophotometrically (Wang et al., 2012). Calcium, Mg and K were analysed by Atomic Absorption (Varian Techtron AA6), following acid digestion (Cantrell et al., 2012). The CEC of the samples was determined by means of sodium chloride adsorption and potassium nitrate displacement method (Silber et al., 2010). The surface acidity of the chars was determined by means of the base adsorption method reported by Cheng and Lehmann (2009). The surface area and porosity were determined according to the Brunauer, Emmet and Teller (BET) method, on the basis of the measurements obtained by N_2 adsorption at 77K using ASAP-2400 Micrometrics apparatus. Each sample was analysed in triplicate.

2.3. Experimental incubation setup

The incubation experiment was carried out in a controlled climate (20 °C, 65% relative humidity) and set-up, as a randomised complete block design, with four replicates. Each experimental unit consisted of a cylindrical glass jar (volume = 3 L, diameter = 7 cm, height =

20 cm). Air-dried and sieved soil (1.5 kg for each jar) was manually mixed with each biochar type at 2% w/w, corresponding to an areal application rate of 20 Mg ha^{-1}, given the filling height of 10 cm of the soil-biochar mixture inside the jar. All the soils were then moistened with deionized water in order to reach 75% saturation. The amount of deionized water that had to be added to each jar in order to reach the desired moisture level was calculated on the basis of soil porosity, and corresponded to 380 ml for Soil A and 430 ml for Soil B. The soil water content was adjusted every two days, both during pre-incubation and the subsequent incubation periods. The jars were pre-incubated until the initial CO_2 flux, due to soil rewetting, had subsided (21 days). All of the soil-biochar mixtures were then fertilized by applying a 170 kg N ha^{-1} (maximum limit set by the Nitrate Directive) of ammonium nitrate solution onto the soil surface.

2.4. Gas sampling and measurement

Gas samples were withdrawn from the jar headspace (1.8 m^3 for soil A, 1.9 m^3 for soil B, corresponding to 60.0% and 63.3% of total jar volume, respectively) twice per week during pre-incubation (before fertilization), daily during the week immediately after fertilization, three times per week during the following week, twice per week thereafter for the following four weeks and once a week for the final four weeks, for a total period of 85 days (21 pre-incubation and 64 incubation days).

During gas sampling, the jars were hermetically sealed with a lid equipped with two stopcocks. Gas samples were taken at 0, 8 and 16 minutes after closing the jars. Approximately 35 mL of gas was extracted from the jars using a polyethylene syringe (60 mL), and was replaced within the headspace by an identical volume of zero-grade air in order

to minimize any pressure reduction due to gas sampling. Of the 35 mL of gas sampled, 5 mL was used to flush the syringe needle and the remaining 30 mL was transferred immediately into pre-evacuated glass vials (EXETAINER®, 12 mL capacity, screw caps and butyl rubber septa). The temperature was recorded during each sampling event to calculate the actual gas concentration. The gas samples were analysed immediately after sampling, using a gas chromatograph (GC, Model 7890A, Agilent Tech., USA), equipped with an electron capture detector to quantify N_2O, and thermal conductivity and flame ionization detectors to quantify the CO_2 and CH_4 concentrations, respectively. Fluxes were calculated from the linear or nonlinear (Hutchinson and Mosier, 1981) increase in concentration (selected according to the emission pattern) in the chamber headspace over time. The cumulative emissions of N_2O, CO_2, and CH_4 were calculated throughout the whole incubation period, assuming a linear flux change between each subsequent sampling event.

2.5. Mineral N, PO_4^{3-}, Cl^- and SO_4^{2-} measurements

A Rhizon soil moisture sampler (diameter = 2.5 mm, length = 10 cm; Rhizon SMS, product no. 19.21.25, www.rhizoshere.com) was inserted horizontally into the soil column of each jar (one per jar) by drilling a hole (diameter = 3.5 mm) 1 cm above the base of the jar. Soil water extraction was undertaken every two weeks, creating a vacuum by means of a syringe (60 mL) connected to the Rhizon sampler. The mineral N, phosphate (PO_4^{3-}), chloride (Cl^-) and sulphate (SO_4^{2-}) concentrations in the soil extracts were then analysed by means of ion chromatography (Dionex, Thermo Scientific ASE 150).

2.6. Post incubation analysis

At the end of the incubation period, a complete chemical characterization of the soil-biochar mixtures was carried out. The soils were analysed for pH, total C, total N, available P and cation exchange capacity. Furthermore, soil mineral N (both NH_4^+ and NO_3^-) was analysed colorimetrically using a continuous flow auto-analyser (System Alliance Evolution II). For this analysis, 30 g of fresh soil was extracted with 150 mL of 1 M KCl (1:5 ratio), and the mixture was shaken for 30 minutes, using a mechanical shaker, and then filtered.

2.7. Data processing and analysis

The data were analysed by two-way ANOVA (IBM SPSS statistics 20), considering the biochar and soil types as the main factors. Statistical significance was tested at $p<0.05$. A Kolmogorov-Smirnov test was used to test normality of the data distribution, and homoscedasticity was verified with Levene's test. Log transformations of any data that did not fulfil the assumptions were carried out prior to the analysis. A Bonferroni post-hoc test was applied to investigate any differences between the biochar types, in terms of cumulative CO_2 and N_2O emissions, mineral N concentration and soil chemical parameters. Moreover, a Pearson bivariate correlation analysis was performed on the different measured variables.

3. Results

3.1. Biochar characterization

A detailed physico-chemical characterization of the different biochars is presented in Table 1. The carbon content of these materials was greater (53–89%), and generally increased as the pyrolysis temperature increased. However, the manure-derived biochar had a lesser C content

(54%, on average) than the standard WC biochar (89% C). The opposite was observed for the N content, which ranged from 5.85% of PL400 to 1.79% of SM600, but was only 0.27% in the WC biochar. The C:N ratio of the WC biochar (335.4) was on average one order of magnitude greater than those of the manure-derived biochars, which ranged from 9.0 for PL400 to 32.4 for SM600. The WC biochar showed the smallest values for P (0.73 g kg^{-1}), K (2.6 g kg^{-1}), Ca (13.6 g kg^{-1}) and Mg (3.2 g kg^{-1}), and, with the exception of P (15.6 g kg^{-1} P in SM600), the greatest values for these elements (58.8, 35.9 and 24.0 g kg^{-1} of K, Ca and Mg respectively) was observed for PL600; all these nutrients were greater within each feedstock type as the pyrolysis temperature increased.

Volatile matter, ash, CEC and surface acidity were greater for the manure-derived biochars (VM: 29%, ash: 30%, CEC: 32 $cmol_c$ kg^{-1} and acidity: 1.2 mmol H^+ kg^{-1}, on average) than for the WC biochar (VM: 15%, ash: 8% and CEC: 15 $cmol_c$ kg^{-1} and acidity: 0.57 mmol H^+ kg^{-1}) (Table 1). The VM, CEC and surface acidity decreased with temperature for the manure-derived biochars, while the opposite was observed for ash. Biochar pH increased slightly with temperature, from 9.5 of PL400 to 10.4 for both PL600 and SM600, while the standard WC biochar had the greatest pH (11.0). The results from the BET analysis showed that both the surface area (SA) and porosity were greater for WC (SA: 187 m^2 g^{-1}, porosity: 115 mm^3 g^{-1}) than the manure-derived biochars (SA: 7.1 m^2 g^{-1}, porosity: 6.2 mm^3 g^{-1}, on average), and increased as the pyrolysis temperature increased.

3.2. Nitrous oxide and methane emissions

The nitrous oxide emissions were initially recorded on day 21, immediately after N fertilization, for all the treatments, with the exception of PL400 (in both soils) and PL600

(only in Soil B), where the emissions had already been observed before N fertilization (Supplementary Fig. S1). The peak N_2O flux values ranged from 6.50 (WC in Soil B) to 189.4 μg $N\text{-}N_2O$ h^{-1} kg^{-1} soil (PL400 in Soil A) (Table 2). The statistical analysis showed significant differences between the type of biochar and the type of soil for the N_2O peaks. Specifically, the PL400 peak (127 μg $N\text{-}N_2O$ h^{-1} kg^{-1}) was significantly greater than those of the control (14 μg $N\text{-}N_2O$ h^{-1} kg^{-1}) and of the other biochar treatments (18 μg $N\text{-}N_2O$ h^{-1} kg^{-1}, on average of PL600, SM400, SM600 and WC). Moreover, SM400 peak (28 μg $N\text{-}N_2O$ h^{-1} kg^{-1}) was greater than the control and WC (10 μg $N\text{-}N_2O$ h^{-1} kg^{-1}), while PL600, SM600 and WC were not significantly different from the control. The peaks were significantly greater in the sandy soil, Soil A, (29 μg $N\text{-}N_2O$ h^{-1} kg^{-1}) than in the silt-loam, Soil B, (18 μg $N\text{-}N_2O$ h^{-1} kg^{-1}).

The total cumulative N_2O emissions, after 85 days of incubation, were affected significantly by the interaction between the biochar and soil type (Table 2). The greatest cumulative emissions were produced by PL400 in Soil A (72 mg $N\text{-}N_2O$ kg^{-1}), and were one-order of magnitude greater than all the other values (between 4–7 mg $N\text{-}N_2O$ kg^{-1}), followed by the same biochar (PL400) in Soil B (35 mg $N\text{-}N_2O$ kg^{-1}). The smallest value was for WC in Soil B (1.85 mg $N\text{-}N_2O$ kg^{-1}), and was significantly lesser than the PL400, PL600 and SM400 treatments in Soil B, which ranged 7–35 mg $N\text{-}N_2O$ kg^{-1}. The N_2O peak values and the cumulative N_2O emissions were significantly positively correlated to both the N and VM contents of the biochars in both soils (Table 5a).

The N_2O emission factor, calculated as the percentage of total N supplied through the NH_4NO_3 fertilizer and biochar, ranged from 0.65 to 3.41% among biochar treatments (Table 2). The emission factor for PL400 (3.41%) was significantly greater in both soils than those of

the other biochar treatments, whose value ranged 0.65–1.29%, with the exception of the control.

For all the treatments in each sampling date, change in CH_4 concentration during jar enclosure fell below the minimum flux detection limit determined for the GC, and thus produced an overall null emission (data not shown).

3.3. C mineralization

The total cumulative CO_2 emissions, after 85 days of incubation, were affected significantly by the interaction between the biochar and soil type (Table 2), and ranged from 0.44 g to 2.02 g C-CO_2 kg^{-1}. The emissions from the PL400 biochar treatments in Soil B were the greatest. These were followed by SM400 (1.26 g C-CO_2 kg^{-1}) in Soil B and a group including PL400 in Soil A, PL600, SM600 and WC in Soil B. The smallest value was that of WC in Soil A. The cumulative CO_2 emissions were significantly positively correlated to both the N and volatile matter contents of the biochars, but negatively correlated with the biochar C:N ratios in both soils (Table 5a).

The net C mineralization, expressed as the percentage of C supplied through added biochar lost as CO_2 during the experiment, varied among treatments, with a significant interaction effect between the biochar and soil types. Values ranged between null to 9.91% (Table 2). The net amount of C mineralised was greatest for PL400 in Soil B, and this was followed by the same treatment in Soil A (4.49%). The smallest value was that of WC in Soil A.

3.4. Soil mineral N, PO_4^{3-}, Cl^- and SO_4^{2-} evolution

The analysis of the mineral N in the soil water extracts collected in porous cups at different dates in all the treatments mainly consisted of N-NO_3^- (Fig. 1), while N-NH_4^+ was below the detection limit for most of the treatments. Significant differences were observed between the biochars and soil types at different dates after the fertilization (Table 3). The effect of the interaction between the biochar amendments and soil types was found significant for the N-NO_3^- content in the soil for most of the dates, except for the last two dates (Day 82 and Day 100). The N-NO_3^- concentration increased from day 24 until day 53 for all the treatments, and then decreased. The N-NO_3^- content in the PL400 (235 mg kg^{-1}, on average) treated Soil B was significantly greater than that of the Control and other biochar treatments (138 mg kg^{-1}, on average) from day 24 until day 67 (Fig. 1 and Table 3). A significantly lesser N-NO_3^- concentration was observed for the WC treatment (97 mg kg^{-1}, on average) than the Control, PL400 and SM400 treatments (181 mg kg^{-1}, on average) from Day 24 until Day 67 in Soil B (Table 3).

Water soluble PO_4^{3-} was too less in Soil A, with values ranging 3–12 mg PO_4^{3-} kg^{-1} for the manure derived biochar treatments, while the values were negligible in Soil B (data not shown). Both the Cl^- and SO_4^{2-} contents in the water extract varied to a great extent between treatments (Fig. 1). The Cl^- contents of SM400, 600 (630 mg kg^{-1}, on average) and PL400, 600 (261 mg kg^{-1}, on average) treatments were significantly greater ($P<0.05$) than the Control and WC treatments (8 mg kg^{-1}, on average) in both soils. Also, the SO_4^{2-} values of PL400 and 600 (220 mg kg^{-1}, on average) were significantly greater ($P<0.05$) than the Control and the other biochar treatments (39 mg kg^{-1}, on average) in both soils. The Cl^- content ranged from 5

(on average for Control Soil A) to 686 mg kg^{-1} (on average for SM600 treated Soil A) throughout the incubation period. Similarly, the SO_4^{2-} value ranged from 13 (on average for control Soil A) to 256 mg kg^{-1} (on average for PL600 treated Soil B) (Fig. 1).

3.5. Post incubation analysis

The chemical properties of the biochar amended soils, at the end of the incubation experiment, are presented in Table 4. A significant interaction was found between the biochar amendments and soil types for all of the parameters, except for the total organic C and nitrate contents. The pH was significantly greater for all the biochar treatments (6.2 unit pH, on average) than for the Control (4.6 unit pH) for sub-acidic Soil B, while in sub-alkaline Soil A, only PL600 (8.8 unit pH) induced a greater pH value than that of the Control (8.0 unit pH). The pH of Soil A (7.7 unit pH) amended with PL400 was significantly lesser than the other treatments (8.3 unit pH, on average). Furthermore, in Soil B, the enhancement of pH, with respect to the Control, was more pronounced for PL600, and this was followed by SM600, SM400 and PL400, WC.

In both soils, the total organic C content of all the biochar treated soils (2% C, on average) was significantly greater than the Control (1% C). Among the biochar-amended soils, the greatest C content was measured for WC (2.4%) and the smallest for PL400 (1.85%). For Soil B, the total N content did not differ from the Control for WC or SM600, where the greatest values were those of the two poultry manure biochars (0.24%, on average), followed by SM400 (0.19%). The WC, SM400 and SM600 in Soil A were similar to the Control, with the greatest values being those of PL600 and PL400 (0.16 and 0.14 %), respectively. A significant negative correlation was found between the biochar C:N ratio and the total N content of the

final amendment mixture, while a positive correlation between the biochar C:N ratio and the C content of the final amendment mixtures (Table 5a). Similarly, biochar VM contents were significantly positively correlated with the total soil N contents, but negatively correlated with the soil C contents.

In contrast to both TOC and TN, the C:N ratio was greater in Soil A (15.4) than in Soil B (11.2), as was the case also observed for the initial soil characterization (Table 1). It was greatest for WC (21.5, on average) in both soils, and this was followed by the two swine manure biochars and finally by the two poultry manure biochars, which were not significantly different from the Control. The soil nitrate content of PL400 treatment (158 mg kg^{-1}), at the end of the incubation, was significantly greater than that of the Control and WC treatments (93 mg kg^{-1}, on average). The average nitrate content, over all treatments, in Soil B was 1.8 times higher than in the Soil A. The ammonium content, while generally less in both soils, was greatest for the Control in Soil B (4.8 mg kg^{-1}). There was a significant positive correlation between the N content of the biochars and the mineral N content of the final amendment mixture for Soil B (Table 5a).

Phosphorous was generally greater for the manure-derived biochars (124 mg kg^{-1}, on average), while WC showed considerably lesser values that were comparable with the Control (22 mg kg^{-1}, on average). Similarly, the K (604 mg kg^{-1}, on average) and Mg (223 mg kg^{-1}, on average) contents were greater for the both soils amended with poultry manure biochars, and this was followed by the swine manure biochar (286 mg kg^{-1}, on average) and finally WC (112 mg kg^{-1}, on average), thus reflecting the mineral contents of the original chars. As far as the Ca content is concerned, all the biochar treatments (1570 mg kg^{-1}, on average) were significantly greater than the Control (1270 mg kg^{-1}) in Soil B, while the greatest value in Soil

A was for the Control (1116 mg kg^{-1}), followed by WC (998 mg kg^{-1}). The CEC values of SM400 and SM600 (4 $cmol_c$ kg^{-1}, on average) in Soil A were significantly lesser than the Control and WC (7.3 $cmol_c$ kg^{-1}, on average), while the CEC did not vary significantly in Soil B but did in Soil A. The biochar- C, N, P, K and Mg contents were significantly and positively correlated to identical nutrients in the final amendment mixture at the end of the experiment, while a negative correlation for Ca emerged for both soils (Tables 5a and 5b).

4. Discussion

Biochars derived from two types of manure feedstock were selected for this experimental study on the basis of their availability and utilization potential. The pyrolysis of poultry litter offers a good option for manure management, as its relatively less moisture content makes it suitable for use as a feedstock, in terms of energy consumption. The drying of swine manure digestate is an important step for manure processing in Europe as it improves the possibility of using it for pyrolysis without any extra energy intake. The pyrolysis of wood residues is common practice on the biochar market in Italy and other countries, and it offers the possibility of using such biochar as a soil conditioner.

In this experiment, the biochar properties varied considerably, according to the feedstock type and pyrolysis temperature. The manure-derived biochars were richer in nutrients (both macro and micro) than the wood one. An increase in pyrolysis temperature led to a decrease in the N and VM contents, and consequently increased ashes and other nutrients (Hossain et al., 2011). The greater volatile matter content of the low temperature manure-derived biochars (PL400 and SM400) is mainly attributed to the increased fraction of labile carbon (Kammann et al., 2012). A smaller pyrolysis temperature results in a greater fixation of volatile

compounds in the aromatic structure (Fuertes et al., 2010). The smaller surface area and lesser porosity of the manure derived biochars can be explained by the presence of the organic molecules on the char surface, which obscure the biochar pores at smaller temperatures (≤600 ºC) or could escape, through volatilisation, from the biochar pores at greater temperatures (1000 ºC), as can be seen from the values obtained for the WC biochar (Fuertes et al., 2010, Clough and Condron, 2010). These are recognised as important properties of the biochar and could be used to improve the environmental benefits after soil application (Budai et al., 2014; Jeong et al., 2015). Unfortunately, these properties did not appear to a significant extent in the manure-derived biochars. The lesser CEC and surface acidity values of the greater temperature manure-derived biochars can be attributed to the loss of the acidic functional groups with increasing pyrolysis temperature and are in line with the findings of other authors (Cheng et al., 2006; Sing et al., 2010, Jeong et al., 2015).

Generally, biochar addition results in a reduction of N_2O from soils through several mechanisms, and modifies the microbial activity in the soil, the concentrations of available NO_3^- and organic C, pH and soil aeration. Instead, in the present study, greater N_2O emissions were observed from the soils amended with low temperature manure-derived biochars in both types of soil. The N_2O emissions (0.65-3.41%) for the manure-derived biochar treatments in this study are greater than any previously observed values associated with wood or plant biomass derived biochars, which have a less N content (Zhang et al., 2010; Taghizadeh-Toosi et al., 2011; Jia et al., 2012). The possible concurrent causes of this behaviour are (i) the creation of a transitory anaerobic condition, (ii) a more labile C content (Zhu et al., 2014; Troy et al., 2014; Martin et al., 2015), (iii) the increased availability of mineral N from the biochar itself (Dalal et al., 2003; Kammann et al., 2012; Cayuela et al., 2014) and (iv) the greater relative abundance of denitrifiers (both bacteria and fungi) associated with increased

soil NO_3^- availability within the system (Richardson et al., 2009, Cayuela et al., 2014; Van Zwieten et al., 2014).

Our experimental conditions could have induced anaerobicity in the soil microsites, since a rather high soil moisture level (75% saturation) was chosen, and the biochar itself (<2 mm particle size) may have clogged the soil pores, thus leading to poor aeration (Troy et al., 2014). As a significant positive correlation was found between N_2O emissions and the volatile matter content of the biochar in both soils, the greater percentage of volatile compounds present in the low temperature biochars may have been available as a potential substrate for the denitrifiers, thereby enhancing the N_2O emissions (Troy et al., 2014; Martin et al., 2015). In addition to this, the greater mineralization, followed by rapid nitrification, in the PL400 and SM400 biochars, associated with more N content, may have resulted in the production of more N-NO_3^- , that is, an easily available substrate for the denitrifies. Similar conclusions were drawn by Cayuela et al. (2014). greater N_2O emissions were observed for the alkaline sandy soil (Soil A with the PL400 treatment) than for the acidic silt-loam (Soil B). This result is in contrast with the findings of other authors (Simek and Cooper, 2002). It is possible that other conditions, such as the induced anaerobicity and greater labile C content of the biochars, might have dominated the influence of the pH in these cases.

On the other hand, the less N_2O emissions from the WC biochar amended soils could be due to (i) the much lesser availability of mineral N from such a biochar (Nelissen et al., 2014; Van Zwieten et al., 2014), (ii) increased soil aeration, due to the greatly porous nature of the biochar (Downie et al., 2009; Jeong et al., 2015; Martin et al., 2015) and (iii) increased N-NO_3^- retention on the biochar surfaces, due to its greater surface area (Cheng et al., 2006; Major et al., 2012). Increased porosity, associated with an increased pyrolysis temperature,

would increase soil porosity and this could eventually suppress N_2O emissions, as corroborated by other authors (Van Zwieten et al., 2010, Yanai et al., 2007; Taghizadeh-Toosi et al., 2011). In the present study, the WC biochar significantly increased $N\text{-}NO_3^-$ retention in both soils after fertilization (Table 3). This short-term retention of $N\text{-}NO_3^-$ and the subsequent reduction in N_2O emissions from the larger surface area biochars confirm the early findings of Karhu et al. (2011), Nelissen et al. (2014) and Van Zwieten et al. (2014).

Our findings show that the greater the pyrolysis temperature, the lesser the N_2O and CO_2 emissions are, due to the lesser N and VM contents of the biochars (Nelissen et al., 2014; Jeong et al., 2015). In this study, a greater net C mineralization was observed from the soils amended with the low temperature manure-derived biochars (PL400 and SM400) than the other biochars. This result could be explained by (i) increased soil microbial respiration associated with soil available C (Martin et al., 2015), and/or (ii) C loss from the biochar through an abiotic process (Ameloot et al., 2014; Foereid et al., 2011). Triggered C mineralization, associated with low temperature biochars, has already been reported by other authors (Ameloot et al., 2013; Zimmerman et al., 2011; Troy et al., 2014). The positive correlation between the CO_2 emissions and volatile matter content of the biochars suggests the consumption of labile biochar C by soil micro-organisms and further supports our study hypothesis (Kammann et al., 2012; Ameloot et al., 2013). The CO_2 emissions were also positively correlated to the N content of the biochars, which suggests that soil microbes could utilize N from biochars as an important food source to break down C. These results highlight the tendency of these biochars to sequester C in soils for long run. Therefore, the WC biochar has shown a greater sequestering tendency, while PL400 less.

The concentration of NH_4^+ in the soil extract was below the detection limit, thus indicating that any produced or supplied NH_4^+ was rapidly nitrified, immobilized or fully adsorbed on the soil-biochar surfaces (Clough and Condron, 2010; Clough et al., 2013). This property of biochar could also reduce the volatilization of applied NH_4^+ via fertilization, and this mechanism suggests the need for further investigations. Reduced $N\text{-}NO_3^-$ concentrations on WC biochar amended soils is mainly associated with an increased $N\text{-}NO_3^-$ retention as a result of a greater surface area plus large C:N ratio (Schomberg et al., 2012; Van Zwieten et al., 2014; Jeong et al., 2015). This property of WC char offers a considerable opportunity to reduce the risk of NO_3^- leaching and to protect groundwater in nitrate vulnerable zones (Liard et al., 2010; Major et al., 2012), while, on the other hand, reduces soil available NO_3^- to the plants (Nelissen et al., 2014).

A greater Cl^- content was found in the manure-derived biochar amended soil extract. The large chlorine (Cl) content in biochar may be due to more salt content (e.g. sodium chloride) in the biochar feedstock, and it could have a salt toxicity effect on the crops as well as lead to the formation of toxic compounds, such as dioxin (Hale et al., 2012). These aspects need to be verified by growing crops on such biochar-amended soils. It was also found that both the PL400 and 600 biochar-amended soils had greater SO_4^{2-} contents, related to the greater sulphur (S) content of these biochars. It has been pointed out that the S present on biochar surfaces as insoluble sulphide during pyrolysis could be altered by the native S present in the biochar-soil matrix after amendment and then become available (Cantrell et al., 2012). As S is an essential plant nutrient, plants could ultimately benefit from its uptake from such biochar-amended soils.

The post amendment transformation of biochar nutrients in soils for plant uptake is influenced a great deal by both biotic and abiotic factors (Berglund et al., 2004; DeLuca et al., 2006; Jeong et al., 2015). The presence of a greater mineral N content in the soils amended with low temperature manure-derived biochars (PL400 and SM400) is due to the fact that the N contained in these biochars is easily mineralizable and could be available for plant uptake (Troy et al., 2014). As pointed out by the significant, positive correlation between the mineral N content of the soil-biochar matrix and the N content of the biochars in the silt-loam soil, this unique property of the PL400 and SM400 biochars could fulfil the crop N demand and could also replace N-fertilizers. The mineral N content in the soil was found to decrease as the biochar C:N ratio increased, as already noted in another study (Kuzyakov et al., 2009; Nelissen et al., 2014). Nitrogen immobilization, associated with the larger C:N ratio biochars, has been reported by several authors (Ameloot et al., 2013; Major et al., 2012, Novak et al., 2010). In the present study, N immobilization via physicochemical adsorption seems to dominate over microbial immobilization for the WC biochar treatment, as it had a large C:N ratio plus greater surface area. This also supports the findings of Major et al. (2012).

The greater soil C content of the WC treated soils at the end of the experiment could be explained by the presence of stable C, which is resistant to microbial degradation, as can be seen from its less CO_2 emissions. It was here found that the post amendment soil C content increased as the pyrolysis temperature of the manure-derived biochars increased. This explains the increased stability of the biochar C content with increased temperature (Zimmerman et al., 2011; Cantrell et al., 2012; Jeong et al., 2015).

The manure-derived biochars were also rich in P, K, Ca and Mg. The significant positive correlation between the nutrient contents of the biochars (P, K and Mg) and the available

fractions of identical nutrients in the final amendment mixtures suggests that the manure-derived biochars could provide essential plant nutrients (Singh et al., 2010; Hossain et al., 2011; Cantrell et al., 2012) and make them available for plant uptake. This could eventually be confirmed by growing crops in such treatments and considering several growth indicators. Although all the manure manure-derived biochars were rich in Ca, the final availability of this element was only increased slightly in Soil B and decreased in Soil A.

The less available P content in the biochar amended acidic Soil B, compared to Soil A, is probably due to P fixation with Fe or Al, as has already been reported in several studies (Johnson and Loeppert 2006; Cui et al., 2011). Phosphorus is mainly found in the mineral fraction of biochars, with pH dependent reactions and the presence of chelating substances controlling its solubilisation (Joseph et al., 2010). These alkaline biochars could enhance P availability and uptake in acidic soils by adjusting the soil pH after a certain time, as reported by other authors (Nelson et al., 2011; Novak et al., 2009). Similarly, the lesser Ca availability in the biochar amended sandy soils (Soil A in this study), compared to silt-loam soils (Soil B), is due to the fixation of Ca, with P being supplied from the biochars on these soils; this result corroborates those of Cui et al. (2011). The relatively lesser exchangeable K in Soil B (for all the biochar treatments) than in Soil A clearly suggests the influence of pH on K availability. The availability of K increases as the soil pH increases and becomes fully available at a pH above 6.5.

All the biochars significantly increased the pH of acidic Soil B. The liming potential of biochars, when applied to acidic soils, is well known (Ippolito et al., 2012; Sohi et al., 2010). In the case of alkaline Soil A, a lesser pH was observed for PL400 than for the other biochar treatments. The reason for this is not completely clear. However, it was hypothesized that this

could be due to more N content of the PL400 biochar and its strong mineralization potential, as can be confirmed from more mineral N content and soil NO_3^- release with time. Furthermore, the acidic functional groups attached to such biochar surfaces, as observed from its greater surface acidity value, might have contributed to lowering the soil pH. Greater surface acidity of low temperature manure-derived biochars has also been recorded in previous studies (Singh et al., 2010; Cheng and Lehmann, 2009). Whatever the reason for this increase, it was able to reduce the pH of the alkaline sandy soil.

The post amendment CEC values of the SM400 and SM600 treated sandy soils were lesser than the Controls. No significant differences were observed in the CEC values in the silt-loam soil. This result is in contrast with the findings of Clough and Condron (2010); Cheng et al. (2006). The reasons for this difference are not fully understood. However, it is believed that the short-term effect of biochar CEC on soil may not be visible (Sing et al., 2010). Moreover, biochar often loses its acidic functional groups at temperature >500 °C (Jeong et al., 2015), but these could be replenished again over time as a result of biochar oxidation in soils, together with the adsorption of organic matter onto the biochar surfaces (Cheng et al., 2006; Glaser et al., 2002). In order to verify this, the long-term effects of biochar on soil CEC should be tested.

5. Conclusions

This study has shown that the N and VM contents of manure-derived biochars are both important characteristics, and that they decreased with increased pyrolysis temperature. These characteristics were positively correlated with both N_2O and CO_2 emissions from such biochar-amended soils. All the manure-derived biochars significantly increased the soil C

content, compared to the Control, at the end of the incubation, even though they were less effective in C sequestration than the standard WC biochar. The mineral N, Cl^- and SO_4^{2-} were all greater in the manure-derived biochar amended soils, compared to the Control and standard WC biochar. Although more nitrates and sulphates can lead to agronomic benefits, large levels of Cl^- could be toxic to plants and needs to be further verified by growing crops on treated soils. A significant interaction effect was observed between the soil types and biochar treatments for most of the measured variables. A significant positive correlation between the contents of the nutrients (N, P, Mg and K) in the biochars and in the final amendment mixture suggests that low temperature manure-derived biochars not only behave like soil amendments, but also like fertilizers enhancing nutrient availability. On the other hand, the standard WC biochar showed a great potential for climate change mitigation, as pointed out by more C content enhancement, but also considerably reduced the $N\text{-}NO_3^-$ availability in the soil. The obtained results could thus be helpful to formulate specific guidelines in order to design new certification schemes for biochars and their associated products.

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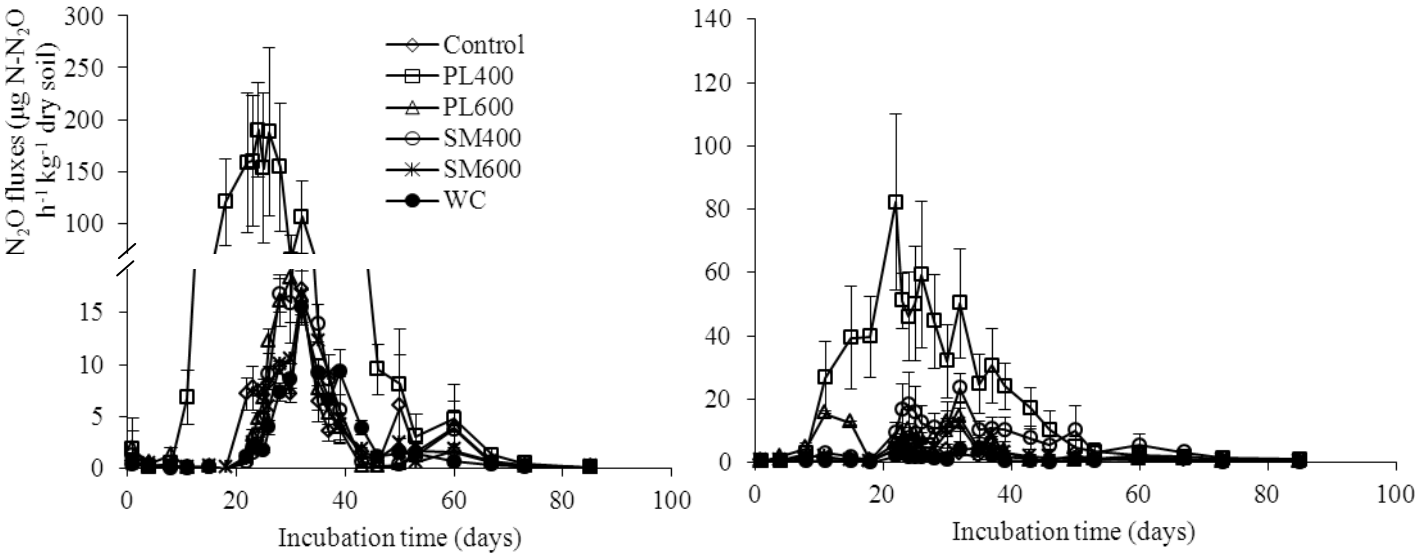

Supplementary Fig. S1. Fluxes of N_2O from soil A (graph on left) and soil B (graph on right) amended with different biochars over 90 days of incubation (Please not different scales of Y-axis). Ammonium nitrate fertilization was started after 21 days of incubation: PL400, PL600- poultry litter biochar at 400 and 600 °C; SM400, SM600- swine manure biochar at 400 and 600 °C; WC- wood chip biochar at 1000 °C. The error bars represent standard errors (n=4). Soil A- sandy soil, Soil B- silt-loam soil.

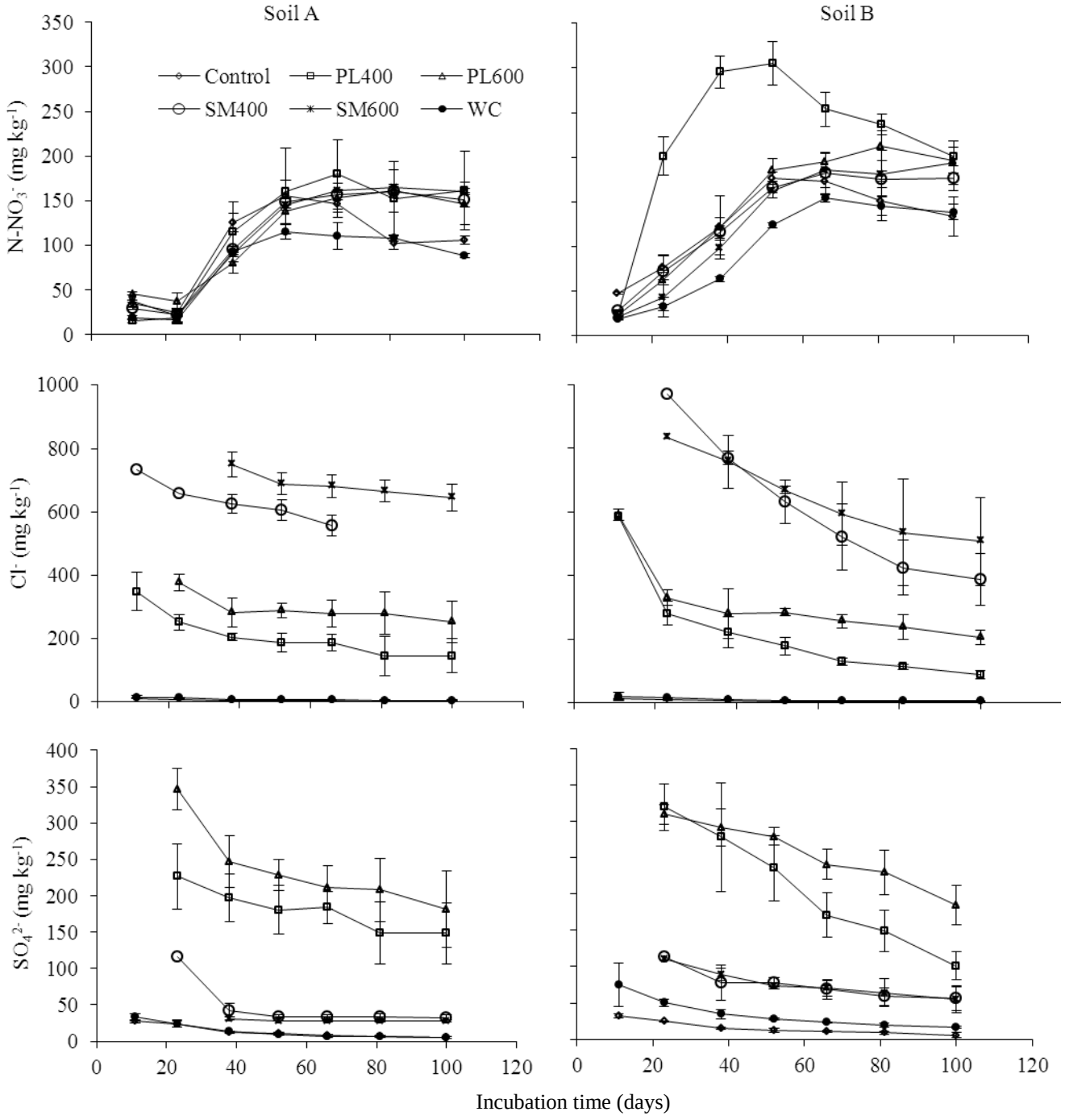

Fig. 1. Effect of the biochar treatment on $N\text{-}NO_3^-$, Cl^- and SO_4^{2-} evolution in sandy soil (soil A) and silt-loam (soil B). Fertilization with NH_4NO_3 was started 21 days after incubation. The error bars represent standard errors (n=4) (Note different scales of Y-axis).

Table 1
Physico-chemical characteristics and elemental compositions (mean values, n=3) of two soils and different biochars utilized in the experiment.

			Biochar types[a]				
Parameters	Soil A	Soil B	PL400	PL600	SM400	SM600	WC
Texture	Sandy	Silt-loam	ND	ND	ND	ND	ND
BD (g cm^{-3})	1.35	1.25	ND	ND	ND	ND	ND
$CaCO_3$ (%)	15.3	0.4	ND	ND	ND	ND	ND
Porosity[b]	45.3	49.2	2.9	3.0	7.6	11.4	115.9
pH	8.3	6.1	9.5	10.4	10.0	10.4	11.0
CEC ($cmol_c$ kg^{-1})	5.4	12.4	30.2	27.5	52.5	18.6	14.8
TOC (%)	0.52	1.2	52.1	52.8	54.9	57.9	89.3
TN (%)	0.057	0.15	5.85	4.0	2.23	1.79	0.27
C:N	9.1	8.0	9.0	13.0	24.6	32.4	335.4
P[c] (g kg^{-1})	0.014	0.023	12.2	15.4	9.7	15.5	0.73
K[d] (g kg^{-1})	0.028	0.042	38.8	58.8	16.2	35.3	2.6
Ca[d] (g kg^{-1})	0.028	0.042	28.3	35.9	20.3	28.9	13.6
Mg[d] (g kg^{-1})	0.981	1.45	17.3	24	15.7	21.3	3.2
S (%)	ND	ND	0.79	0.8	0.24	0.39	0
Moisture (%)	ND	ND	3.2	4.6	3.8	2.9	6.8
VM (%)	ND	ND	44.9	24.7	29.9	17.8	15.3
Ash (%)	ND	ND	25.3	35.4	27.5	34.5	7.8
Acidity (mmol H^+ g^{-1})	ND	ND	1.91	0.72	1.12	0.98	0.57
Surface area (m^2 g^{-1})	ND	ND	5.4	6.3	5.8	10.6	178.3

Abbreviations: ND- not determined, BD- bulk density, TOC- total organic carbon, TN- total nitrogen, CEC- cation exchange capacity, VM- volatile matter, SA- surface area.
[a] Letters refer to feedstock material as poultry litter (PL), swine manure (SM) and wood chip (WC), numbers refer to pyrolysis temperature in ºC, with addition to WC at 1000 ºC.
[b] expressed as % in soil, and as mm^3 g^{-1} in biochar.
[c] available P.
[d] Exchangeable cations in soil, while total in biochar

Table 2
The N_2O (µg N-N_2O h^{-1} kg^{-1} soil) peak, cumulative N_2O (mg N-N_2O kg^{-1} soil) and CO_2 (g C-CO_2 kg^{-1} soil) emissions at day 85, N_2O emission factor (EF) (%) and net C mineralisation (%) at the end of the incubation experiment (n=4).

Source	N_2O peak	Cum. N_2O	N_2O EF [a]	Cum. CO_2	Net C min.[b]
Biochar type					
Control	13.7 c	3.85	2.68 ab	0.69	
PL400	127.6 a	54.14	3.41 a	1.44	6.74
PL600	20.5 bc	6.39	0.65 c	0.77	0.98
SM400	28.6 b	8.05	1.29 bc	0.96	2.45
SM600	14.9 bc	4.56	0.85 c	0.85	1.47
WC	9.91 cd	2.92	1.28 bc	0.65	-0.15
SEM[†]	0.16	0.13	0.47	0.04	0.16
P (F)	<0.001	<0.001	<0.001	<0.001	<0.001
Soil type					
Soil A	29.4 a	16.30	1.59	0.61	1.39
Soil B	18.1 b	10.60	1.28	1.21	3.57
SEM	3.09	0.07	0.33	0.02	0.10
P (F)	0.001	0.012	0.117	<0.001	0.012
Soil type x biochar					
Soil A x Control	18.4	5.27 cd	3.01	0.52 fg	
Soil A x PL400	189.4	72.7 a	4.56	1.02 bc	4.49 b
Soil A x PL600	20.4	5.75 cd	0.58	0.49 fg	-0.08 de
Soil A x SM400	34.1	6.87 cd	1.11	0.72 de	1.85 cd
Soil A x SM600	17.0	4.91 cd	0.92	0.62 ef	1.12 cde
Soil A x WC	15.2	4.58 cde	2.01	0.44 g	-0.37 e
Soil B x Control	10.2	2.81 de	2.38	0.95 cd	
Soil B x PL400	82.1	35.3 ab	2.56	2.02 a	9.91 a
Soil B x PL600	20.6	7.09 cd	0.73	1.19 bc	2.45 cd
Soil B x SM400	24.1	9.42 bc	1.52	1.26 b	3.26 c
Soil B x SM600	13.1	4.23 cde	0.79	1.15 bc	2.01 cde
Soil B x WC	6.50	1.85e	0.81	0.92 c	0.16 de
SEM	0.23	0.18	0.52	0.05	0.23
P (F)	0.342	0.009	0.124	0.029	0.005

[†]Standard error of the means.
Values in the same column followed by the same letters indicate no significant differences at the P<0.05 level.
[a](N-N_2O emitted)/(total N supplied from NH_4NO_3 and biochar)*100.
[b](Cum. C-CO_2 emissions of biochar treatment – Cum. C-CO_2 emissions of Control)/(C supplied through biochar)*100.

Table 3

Effects of amendments, soil type and their interaction on the soil NO_3^- concentrations throughout the experiment (n=4). Fertilization with NH_4NO_3 was started at day 21.

	N-NO_3^- (mg kg^{-1})						
Source	Day 12	Day 24	Day 40	Day 53	Day 67	Day 82	Day 100
Biochar type							
Control	41.1	53.0	132.3	175.5	168.1	120.2 c	114.2 b
PL400	19.8	121.3	205.1	237.3	224.5	196.6 a	173.2 a
PL600	34.7	67.5	127.1	163.1	182.2	199.1 a	174.1 a
SM400	28.9	50.2	108.3	159.8	172.1	167.7 b	161.5 a
SM600	29.2	42.5	98.9	155.5	176.1	182.6 ab	182.1 a
WC	20.2	29.8	78.4	118.6	138.5	130.1 c	111.3 b
SEM[†]	1.27	2.94	3.30	2.81	2.32	6.38	5.65
P (F)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Soil type							
Soil A	29.9	27.6	105.2	150.3	160.3	145.4 b	137.1 b
Soil B	28.1	93.8	144.9	186.2	193.6	186.7 a	168.3 a
SEM	0.73	1.70	1.93	1.62	1.33	3.68	3.26
P (F)	0.071	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Soil type x biochar							
Soil A x Control	33.9 cd	25.1 ef	136.6 c	169.8 bc	158.2 e	102.6	106.0
Soil A x PL400	15.1 f	26.1 ef	123.9 cd	175.5 b	194.4 bc	162.4	156.7
Soil A x PL600	45.7 ab	43.1 de	91.7 e	149.3 cd	169.1 de	174.7	160.1
Soil A x SM400	29.0 cde	27.6 ef	95.1 e	149.1 cd	159.1 e	159.9	150.9
Soil A x SM600	37.3 bc	27.2 ef	90.8 e	144.9 d	160.8 e	164.8	160.4
Soil A x WC	18.7 f	16.2 f	92.7 e	113.2 e	120.0 f	108.2	88.5
Soil B x Control	48.2 a	80.9 b	128.1 cd	181.2 b	178.1 cd	137.9	122.4
Soil B x PL400	24.5 de	216.4 a	286.1 a	299.1 a	254.5 a	230.7	189.6
Soil B x PL600	23.7 ef	91.9 b	162.5 b	176.7 b	195.4 b	223.6	188.2
Soil B x SM400	28.8 cde	72.7 bc	121.6 cd	170.4 b	184.9 bcd	175.5	172.1
Soil B x SM600	21.1 ef	57.7 cd	107.0 de	166.2 bc	191.5 bc	200.4	203.9
Soil B x WC	21.7 ef	43.4 de	64.1 f	124 e	157.1 e	151.9	134.1
SEM	1.79	4.17	4.74	3.98	3.28	9.10	8.00
P (F)	<0.001	<0.001	<0.001	<0.001	<0.001	0.120	0.391

[†]Standard error of the means.

Values in the same column followed by the same letters indicate no significant differences at the P<0.05 level.

Table 4
Effects of amendments, soil types and their interaction on the chemical properties of the soils at the end of the incubation experiment (n=4).

	Contents (%)			Mineral N (mg kg^{-1})			Macronutrients (mg kg^{-1})				CEC
Source	TOC	TN	C/N	$N\text{-}NH_4^+$	$N\text{-}NO_3^-$	pH	P[a]	K[b]	Ca[b]	Mg[b]	($cmol_c$ kg^{-1})
Biochar type											
Control	1.01 d	0.11	9.5	4.78	95.1 b	6.33	21.5	67.7	1194.1	67.5	9.94
PL400	1.85 c	0.2	9.7	2.96	158.1 a	6.78	119.4	550.8	1218	206.2	7.91
PL600	2.07 b	0.19	10.8	2.76	126.6 ab	7.67	134.6	656.8	1099.2	240.9	8.15
SM400	1.89 bc	0.15	13.5	2.91	133.1 ab	7.13	120.9	260.6	1146.2	209.8	6.09
SM600	2.01 bc	0.14	14.7	2.85	114.1 ab	7.21	122.8	268.6	1165.9	183.4	7.83
WC	2.41 a	0.12	21.5	2.78	90.7 b	6.87	20.8	111.7	1298.2	80.3	9.6
SEM†	0.04	0.003	1.68	0.12	12.7	0.04	9.12	11.8	17.2	10.7	1.46
P (F)	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Soil type											
Soil A	1.67 a	0.11	15.4	2.56	85.9 b	8.21	93.1	302.1	849.5	79.6	5.36
Soil B	2.08 b	0.19	11.2	3.79	153.4 a	5.79	87.7	190.8	1524.4	273.7	12.3
SEM	0.02	0.002	1.05	0.07	7.38	0.02	7.31	13.6	9.98	8.37	1.87
P (F)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.072	<0.001	<0.001	<0.001	<0.001
Soil type x biochar											
Soil A x Control	0.84	0.08 f	10.8 cd	2.67 bc	65.5	8.01 bc	16.8 f	61.2 g	1116.5 d	29.2 e	7.31 b
Soil A x PL400	1.58	0.14 cd	11.1 cd	2.55 bc	90.3	7.71 c	123.1 bc	823.5 a	771.4 e	137.3 c	5.27 bc
Soil A x PL600	1.92	0.16 d	11.9 cd	2.45 c	97.8	8.81 a	149.9 a	830.1 a	617.7 f	137.7 c	5.78 bc
Soil A x SM400	1.62	0.11 e	15.5 b	2.55 bc	99.7	8.23 b	148.1 a	391.9 c	730.8 ef	131.6 c	3.22 d
Soil A x SM600	1.83	0.11 e	17.2 b	2.62 bc	95.9	8.24 b	139.3 cd	394.6 c	861.8 e	93.7 d	4.59 cd
Soil A x WC	2.17	0.09 ef	26.2 a	2.5 bc	66.3	8.21 b	17.6 f	117.5 e	998.5 d	37.5 e	7.21 b
Soil B x Control	1.18	0.14 cd	8.1 e	6.9 a	124.7	4.64 g	27.6 e	74.9 f	1271.4 c	155.8 c	13.53 a
Soil B x PL400	2.12	0.25 a	8.5 e	3.37 b	225.9	5.85 f	115.9 cd	368.4 c	1664.6 a	309.7 b	11.89 a
Soil B x PL600	2.21	0.23 a	9.7 de	3.07 bc	155.5	6.54 d	120.9 bc	519.7 b	1580.6 ab	421.5 a	11.46 a
Soil B x SM400	2.16	0.19 b	11.5 cd	3.27 bc	166.5	6.03 ef	98.7 d	173.3 d	1561.6 ab	334.6 ab	11.52 a
Soil B x SM600	2.16	0.17 bc	12.2 c	3.07 bc	132.2	6.19 e	108.4 ab	182.8 d	1470.0 b	359.1 ab	13.32 a
Soil B x WC	2.65	0.16 cd	16.8 b	3.07 bc	115.2	5.54 f	24.8e	106.2 e	1597.9 ab	172.1 c	12.76 a
SEM	0.06	0.005	1.93	0.17	18.1	0.06	10.83	12.3	24.4	11.7	1.96
P (F)	0.09	0.002	<0.001	<0.001	0.123	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

†Standard error of the means.
Values in the same column followed by the same letters indicate no significant differences at the P<0.05 level.
[a] Available P (Olsen), [b] Exchangeable K, Ca and Mg.

Table 5a
Pearson bivariate correlations (n = 15) between the biochar and soil properties and emissions at the end of the experiment.

		Biochar properties			
Soil type	Property	C	N	VM	C/N
Soil A	C	0.729**		-0.766**	0.734**
	N		0.836**	0.529*	-0.642**
	N_2O peak	-0.321	0.755**	0.836**	-0.306
	Cum. CO_2	-.559*	0.754**	0.864**	-0.563*
	Cum. N_2O	-0.345	0.794**	0.865**	-0.331
	N_{min}[a]	-0.367	0.115	0.002	-0.361
Soil B	C	0.889**		-0.561*	0.900**
	N		0.967**	0.815**	-0.666**
	N_2O peak	-0.515*	0.800**	0.902**	-0.504
	Cum. CO_2	-0.576*	0.879**	0.939**	-0.562*
	Cum. N_2O	-0.508	0.693**	0.810**	-0.493
	N_{min}[a]	-0.568*	0.722**	0.826**	-0.542*

*P<0.05, **P<0.01.
[a] soil mineral N content.

Table 5b
Pearson bivariate correlations (n = 15) between the biochar nutrient contents, pH, CEC and the same for the soil-biochar matrix at the end of the experiment.

	Biochar/soil properties					
Variables	P	K	Ca	Mg	pH	CEC
Soil A	0.816**	0.883**	-0.730**	0.764**	0.475	-0.705**
Soil B	0.982**	0.906**	-0.091	0.954**	-0.191	-0.346

*P<0.05, **P<0.01.