The Impact of Woody Biochar on Microbial Processes in Conventionally and Organically Managed Arable soils

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ABSTRACT
Although environmental impacts of biochar are well characterized, impacts on soil quality, nutrient availability and crop productivity, still remain a challenge due to the diverse response of different soil types to different types of biochar, namely those obtained at low temperature. The impact of an alkaline woody biochar (two doses 5% and 10%) obtained at 280°C, on soil enzyme activity, soil microbial respiration rate, mineral nitrogen (N) availability and ammonia volatilization was studied in one conventionally and one organically managed soils, with and without the addition of urea or composted farmyard manure. Biochar additions had different effects on soil enzyme activity in both soils, suggesting lower decomposing microbial activity processes promoted by biochar. Both soils showed a similar decreasing trend regarding soil respiration rates for all treatments, and significant relationships were observed between the treatments with different rates of applied biochar, but not constant for the entire incubation period. Urea application increased soil mineral N concentrations, especially nitrate concentrations when biochar was applied as well. Biochar decreased ammonia volatilization from conventionally managed soil fertilized with urea, but did not have a significant effect when compost was added to the organically managed soil. Biochar altered microbial behavior in soil, and was affected by previous soil management. So, the impact of biochar produced at low temperatures on soil biological processes is similar to those obtained at high temperature, thus proving that there is no need to increase the energy expenditure to produce biochar, to obtain a good product.

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Introduction
A growing concern of environmental quality has been a major driver for agro-ecosystems to develop strategies to reduce soil nutrient losses and the bioavailability of environmental contaminants, to sequester carbon (C) and to mitigate emissions of greenhouse gases and at the same time improve soil quality and crop productivity (Rockström et al. 2009). As a response to these challenges, biochar application to improve soil conditions and reduce mineral nitrogen (N) fertilizer use in agriculture has been investigated. However, there are still considerable knowledge gaps in some areas as highlighted by Sakrabani et al. (2017) and Tammeorg et al. (2016).

Biochar is a carbon-rich by-product of pyrolysis at low oxygen concentrations (Lehmann, Gaunt, and Rondon 2006). The chemical and physical composition of biochar is highly variable, depending on the type of feedstock, pyrolysis conditions, namely the temperature, and postproduction handling. Biochars have complex porous structures with large surface areas, an affinity for charged
particles, an ability to increase the soil water holding capacity (WHC) (Ulyett et al. 2014), and to retain nitrate-N (NO$_3$-N) (Kammann et al. 2015) and ammonia-N (NH$_3$-N) (Taghizadeh-Toosi et al. 2012). Thereby biochar affects nutrient cycling and organic matter (OM) decomposition (Biederman and Harpole 2013) with both environmental and agronomical implications. Most of the biochar products investigated in biogeochemical research are produced at high temperatures (>500 °C) providing highly recalcitrant and decay-resistant products (Sakrabani et al. 2017). Contrary low temperature biochars (~300 ºC) have not received the same attention, in spite of the fact that they have higher bioavailability and provide C and other nutrients to the microbial community and thereby potentially can increase mineralization rates and nutrient supply to plant roots and soil microorganisms (Kumar et al. 2013). To fill this knowledge gap we have investigated the effect of a commercial alkaline low-temperature (280 ºC) biochar, made from conifer wood chips, on soil microbial processes indicative of changes in soil nutrient cycling in soils from an organic vegetable farm using composted manure as N fertiliser and a conventional vegetable farm using urea as N source. To compare the impact of a low temperature biochar addition in these two systems commonly used biological indicators of change were investigated (1) soil CO$_2$ respiration rates, as indicator of the soil microbial community composition (Degens and Harris 1997), (2) soil enzyme activities indicative of key processes involved in nutrient cycling (Chen et al. 2013; Paz-Ferreiro et al. 2012) and (3) ammonia emissions, as the presence of biochar has been reported to reduce emissions of NH$_3$ after N fertiliser application (Mandal et al. 2016).

**Materials and methods**

Incubation experiments were set up using two different Cambisols (WRB 2006) collected from two vegetable production farms with previous contrasting fertilization management: Farm A uses urea as fertilizer and Farm B uses composted farmyard manure (FYM) for the same purpose. Both farms are located in Sintra (Portugal) (38°53′52.0″N 9°25′12.2″W; 38°53′23.3″N 9°22′57.1″W). Soils from both farms were collected from the topsoil (0–20 cm depth).

The biochar used was produced from conifer wood chips in a fast pyrolysis process by the Polish company “Fluid Spółka Akcyjna”. The temperature of pyrolysis ramped up at 10 °C/min and had a residence time of 10 min after reaching the 280 °C maximum temperature. Soils, biochar and composted FYM provided by Farm B, were fully characterized. Methods used are described in Sparks et al., (1996): pH was measured by a glass electrode using a 1:2.5 (material: water) ratio; TKN was determined by Kjeldahl method after sample digestion; N$_{\text{min}}$ (N-NH$_4^+$ + N-NO$_3^-$) was determined by molecular absorption spectrophotometry using a segmented flow auto-analyzer, after extraction with 2M KCl at a 1:10 (soil:water) ratio; available P and K concentrations were determined through Egner-Riehm procedure; total P and K concentration determined by Ammonium Vanadate method (Póvoas and Barral 1992).

The same biochar treatments were applied to both soils and to all experiments (Table 1). Biochar was mixed with both soils, previously air dried at room temperature and sieved in a 2 mm mesh, to achieve even distribution. The rate of biochar applied (5% and 10%) was similar to those used in other studies (Ameloot et al. 2014; Jeffery et al. 2011; Ouyang et al. 2014a; Paz-Ferreiro et al. 2012). Urea and composted FYM were added to the soils A and B, respectively, with and without biochar addition, in accordance to their previous management, at rates equivalent to an application of 170 kg N ha$^{-1}$ (91/676/EEC Directive) (EC 2017).

| Table 1. Treatments applied to the conventionally managed soil A and to the organically managed soil B. |
|:----------------------------------------------------------------------------------------------------------------------------------|
| Treatments of soil A                                                                                                                                            | Treatments of soil B                                                                                     |
| A   | Soil A control (no fertilizer added)                                                                                                                          | B   | Soil B control (no fertilizer added)                                                                 |
| AU  | A + Urea                                                                                                                                                    | BC  | B + Compost                                                                                           |
| A5  | A + 5% Biochar                                                                                                                                             | B5  | B + 5% Biochar                                                                                          |
| A10 | A + 10% Biochar                                                                                                                                             | B10 | B + 10% Biochar                                                                                          |
| U5  | A + 5% Biochar + Urea                                                                                                                                          | C5  | B + 5% Biochar + Compost                                                                                |
| U10 | A + 10% Biochar + Urea                                                                                                                                         | C10 | B + 10% Biochar + Compost                                                                                |


The impact of biochar rate on enzyme activity, microbial respiration rate and mineral n concentration

Batches of 300 g of each treatment mixture (Table 1) were placed into round polyethylene containers (500 cm$^3$) and incubated aerobically in an Aqua Tag incubation chamber at 24 ± 2 °C for 60 days (D60). Enough batches were prepared to allow destructive sampling in triplicates every 15 days, including at day 0 (D0). The mixtures were maintained at 60% soil water holding capacity (WHC) by monitoring the weight of the soil-filled containers every two days and correcting with distilled water whenever needed. WHC was determined (Póvoas and Barral 1992), in triplicates, by saturating the samples with water and weighing once equilibrium of the system was reached, for all the treatments as the application of biochar might alter this parameter.

Soil pH

The pH of soil with and without biochar was determined at the beginning (D0) and at the end (D60) of the incubation experiment in a 1:2.5 soil and distilled water mixture, stirred for 1 h prior measurement using a Thermo Electron Corporation potentiometer, with a detection limit of 0.01 pH units.

Enzyme activity

Enzyme activities were determined for all the treatments mixtures at the beginning of the incubation experiment (D0), after 30 (D30) and 60 days (D60). For dehydrogenase activity, soil samples (3 g) from each destructive triplicate were mixed with 0.1% (w/v) triphenyltetrazolium chloride in Tris-buffer (0.1M; pH 7.6; 3 mL) and incubated at 25 °C for 16 h, followed by the quantification of the triphenylformazan (TPF) formed by spectrophotometry (546 nm) as described by Deng and Tabatabai (1997).

β-glucosidase activity was obtained through the determination by spectrophotometry (400nm) of the $p$-nitrophenol (p-NP) released after the incubation of the soil samples (1 g) with a buffered solution (pH 6; 4 mL), toluene (0.25 mL) and $p$-nitrophenyl-β-d-glucopyranoside (1 ml) for 1 h at 37 °C. Soil phosphatase activity was assayed by colorimetric estimation of the $p$-nitrophenol released by spectrophotometry (400nm) after the soil samples (1 g) were incubated with a buffered solution (pH 6.5; 4 mL), toluene (0.25 mL) and sodium $p$-nitrophenyl phosphate (p-NPP) (1 mL) at 37 °C for 1 h (Tabatabai and Bremmer 1969; Tabatabai and Bremner 1972). The spectrophotometer used was a segmented flow analyzer from Skalar.

Soil microbial respiration

The physiological profiles of the microbial communities (CLPP) were determined at the beginning (D0) and every 15 days (D15, D30, D45, D60) using the MicroResp method (Campbell et al. 2003), which is a colorimetric detection (with cresol red in the detection plate) to measure soil respiration in the presence of three different C sources. Three carbon substrates (D-glucose, citric acid and N-acetyl-D-glucosamine) were prepared at 1% (m/v) in deionized water to determine substrate-induced respiration (SIR) (Cordovil et al. 2011). Basal respiration (BR) was determined by using 200 µL of distilled water as substrate. The substrates (200 µL) were added to the wells in the microtiter deep-well plate containing the soil mixtures (approximately 0.5 g to fill the wells), and a total of 36 replicates per sampling date × 4 (3C sources + water) were generated. The detection plate was read at 600 nm in a microplate reader before the beginning of the incubation and after 6 h of incubation at 24 °C ± 2 °C. Data were normalized for time zero, to eliminate differences in color between wells due to uneven gel density.

Mineral nitrogen

The mineral N (NH$_4^+$-N and NO$_3^-$N) content was determined by segmented flow spectrophotometry (Skalar) at set-up (D0) and every 15 days thereafter (D15, D30, D45, and D60). Fresh soil samples (5 g) were shaken for 1 h with 2 M KCl (potassium chloride) solution (1:10) at room temperature, and centrifuged at 3000 rpm for 5 min as adapted by Cordovil et al. (2005). Prior to analysis, KCl extracts were stored in the fridge until the next day.
The impact of biochar rate on \( \text{NH}_3 \) emissions

The setup of soil cores was the same as in experiment 1 (section 2.1), and all were brought to a WHC of 60% on day 1 but not rewetted again. Thereafter, air temperature, soil moisture, and \( \text{NH}_3 \) emissions were measured until the moisture content had dropped drastically, which occurred after 10 days.

Ammonia volatilization (Alves et al. 2011) was determined every two days (D2, D4, D6, D8 and D10) by passive diffusion using polyurethane density foams (20 kg m\(^{-1}\); 5 × 5 × 2 cm) soaked in 7 ml of phosphoric acid (0.5 M) and then fixed to acrylic plates (7 × 7 × 0.3 cm) with polytetrafluoroethylene tape, which is permeable to \( \text{NH}_3 \) but not to water. The foams were placed 1 cm above each of the 12 × 3 plastic containers supported by four plastic rods to fully cover the container. This procedure was the one that proved to be more efficient among several combinations tested (Alves et al. 2011). To ensure that there was no contamination between containers they were arranged randomly and spaced 30 cm apart from each other. Foams were collected every two days and washed with 200 ml of deionized water on a Buckner funnel attached to a vacuum pump. \( \text{NH}_3 \) was then determined using the segmented-flow analyzer detailed above.

Statistical analysis

The equality of the means of each parameter when different treatments were applied was tested using the Kruskal–Wallis test (Sokal and Rohlf 1995) for each sampling date. Differences between the parameters analyzed were considered statistically significant at \( p \leq 0.05 \), and the \( p \)-values for pairwise comparisons between specific levels of the treatments were adjusted for multiple comparisons using the method of Benjamini, Hochberg, and Yekutieli for controlling the false discovery rate (Benjamini and Hochberg 1995; Benjamini and Yekutieli 2001). All statistical inferences were performed using the software R (R Core Team 2013).

Results

The characterization of biochar, composted farmyard manure, and soils A and B are shown in Table 2. An addition of biochar to both conventionally and organically managed soils increased the WHC of the soils (Table 3). The WHC in the treatments with organically managed soil was higher than in the conventionally managed soil treatments when comparing the same biochar treatments and controls (\( p \leq 0.05 \)). Additionally, the WHC increased with increasing biochar application rate. Biochar presented a high adsorption capacity due to its specific porosity (0.008 cm\(^3\).g\(^{-1}\) total pores and 0.0007 cm\(^3\).g\(^{-1}\) micropores <20 Å). For the conventionally managed soil A, this increase relative to the control was 18% and 22% for the 5% and 10% biochar application rate, respectively. The increase in WHC for the organically managed soil B was lower with only ~14% for the 5% biochar application rate and 18% for the 10% rate, relative to the control. Contrary, compost addition did not affect the WHC (Table 3).

| Parameter                  | Biochar | Composted FYM | Conventionally managed soil A | Organically managed soil B |
|----------------------------|---------|---------------|-------------------------------|----------------------------|
| pH                         | 8.4     | 7.0           | 7.2                           | 7.4                        |
| Dry Matter (%)             | 88.1    | 98.1          | 85.4                          | 76.3                       |
| Organic Matter (g 100g\(^{-1}\)) | 81.4   | 40.2          | 2.38                          | 6.64                       |
| Texture                    | n.a.    | n.a.          | Silt loamy                    | Clay loamy                 |
| Bulk Density (g cm\(^{-3}\)) | n.a.   | n.a.          | 1.32                          | 1.08                       |
| \( N_{Kj} \) (g 100 g\(^{-1}\)) | 0.64   | 1.90          | 0.10                          | 0.14                       |
| \( P \) (g 100 g\(^{-1}\))  | 0.12    | 0.58          | 0.07                          | 0.38                       |
| \( K \) (g 100 g\(^{-1}\))  | 0.62    | 0.29          | 0.03                          | 0.16                       |
| \( Ca \) (g 100 g\(^{-1}\)) | 1.80    | 7.60          | 0.25                          | 1.44                       |
| \( Mg \) (g 100g\(^{-1}\)) | 0.10    | 0.50          | 0.07                          | 2.19                       |

n.a. not applicable. Nkj_ Kjeldahl nitrogen
At the beginning of the 60-day incubation period, the conventionally managed soil with biochar and with biochar + urea, showed slightly higher pH values than the controls, especially in treatments with the higher rate of biochar ($p \leq 0.05$) (Table 3). Organically managed treatments exhibited opposite results, as biochar addition to soil B had lower pH values compared to the controls but in this case the lower the rate of biochar addition the lower the pH decrease. When compost addition was combined with biochar, pH did not change.

After 60 days (D60), a slight increase in pH was noticeable in all treatments, with the exception of those receiving urea + 10% biochar (U10). The maximum pH increase (~0.7 pH units) occurred in B5 followed closely by the other organically managed soil with biochar at 5% and 10% and with biochar + compost (B5, B10, and C10). For the conventionally managed soils, the highest pH increase (~0.6 pH units) after 60 days incubated was measured for the A and U5 and U10 treatments.

The organically managed soil had considerably larger organic matter contents than conventionally managed soils, both at D0 and D60 (Table 3), as expected. Additions of biochar to the conventionally farmed soil A raised the organic matter contents relative the control ($p \leq 0.05$) by about 58% and 73% for the 5% and 10% biochar treatments, respectively, and remained practically constant over the 60-day incubation period. Contrary, in the organically managed soils the increase in SOC at the start of the incubation period (D0) was 60% for the 5% biochar and 67% for the 10% biochar treatments. This increase had declined to 33% and 46% for 5% and 10% biochar treatments, respectively, by day 60.

**Enzyme activity**

The three soil enzymes investigated behaved differently throughout the experimental period (Table 4). Dehydrogenase activity was higher in conventionally managed soil treatments (soil A) compared to organically managed soil treatments (soil B) at the start of the experiment (D0). Conventionally managed treatments (see Table 1) ranged from 0.33 ± 0.01 (U10) to 3.52 ± 0.13 µg TPF g$^{-1}$ h$^{-1}$ (AU), whereas organically managed treatments (see Table 1) only reached 1.58 ± 0.55 µg TPF g$^{-1}$ h$^{-1}$ in the B soil, with and without compost (B, BC). All treatments showed a decreasing trend over time. Biochar treatments tended to show lower dehydrogenase activity than the controls ($p \leq 0.05$). This difference was more pronounced at the beginning (D0) of the incubation period, especially for the higher rate of biochar ($p \leq 0.05$), with the exception of the treatments where biochar was also mixed.

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**Table 3.** Water holding capacity (WHC), pH and soil organic matter content (SOM) in all the treatments (Table 1).

| Treatments         | WHC (%) | pH  | SOM (%) |
|--------------------|---------|-----|---------|
|                    | D0      | D0  | D60     | D0      | D60     |
| Conventionally farmed soil A |         |     |         |         |         |
| A                  | 27 ± 2.3 c | 7.20 ± 0.2 c | 7.21 ± 0.2 c | 1.40 ± 0.1 c | 1.37 ± 0.2 c |
| A5                 | 32 ± 1.8 b | 7.46 ± 0.3 a | 7.53 ± 0.3 a | 2.21 ± 0.2 b | 2.24 ± 0.2 b |
| A10                | 36 ± 3.1 a | 7.49 ± 0.7 a | 7.52 ± 0.2 a | 2.42 ± 0.2 a | 2.39 ± 0.2 a |
| AU                 | 28 ± 5.1 c | 7.19 ± 0.4 bc | 7.20 ± 0.3 c | 1.43 ± 0.08 c | 1.41 ± 0.2 c |
| U5                 | 33 ± 2.3 ab | 7.38 ± 0.4 b | 7.44 ± 0.2 b | 2.23 ± 0.2 b | 2.28 ± 0.3 b |
| U10                | 42 ± 5.8 a | 7.42 ± 0.2 b | 7.43 ± 0.3 b | 2.43 ± 0.3 a | 2.42 ± 0.2 a |
| Organically farmed soil B |         |     |         |         |         |
| B                  | 44 ± 4.3 b | 7.43 ± 0.1 a | 7.50 ± 0.2 a | 3.83 ± 0.2 d | 4.07 ± 0.2 d |
| B5                 | 50 ± 1.8 a | 7.34 ± 0.2 b | 7.33 ± 0.3 c | 6.12 ± 0.3 b | 5.41 ± 0.3 b |
| B10                | 52 ± 3.3 a | 7.26 ± 0.1 b | 7.43 ± 0.3 b | 6.39 ± 0.4 a | 5.94 ± 0.3 b |
| BC                 | 48 ± 2.4 b | 7.47 ± 0.4 a | 7.51 ± 0.2 a | 4.15 ± 0.2 c | 4.04 ± 0.2 d |
| C5                 | 47 ± 1.7 b | 7.47 ± 0.1 a | 7.51 ± 0.2 a | 6.13 ± 0.2 b | 6.08 ± 0.4 a |
| C10                | 48 ± 2.3 b | 7.44 ± 0.3 a | 7.50 ± 0.3 a | 6.29 ± 0.2 a | 6.14 ± 0.3 a |

*For each sampling date, significant differences ($p \leq 0.05$) are indicated by different letters within each column. Note that the statistical analysis was performed separately for soil A and soil B.

The data shows the average and standard deviations of three replicates measured at the beginning (D0) and end (D60) of the 60-day incubation experiment.
with compost (C5 and C10, at setting date D0). After 60 days, dehydrogenase activity had declined substantially in all treatments. The two treatments where some dehydrogenase activity was still detected at the end of the experiment were B (control) and BC (compost) treatments. Their rates were significantly larger ($p \leq 0.05$) compared to the remaining treatments.

$\beta$-glucosidase activity $>1 \text{µmol } p$-NP g$^{-1}$ h$^{-1}$ was only found in the controls of the conventionally (A) and organically managed soils (B, BC) at D0 and D30 (Table 4). The highest $\beta$-glucosidase activity of $15.88 \pm 1.79 \text{µmol } p$-NP g$^{-1}$ h$^{-1}$ was measured for the conventionally managed control soil (A) followed by similar activities of $5.29 \pm 0.97$ and $4.88 \pm 1.42 \text{µmol } p$-NP g$^{-1}$ h$^{-1}$ for BC and B, respectively, at the beginning of the experiment (D0). At D30, $\beta$-glucosidase activities significantly decreased for A and B soil treatments in general, but increased for BC to $7.30 \pm 0.44 \text{µmol } p$-NP g$^{-1}$ h$^{-1}$ ($p \leq 0.05$); and at D60 their activities were reduced to $<1 \text{µmol } p$-NP g$^{-1}$ h$^{-1}$.

For soils amended with biochar or urea, $\beta$-glucosidase activity remained below $1 \text{µmol } p$-NP g$^{-1}$ h$^{-1}$ throughout the measurement period. After 60 days, no significant difference ($p \leq 0.05$) was found between different biochar application rates for the organically managed treatments with and without compost. On the other hand, treatments with biochar (5% and 10%), with (U5, U10) and without urea (A5, A10) for the conventionally managed soil A were significantly different ($p \leq 0.05$) than the other treatments but not amongst themselves.

Phosphatase activities were $>6.6 \text{µmol } p$-NPP g$^{-1}$ h$^{-1}$ for the conventionally managed treatments with urea and biochar (U5, U10) and for the two organically managed controls (B, BC) at D0 (Table 4). For the remaining treatments phosphatase activities only ranged between $0.42 \pm 0.03$ and $1.06 \pm 0.12 \text{µmol } p$-NPP g$^{-1}$ h$^{-1}$ and were not significantly different from each other ($p \leq 0.05$). After 30 days of incubation, phosphatase activity had increased for all treatments. Largest increases, 8 to 39 fold, were observed for the treatments that had the low phosphatase activities at D0. By day 60 (D60), all treatments had declined significantly to an average rate of $0.47 \text{µmol } p$-NPP g$^{-1}$ h$^{-1}$, with no significant difference between treatments ($p \leq 0.05$).

### Soil microbial respiration rates

Figure 1 show soil respiration rates from the controls (water only) and those induced by addition of three different carbon substrates: glucose, citric acid and N-acetyl glucosamine for the conventionally (A) and organically (B) managed soils, respectively. In general, largest soil respiration rates were measured at the beginning of the incubation period and declined in a similar manner during the first 15 days for all treatments. Thereafter, treatments remained constant until the end of the incubation.

### Table 4. Soils enzyme activities in all the treatments performed (Table 1).

| Treatments | Dehydrogenase ($\mu$g TPF g$^{-1}$ h$^{-1}$) | $\beta$-Glucosidase ($\mu$mol p-NP g$^{-1}$ h$^{-1}$) | Phosphatase ($\mu$mol p-NPP g$^{-1}$ h$^{-1}$) |
|------------|---------------------------------------------|-------------------------------------------------|---------------------------------------------|
|            | D0   | D30  | D60  | D0   | D30  | D60  | D0   | D30  | D60  |
| A          | 3.16a* | 0.91a | 0.05b | 15.88a | 7.98a | 0.15ab | 0.86c | 5.99c | 0.56a |
| A5         | 0.82b  | 0.18c | 0.03b | 0.27c | 0.12cd | 0.09b | 0.73c | 9.46b | 0.49c |
| A10        | 0.48cd | 0.11cd | 0.05b | 0.26c | 0.08d | 0.11ab | 1.06c | 9.08b | 0.41a |
| AU         | 3.52a  | 0.67b | 0.11a | 0.42b | 0.11cd | 0.21a | 0.85c | 5.65c | 0.44a |
| U5         | 0.86b  | 0.20c | 0.03b | 0.26c | 0.29b | 0.08b | 7.61b | 11.76a | 0.63a |
| U10        | 0.33d  | 0.09d | 0.02b | 0.23c | 0.23b | 0.06b | 8.96ab | 10.17ab | 0.56a |
| B          | 1.58a  | 0.74a | 0.53a | 4.88a | 4.06b | 0.35ab | 6.62a | 8.56c | 0.72a |
| BS         | 0.46b  | 0.19b | 0.08c | 0.59bc | 0.79c | 0.28bc | 0.42b | 16.45a | 0.25bc |
| B10        | 0.18d  | 0.08c | 0.01c | 0.47c | 0.11d | 0.15c | 0.45b | 9.87bc | 0.19c |
| BC         | 1.58a  | 0.94a | 0.29b | 5.29a | 7.30a | 0.56a | 7.89a | 10.77b | 0.84a |
| C5         | 0.40c  | 0.17b | 0.06c | 0.72bc | 0.24cd | 0.21bc | 0.47b | 15.60a | 0.22bc |
| C10        | 0.47b  | 0.10c | 0.02c | 0.51c | 0.19cd | 0.16c | 0.52b | 16.69a | 0.37bc |

*For each sampling date, significant differences ($p \leq 0.05$) are indicated by different letters within each row. Note that the statistical analysis was performed separately for conventionally managed soil A and organically managed soil B. The data show the average and standard deviations ($n = 3$) at D0, D30, D60 = 0, 30, 60 days after setup.
Figure 1. Soil A and B respiration rates: additions of (a) control water; (b) glucose; (c) citric acid and (d) N-acetyl glucosamine in all the treatments (Table 1) (n = 3 × 4). For each sampling date, significant differences (p ≤ 0.05) are indicated by different letters.
period, with exception of the conventionally managed soils (A) on the last measurement date (D60). For these soil treatments, respiration rates had increased slightly in all treatments between D45 and D60.

At D0, control respiration rates ranged from $4.05 \pm 0.48 \mu g \ CO_2-C \ g^{-1} \ h^{-1}$ (10% biochar + urea U10) to $7.42 \pm 0.52 \mu g \ CO_2-C \ g^{-1} \ h^{-1}$ (5% biochar A5) in conventionally managed soil A, and from $4.36 \pm 1.05 \mu g \ CO_2-C \ g^{-1} \ h^{-1}$ (10% biochar + compost C10) to $6.50 \pm 1.84 \mu g \ CO_2-C \ g^{-1} \ h^{-1}$ (5% biochar B5) in organically managed soil B (Figure 1a). Glucose (Figure 1b) and N-acetyl glucosamine (Figure 1d) induced respiration rates were similar to the control respiration rates (Figure 1a). Citric acid induced respiration rates (Figure 1c), on the other hand, were more than twice as large compared to the control and ranged between $4.18 \mu g \ CO_2-C \ g^{-1} \ h^{-1}$ (10% biochar+urea, U10, D30) and $16.76 \mu g \ CO_2-C \ g^{-1} \ h^{-1}$ (5% biochar, D0) in conventionally managed soil A5, and from $4.57 \mu g \ CO_2-C \ g^{-1} \ h^{-1}$ (10% biochar+compost C10, D45) to $15.22 \mu g \ CO_2-C \ g^{-1} \ h^{-1}$ (5% biochar B5, D0) in organically managed soil B.

For both the conventionally (A) and organically (B) managed soils, respiration rates in the 5% biochar application rate treatments, were significantly different from the remaining treatments ($p \leq 0.05$) throughout the incubation period for the control (water only) and the three selected carbon substrates (Figure 1). Significant relationships specific to each sampling date were as follows. At D0 control respiration rates did not differ ($p \leq 0.05$) between U5 and U10 treatments, and also between C5 and C10, AU and A10, and BC and B10. These relationships were also found for glucose (AU = A10; U5 = U10), citric acid (BC = B10) and for N-acetyl glucosamine (AU = A10) induced respiration rates. After the 60-day incubation period, control respiration rates were not significantly different between BC and B10 treatments, and between C5 and C10 ($p \leq 0.05$). N-acetyl glucosamine induced respiration rates did not differ ($p \leq 0.05$) between BC, C5, and U10, between B and U10, and between C5 and C10.

**Soil mineral nitrogen**

Much larger concentrations of available N were measured from the conventionally managed treatments than the organically managed treatments (Figure 2).

NH$_4^+$-N concentrations were higher at the beginning of the experiment (D0) for both conventionally (A) and organically (B) managed treatments (Figure 2a), ranging between 45.14 ± 6.68 mg kg$^{-1}$ (A10) and 111.24 ± 1.33 mg kg$^{-1}$ (AU) in the former, and between 16.63 ± 0.25 mg kg$^{-1}$ (B) and 18.20 ± 0.08 mg kg$^{-1}$ (B10) in the latter. NH$_4^+$-N concentrations declined after 15 days (D15) in all treatments and were below the detection limit for most of the remaining study period (D30 and D45) which is why data for D30 and D45 are not shown in the graphs. For both farm management systems, NH$_4^+$-N concentrations decreased with time. This decline was greater for the conventionally managed treatments, particularly the urea treatments, as they produced very large NH$_4^+$-N concentrations at the start of the incubation period. At D0 biochar amendments significantly reduced NH$_4^+$-N concentrations in the A and AU treatments, whereas after 60 days, biochar addition to soils did not significantly affect NH$_4^+$-N concentrations ($p \leq 0.05$) regardless of the application rate, but the presence of urea did. Conversely, organically managed treatments with and without compost plus 5% of biochar (B5 and C5) did not differ throughout the entire incubation period ($p \leq 0.05$), whereas the higher rate of applied biochar with and without compost varied (D0 and D60).

NO$_3^-$N concentrations increased in all conventionally managed treatments during the first 45 days (Figure 2b) and declined at the end of the incubation experiment (D60). At all sampling dates, NO$_3^-$N concentrations were significantly larger when biochar was added. The impact of biochar was the largest at D45, when A5 and A10 treatments were around 83% and 82% larger than the control (A), and U5 and U10 were 85% and 87% higher than the urea control (AU). Organically managed treatments, however, revealed a different behavior with time. Initially (D0), the non-biochar treatments (B and BC) had significantly larger NO$_3^-$N concentrations compared to the biochar
treatments (B5 = C5; B10 = C10) (p ≤ 0.05). However, after 45 days, the NO$_3^-$ concentrations in B10 treatment were 54.9% lower than the control (B), whereas the concentrations in B5 were 23.4% higher. Conversely, the concentrations of NO$_3^-$ in both biochar and compost treatments (C5 and C10) were 17% and 56.3% lower when compared to BC, respectively. Even though no difference was found between B10 and C10 at days 30 and 45, after the 60-day incubation period all biochar treatments significantly differed (p ≤ 0.05).

Ammonia emission

In general, larger fluctuations of NH$_3$ concentrations were observed throughout the 10-day incubation period for the conventionally managed soil (A) compared to organically managed soil (B) treatments (Figure 3). During the incubation period, air temperature increased with time, from 22 °C at the start (data not shown) to 25 °C after 10 days. Largest NH$_3$ concentrations were measured from the soil A with urea addition only (AU, 1.03 ± 0.59 NH$_3$ mg kg$^{-1}$) at day 2, decreasing in total 55% after 10 days. The effect of biochar on NH$_3$ concentrations in conventionally managed treatments varied throughout the experiment. The treatments with the higher rate of biochar (A10 and U10) had smaller NH$_3$ concentrations compared to the treatments with the lower biochar rate (A5 and U5) at days 2, 6 and 8, while the opposite occurred on day 4. However, lower NH$_3$ concentrations
were in general measured with the higher rate of biochar for all sampling dates, except the final date (D10).

A differing range in biochar effects on NH$_3$ emissions was also observed for organically managed treatments (Figure 3). After two days, the treatments with the lower biochar rate (B5 and C5) tended to release larger amounts of NH$_3$ compared to the treatments with 10% biochar (B10 and C10). After 10 days, no difference was observed between the treatments with both compost and biochar (C5 and C10) and between conventionally managed control and treatments with biochar and organically managed controls and treatments with just biochar (A, A5, A10, B, BC, B5, and B10).

**Figure 3.** Ammonia (NH$_3$) emission in all the treatments (Table 2) (n=3×2) (left axis), and air temperature (Temp) (right axis). For each sampling date, significant differences (p ≤ 0.05) are indicated by different letters (note that soil from each farm was analysed separately).
**Discussion**

Discrepancies in the biogeochemical response to biochar amendments to soils are frequently reported and demonstrate that there are no universal responses to biochar use (Kolb, Fermanich, and Dornbush 2009; Prayogo et al. 2014). Different behaviors may result from variations in biochar types (e.g., feedstock, pyrolysis conditions), application rates, soil types and properties, farming practices and climatic conditions.

As expected the WHC increased as a result of biochar addition to soil. The WHC in the organically farmed soil B was larger than in the conventionally farmed soil A, due to an almost three times larger soil organic matter content. Biochar, as well as soil organic matter, can improve soil pore structure and enhance water retention due to its highly porous structure and large surface area (Ouyang and Zhang 2013; Ulyett et al. 2014; Verheijen et al. 2009). The porous nature of biochar primarily affects the physical properties of the topsoil and is a source of organic carbon (Marousek et al. 2017; Tammeorg et al. 2016). The magnitude of this effect in our study depended on the biochar application rate. The larger biochar application rate of 10% supplied more organic carbon and thereby increased the WHC to a greater extent than the 5% rate. It is interesting to observe that biochar additions increased the WHC in the conventionally farmed low organic matter soil by 46%, but in the organically farmed soil with a higher organic matter only by 28%. Equally, the soil organic matter content increased when biochar was added, and this increase was slightly lower with the larger biochar application rate of 10%. The stable percentage of SOM in the conventionally managed soil compared to a decline in SOM in the organically managed soils, suggests larger mineralization rates. This difference did not translate into the impact of biochar on soil enzyme activities or microbial respiration rates.

Changes in soil pH after biochar additions have been shown to increase the soil pH for a range of biochar products (Ouyang et al. 2014a). The rate of increase may be very different, depending on the product or soil conditions. In our study, the soil pH slightly increased when biochar was added to the conventionally managed soils by 0.3 pH units. Similar small rates of increase to those (0.1–0.2 pH units) were also reported by Taghizadeh-Toosi et al. (2011) for a perennial grassland treated with woody biochar in New Zealand. But the opposite was the case for the organically managed soils, for which the pH decreased by 0.2. This may happen in the short term due to the microbial decomposition of easily mineralizable small organic molecules, that produce CO₂, organic acids and initial ammonia, what decrease soil pH. Soil B had a higher content of SOM and thus more mineralizable compounds.

The soil enzymes included in this study represent key processes of soil organic matter turnover, which may be different in soils treated with biochar. Dehydrogenase enzyme activity is often used as a biological activity indicator of soil fertility, as it facilitates soil organic matter oxidation (Makoi and Ndakidemi 2008). In our study dehydrogenase activities declined with time and were much reduced in the presence of biochar. The enzyme activity could have increased right after the biochar addition, but the first sampling was at day 30 which may have masqueraded earlier effects. Similar trends were observed by Ouyang et al. (2014a), investigating the impact of biochar dehydrogenase activity in a silt loamy soil, and also after applying sewage sludge-derived biochar to a forest soil (Paz-Ferreiro et al. 2012). The latter authors implied that the high heavy metal concentration in the sewage-derived biomass may have been responsible for the reduction in dehydrogenase activity.

Similar to dehydrogenase, also β-glucosidase activity in biochar treatments was significantly lower during the entire experimental period when compared to the controls, as also reported previously (Paz-Ferreiro et al. 2012). This decrease may be partially due to the fact that the optimum pH for β-glucosidase activity is in general acidic, and the pH of the soils in our study was neutral to alkaline, and on average slightly increased during the incubation period, in some cases significantly. Contrary Ventura et al. (2014) reported that the addition of an alkaline wood biochar to an apple orchard did not effect β-glucosidase activity. Also in our study, β-glucosidase behavior in the conventionally managed control soil treated with urea (AU) was significantly higher than in biochar treatments (A5,
A10) at the beginning (D0) and at the end (D60) of this study. The effect of biochar on β-glucosidase activity in our study is inconclusive and needs further research.

The lower dehydrogenase and β-glucosidase activities in the biochar treatments may be caused by the condensed aromatic structures and physically resistant to degradation of the wood-based biochar in contrast to manure-based biochars (Ouyang, Yu, and Zhang 2014b). Indeed, woody biochar tends to adsorb more substrate than manure based ones, reducing their availability and thus inhibiting enzyme activity. Even though biochar increases the soil absorption capacity and stabilizes soil–enzyme interactions in some cases (Sun et al. 2014), further long-term studies need to verify this, especially as woody biochar tends to exhibit beneficial effects on soil microbial abundance much later (>60 days) (Gul et al. 2015) than manure based biochar.

Phosphatase activity is associated with the demand for P by microorganisms and plants (Piotrowska-Długosz 2014) and is inversely proportional to plant available P (Amador et al. 1997; Sinsabaugh et al. 1993). In our study phosphatase activity was largest after the 30-day incubation period, unlike the dehydrogenase and β-glucosidase activities which were largest at the start of the incubation period and declined thereafter. In general, microbial biomass increases after biochar application (Liu et al. 2016) because biochar may promote nutrient cycling in soil. This includes phosphorus (P) mobilization by stimulation of the soil microbial activity; and the response is strongly dependent on soil type (Deb et al. 2016). The reason for the decline in phosphatase activity between day 30 and day 60 may be a decline in substrate availability. Addition of biochar increased phosphatase activity, especially when urea and biochar (U5, U10) were added. Our results are in line with the observation that phosphatase activity is mainly promoted by a low inorganic phosphorus content or by an increase in organic matter and hence organic P (Nannipieri et al. 2011). Bell, Robinson, and Schwartz (2006) also observed increases in phosphatase activity after manure application which may be explained by enhanced P mineralization.

Contrasting microbial responses to biochar addition can be found in the literature (Dempster et al. 2012; Kolb, Fermanich, and Dornbush 2009; Zimmerman, Gao, and Ahn 2011). Differences in physiochemical properties of the soil and biochar products are an important driver of such contrasting results (Gul et al. 2015). The present study showed a decline in soil respiration rates after 60 days in all treatments, with the addition of glucose, citric acid, N-acetyl glucosamine or no carbon addition (Figure 1, 2). Comparing the biochar treatments (5% and 10%) with the respective non-biochar controls for each sampling date, we concluded that the general trend was that biochar additions reduced soil respiration rates. This is in agreement with Prayogo et al. (2014) and Weyers et al. (2010) who reported that an increase in the biochar application rate caused a progressive reduction in soil respiration rate. Contrary, Kolb, Fermanich, and Dornbush (2009) found that background and substrate-induced respiration rates increased the most following a 10% manure based biochar application rate. Background respiration increased throughout their 96-day incubation period but remained constant in the other treatments throughout the experiment, whereas induced respiration generally decreased in the unamended and lower biochar amended treatments.

Biochar is known to influence N availability in soils (Spokas et al. 2012) thus affecting crop growth and soil N losses. Our results for the conventionally managed soil treatments showed that NH$_4^+$-N concentrations were lower in the biochar treatments compared to the controls whilst NO$_3^-$ N concentrations were higher. Nutrients such as nitrogen (N) are known not to be immediately available for plant uptake in the presence of biochar because their mineralization rate will be reduced by covalent bonding to biochar particles (Tammeorg et al. 2016). So, direct nutrient supply via biochar mineralization was considered less important than indirect processes, such as enzyme activities. Also, alkaline biochar additions to agricultural soils, such as reported in this study, are likely to promote nitrification of NH$_4^+$ to NO$_3^-$ by promoting soil water holding capacity and aeration and raising soil pH from neutral to alkaline (Gul and Whalen 2016). As demonstrated by a study using low-temperature biochar (Deenik et al. 2010), our results also showed that both conventionally and organically managed treatments had a higher NH$_4^+$-N content at the start of the
experiment, especially in urea treatments and in non-biochar treatments, but declined considerably afterwards, possibly followed by nitrification or $\text{NH}_4^+$ adsorption to biochar, clay particles or other types of organic matter. The extent of $\text{NH}_4^+$-N decline was greater in the conventionally managed treatments (A), presumably attributable to the larger initial N content in relation to the organically managed ones (B) (Kelly et al. 2015).

Ippolito et al. (2014) described progressive $\text{NO}_3$-N increases in biochar amended soils during 12 months for all biochar application rates used. This means that mineral N release is slower, and there is less nitrate losses. However, the largest increase occurred with the lower biochar application rate. Although on a much shorter timescale, the results from the present study also showed a continuous increase in $\text{NO}_3$-N concentrations in biochar treatments during the first 45 days, especially in the conventional farming treatments, declining in the last 15 days of incubation, while non-biochar treatments showed almost a constant behavior. Additionally, in the conventionally managed soil treatments, $\text{NO}_3$-N concentrations were in general higher in the treatments with the higher biochar application rate. Conversely, the organically managed soil treatments showed lower concentrations of $\text{NO}_3$-N with the higher rate of biochar. These results relate to those of Prayogo et al. (2014), who found $\text{NH}_4^+$-N levels became reduced between day 30 and day 90, suggesting net immobilization. Other authors (Deenik et al. 2010; Rondon et al. 2007) observed a decrease in soil mineral N (N immobilization) in the presence of low-temperature biochars with high volatile matter content and high C/N ratio, supporting thus our findings in the later incubation stage. Once the available C is exhausted, the immobilized N may be remineralized, therefore supporting biochar’s potential ability to act as a slow-release fertilizer (Kammann et al. 2015).

$\text{NH}_3$ volatilization is promoted by high soil pH, temperature and low soil moisture content, provided there is a suitable N source, such as $\text{NH}_4^+$-N or urea (Taghizadeh-Toosi et al. 2012). These conditions were met by some of our treatments, particularly AU. In the organically managed soil treatments $\text{NH}_3$ volatilization was almost constant during the 10-day incubation period, whereas in the conventionally managed soils the urea treatments produced considerably higher losses. However, when comparing the controls (A and AU) with the biochar treatments (A5, A10, U5 and U10), it can be seen that biochar, in general, caused a decrease in $\text{NH}_3$ emissions, which may be attributable to $\text{NH}_3$ hydrolysis to $\text{NH}_4^+$ followed by adsorption to biochar, immobilization or nitrification, as suggested by Mandal et al. (2016). Taghizadeh-Toosi et al. (2012) also found that biochar could adsorb $\text{NH}_3$ and significantly decrease its volatilization from ruminant urine.

**Conclusions**

Our short-term 60-day experiments suggest that different previous farm managements (conventional vs. organic) as well as different fertilization practices (mineral vs. organic) should be considered when adding biochar, as these variables affect biochar impacts in soil. The impact of biochar produced at low temperatures on soil biological processes, such as enzymatic and microbial activities, is similar to those obtained at high temperature, thus proving that there is no need to increase the energy expenditure to produce biochar, to obtain a good product. The benefit of low-temperature biochar production is the lower energy requirements, while improving water holding capacity of the soil, and in some cases increasing microbial respiration. This, in turn, can increase soil organic matter mineralization in the short term.

Biochar addition significantly decreased dehydrogenase and $\beta$-glucosidase activities in both conventionally and organically managed soils. However, this effect was more pronounced in the conventionally managed soil and when urea was added. The largest phosphatase activity was observed in treatments with biochar addition, especially for the organically managed soil treatments. This is most likely due to a greater release and availability of organic phosphorus. Biochar decreased $\text{NH}_4^+$-N content in the conventionally managed soil and a progressive increase in $\text{NO}_3$-N, while in the organically managed soil, biochar had no effect on $\text{NH}_4^+$-N concentration, but promoted a decrease in $\text{NO}_3$-N, that happened probably through denitrification. Volatilization of $\text{NH}_3$ was
higher in urea treatments than in treatments with compost, and decreased with biochar addition in all situations.

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