

1 The impact of woody biochar on microbial processes in conventionally
2 and organically managed arable soils

3 Cláudia M d S Cordovil^{1*}, Renata Pinto¹, Beatriz Silva¹, Lidia Sas-Paszt²,
4 Ruben Sakrabani³ and Ute M Skiba⁴

5 ¹University of Lisbon, School of Agronomy, LEAF, Tapada da Ajuda 1349-017 Lisbon,
6 Portugal

7 ²Research Institute of Horticulture, 96-100 Skierniewice, Poland

8 ³School of Energy, Environment and Agrifood, Cranfield University, Cranfield MK43
9 OAL, UK

10 ⁴Centre for Ecology and Hydrology, Edinburgh, Bush Estate, Penicuik, Midlothian EH26
11 0QB, UK

12 * Corresponding author – cms@isa.ulisboa.pt

13 Instituto Superior de Agronomia, LEAF, Tapada da Ajuda, 1349-017 Lisboa, Portugal

The impact of woody biochar on microbial processes in conventionally and organically managed arable soils

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 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 nitrogen 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 behaviour 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.

Keywords: ammonia volatilization, enzyme activity, low temperature biochar, mineral nitrogen, soil respiration.

1. 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) fertiliser 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. (2017).

Biochar is a carbon-rich by-product of pyrolysis at low oxygen concentrations (Lehmann et al., 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 ($\text{NO}_3\text{-N}$) (Kammann et al., 2015) and ammonia-N ($\text{NH}_3\text{-N}$) (Taghizadeh-Toosi et al., 2012). Thereby biochar affects nutrient cycling and organic matter 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 ($>500^\circ\text{C}$) providing highly recalcitrant and decay-resistant products (Sakrabani et al., 2017). Contrary low temperature biochars ($\sim 300^\circ\text{C}$) have not received the same attention, in spite of the fact that they have higher bioavailability and provide carbon 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°C) 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 composed 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 CO₂ 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 (Paz-Ferreiro et al., 2012; Chen et al., 2013) and (3) ammonia emissions, as the presence of biochar has been reported to reduce emissions of NH₃ after N fertiliser application (Mandal et al., 2016).

2. Materials and Methods

Incubation experiments were set up using two different Cambisols (WRB 2006) collected from two vegetable production farms with previous contrasting fertilisation 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_{min} (N-NH₄⁺ + N-NO₃⁻) 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 (Jeffery *et al.*, 2011; Paz-Ferreiro *et al.*, 2012; Ameloot *et al.*, 2014; Ouyang *et al.*, 2014a). 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).

2.1 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 cm³) 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.

2.1.1 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 one hour prior measurement using a Thermo Electron Corporation potentiometer, with a detection limit of 0.01 pH units.

2.1.2 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 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 hour 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 hour (Tabatabai and Bremner, 1969). The spectrophotometer used was a segmented flow analyser from Skalar.

2.1.3 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 μ L

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 $\times$ 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 $24^{\circ}\text{C} \pm 2^{\circ}\text{C}$. Data was normalized for time zero, to eliminate differences in colour between wells due to uneven gel density.

2.1.4 Mineral Nitrogen

The mineral N ($\text{NH}_4^+\text{-N}$ and $\text{NO}_3^-\text{-N}$) 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 hour with 2M KCl solution (1:10) at room temperature, and centrifuged at 3000 rpm for 5 minutes as adapted by Cordovil *et al.* (2005). Prior to analysis, KCl extracts were stored in the fridge until the next day.

2.2 The impact of biochar rate on 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 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 $\times$ 5 $\times$ 2 cm) soaked in 7 ml of phosphoric acid (0.5M) and then fixed to acrylic plates (7 $\times$ 7 $\times$ 0.3 cm) with polytetrafluoroethylene tape, which is permeable to NH_3 but not to water. The foams were placed 1 cm above each of the 12 $\times$ 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. NH_3 was then determined using the segmented-flow analyser detailed above.

2.3 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 analysed 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).

3. 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 ($0.008 \text{ cm}^3 \cdot \text{g}^{-1}$ total pores and $0.0007 \text{ cm}^3 \cdot \text{g}^{-1}$ micro pores $< 20 \text{ \AA}$). 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).

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 considerable 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.

3.1 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 $\mu\text{g TPF g}^{-1} \text{h}^{-1}$ (AU), whereas organically managed treatments (see Table 1) only reached 1.58 ± 0.55 $\mu\text{g TPF g}^{-1} \text{h}^{-1}$ 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 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.

β -glucosidase activity > 1 $\mu\text{mol } p\text{-NP g}^{-1} \text{h}^{-1}$ was only found in the controls of the conventionally (A) and organically managed soils (B, BC) at D0 and D30 (Table 4). The highest β -glucosidase activity of 15.88 ± 1.79 $\mu\text{mol } p\text{-NP g}^{-1} \text{h}^{-1}$ was measured for the conventionally managed control soil (A) followed by similar activities of 5.29 ± 0.97 and 4.88 ± 1.42 $\mu\text{mol } p\text{-NP g}^{-1} \text{h}^{-1}$ for BC and B, respectively, at the beginning of the experiment (D0). At D30, β -glucosidase activities significantly decreased for A and B

soil treatments in general, but increased for BC to $7.30 \pm 0.44 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ ($p \leq 0.05$); and at D60 their activities were reduced to $< 1 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$.

For soils amended with biochar or urea, β -glucosidase activity remained below $1 \mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1}$ 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 $> 6.6 \mu\text{mol } p\text{-NPP g}^{-1} \text{ h}^{-1}$ 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 ± 0.03 and $1.06 \pm 0.12 \mu\text{mol } p\text{-NPP g}^{-1} \text{ h}^{-1}$ 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 $0.47 \mu\text{mol } p\text{-NPP g}^{-1} \text{ h}^{-1}$, with no significant difference between treatments ($p \leq 0.05$).

3.2 Soil Microbial Respiration Rates

Figure 1 and Figure 2 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 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 $4.05 \pm 0.48 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ (10% biochar+urea U10) to $7.42 \pm 0.52 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ (5% biochar A5) in conventionally managed soil A, and from $4.36 \pm 1.05 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ (10% biochar+compost C10) to $6.50 \pm 1.84 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ (5% biochar B5) in organically managed soil B (Figures 1a, 2a). Glucose (Figures 1b, 2b) and N-acetyl glucosamine (Figures 1d, 2d) induced respiration rates were similar to the control respiration rates (Figures 1a, 2a). Citric acid induced respiration rates (Figures 1c, 2c), on the other hand, were more than twice as large compared to the control and ranged between $4.18 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ (10% biochar+urea, U10, D30) and $16.76 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ (5% biochar, D0) in conventionally managed soil A5, and from $4.57 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ (10% biochar+compost C10, D45) to $15.22 \mu\text{g CO}_2\text{-C g}^{-1} \text{ h}^{-1}$ (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 (Figures 1, 2). 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.

3.3 Soil Mineral Nitrogen

Much larger concentrations of available N were measured from the conventionally managed treatments than the organically managed treatments (Figure 3).

$\text{NH}_4^+\text{-N}$ concentrations were higher at the beginning of the experiment (D0) for both conventionally (A) and organically (B) managed treatments (Figure 3a), ranging between $45.14 \pm 6.68 \text{ mg kg}^{-1}$ (A10) and $111.24 \pm 1.33 \text{ mg kg}^{-1}$ (AU) in the former, and between $16.63 \pm 0.25 \text{ mg kg}^{-1}$ (B) and $18.20 \pm 0.08 \text{ mg kg}^{-1}$ (B10) in the latter. $\text{NH}_4^+\text{-N}$ 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, $\text{NH}_4^+\text{-N}$ concentrations decreased with time. This decline was greater for the conventionally managed treatments, particularly the urea treatments, as they produced very large $\text{NH}_4^+\text{-N}$ concentrations at the start of the incubation period. At D0 biochar amendments significantly reduced $\text{NH}_4^+\text{-N}$ concentrations in the A and AU treatments, whereas after 60 days, biochar addition to soils did not significantly affect $\text{NH}_4^+\text{-N}$ 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).

NO₃⁻-N concentrations increased in all conventionally managed treatments during the first 45 days, (Figure 3b), and declined at the end of the incubation experiment (D60). At all sampling dates, NO₃⁻-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 behaviour with time. Initially (D0), the non-biochar treatments (B and BC) had significantly larger NO₃⁻-N concentrations compared to the biochar treatments (B5=C5; B10=C10) ($p \leq 0.05$). However, after 45 days, the NO₃⁻-N 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 NO₃⁻-N 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 ($p \leq 0.05$).

3.4 Ammonia emission

In general, larger fluctuations of NH₃ concentrations were observed throughout the 10-day incubation period for the conventionally managed soil (A) compared to organically managed soil (B) treatments (Figure 4). 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 NH₃ concentrations were measured from the soil A with urea addition only (AU, 1.03 ± 0.59 NH₃ mg kg⁻¹) at day 2, decreasing in total 55% after 10 days. The effect of biochar on NH₃ 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 4). 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).

4. 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 *et al.*, 2009; Prayogo *et al.*, 2014). Different behaviours 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 (Verheijen *et al.*, 2009; Ouyang and Zhang, 2013; Ulyett *et al.*, 2014). 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.*, 2017). 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 has 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 Anderson *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 *et al.*, 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 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 were 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 behaviour 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 *et al.*, 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 *et al.* (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 (Kolb *et al.*, 2009; Zimmerman *et al.*, 2011; Dempster *et al.*, 2012). 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

434 a decline in soil respiration rates after 60 days in all treatments, with the addition of
 435 glucose, citric acid, N-acetyl glucosamine or no carbon addition (Figures 1, 2).
 436 Comparing the biochar treatments (5 and 10%) with the respective non-biochar controls
 437 for each sampling date, we concluded that the general trend was that biochar additions
 438 reduced soil respiration rates. This is in agreement with Prayogo *et al.* (2014) and
 439 Weyers *et al.* (2010) who reported that an increase in the biochar application rate caused
 440 a progressive reduction in soil respiration rate. Contrary, Kolb *et al.* (2009), found that
 441 background and substrate induced respiration rates increased the most following a 10%
 442 manure based biochar application rate. Background respiration increased throughout
 443 their 96-day incubation period but remained constant in the other treatments throughout
 444 the experiment, whereas induced respiration generally decreased in the unamended and
 445 lower biochar amended treatments.

446 Biochar is known to influence N availability in soils (Spokas *et al.*, 2012) thus
 447 affecting crop growth and soil N losses. Our results for the conventionally managed soil
 448 treatments showed that NH_4^+ -N concentrations were lower in the biochar treatments
 449 compared to the controls whilst NO_3^- -N concentrations were higher. Nutrients such as
 450 nitrogen (N) are known not to be immediately available for plant uptake in the presence
 451 of biochar because their mineralisation rate will be reduced by covalent bonding to
 452 biochar particles (Tammeorg, *et al.*, 2017). So, direct nutrient supply via biochar
 453 mineralization was considered less important than indirect processes, such as enzyme
 454 activities. Also, alkaline biochar additions to agricultural soils, such as reported in this
 455 study, are likely to promote nitrification of NH_4^+ to NO_3^- by promoting soil water
 456 holding capacity and aeration and raising soil pH from neutral to alkaline (Gul and
 457 Whalen, 2016). As demonstrated by a study using low-temperature biochar (Deenik *et*
 458 *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 NH_4^+ adsorption to biochar, clay particles or other types of organic matter. The extent of 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 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 time scale, the results from the present study also showed a continuous increase in 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 behaviour. Additionally, in the conventionally managed soil treatments, 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 NO_3^- -N with the higher rate of biochar. These results relate to those of Prayogo *et al.* (2014), who found NH_4^+ -N levels became reduced between day 30 and day 90, suggesting net immobilization. Other authors (Rondon *et al.*, 2007; Deenik *et al.*, 2010) 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 remineralised, therefore supporting biochar's potential ability to act as a slow-release fertiliser (Kammann *et al.*, 2015).

NH₃ volatilization is promoted by high soil pH, temperature and low soil moisture content, provided there is a suitable N source, such as NH₄⁺-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 NH₃ 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 NH₃ emissions, which may be attributable to NH₃ hydrolysis to NH₄⁺ 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 NH₃ and significantly decrease its volatilization from ruminant urine.

5. Conclusions

Our short-term 60 day experiments suggest that different previous farm managements (conventional vs. organic) as well as different fertilisation 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 SOM mineralization in the short term.

Biochar addition significantly decreased dehydrogenase and β -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 NH_4^+ -N content in the conventionally managed soil and a progressive increase in NO_3^- -N, while in the organically managed soil, biochar had no effect on NH_4^+ -N concentration, but promoted a decrease in NO_3^- -N, that happened probably through denitrification. Volatilization of NH_3 was higher in urea treatments than in treatments with compost, and decreased with biochar addition in all situations.

Acknowledgements

The authors thank the NitroPortugal project, H2020-TWINN-2015, EU coordination and support action n. 692331 for funding. The support of FCT, Portugal, through the research unit LEAF (UID/AGR/04129/2013) is acknowledged.

References

- Alves, A., Oliveira, P., Herling, V., Trivelin, P., Luz, P., Alves, T., Rochetti, R., Barioni Jr, W. 2011. New methods to quantify NH_3 volatilization from fertilized surface soil with urea. *Revista Brasileira Ciência do Solo* 35, 133–140.
- Amador, J.A., Glucksman, A.M., Lyons, J.B., Görres, J.H. 1997. Spatial distribution of soil phosphatase activity within a riparian forest. *Soil Science*, 162, 808-825.

- 529 Ameloot, N., Sleutel, S., Case, S.D.C., Alberti, G., McNamara, N.P., Zavalloni, C.,
 530 Vervish, B., Vedove, G., De Neve, S. C. 2014. Mineralization and microbial activity in
 531 four biochar field experiments several years after incorporation. *Soil Biology and*
 532 *Biochemistry*, 78, 195-203.
- 533 Bell, J.M., Robinson, C.A., Schwartz, R.C. 2006. Changes in soil properties and enzyme
 534 activities following manure applications to a rangeland. *Rangeland Ecological*
 535 *Management*, 59, 314-320.
- 536 Benjamini, Y., Hochberg, Y. 1995. Controlling the false discovery rate: a practical and
 537 powerful approach to multiple testing. *J Royal Statistical Soc Series B*, 57, 289–300.
- 538 Benjamini, Y., Yekutieli, D. 2001. The control of the false discovery rate in multiple
 539 testing under dependency. *Annals Stats*, 29, 1165–1188.
- 540 Biederman, L.A., Harpole, W.S. 2013. Biochar and its effects on plant productivity and
 541 nutrient cycling: a meta-analysis. *GCB Bioenergy*, 5, 202–214.
- 542 Campbell, C.D., Chapman, S.J., Cameron, C.M., Davidson, M.S., Potts, J.M., 2003. A
 543 rapid microtiter plate method to measure carbon dioxide evolved from carbon substrate
 544 amendments so as to determine the physiological profiles of soil microbial communities
 545 by using whole soil. *Appl Environ Microbiol* 69, 3593-3599.
- 546 Chen J., Liu X., Zheng, J., Zhang, B., Lu, H., Chi, Z., Pan, G., Li, L., Zheng, J., Zhang,
 547 X., Wang, J., Yu, X., 2013. Biochar soil amendment increased bacterial but decreased
 548 fungal gene abundance with shifts in community structure in a slightly acid rice paddy
 549 from Southwest China. *Applied Soil Ecol*, 71, 33-44.

- 550 Cordovil, C.M.d.S., Coutinho, J., Goss, M., Cabral, F., 2005. Potentially mineralizable
 551 nitrogen from organic materials applied to a sandy soil: fitting the one-pool exponential
 552 model. *Soil Use Manage*, 21, 65-72.
- 553 Cordovil C.M.d.S., Varennes A. de, Pinto R., Fernandes R.C., 2011. Changes in mineral
 554 nitrogen, soil organic matter fractions and microbial community level physiological
 555 profiles after application of digested pig slurry and compost from municipal organic
 556 wastes to burned soils. *Soil Biol. Biochem.*, 43, 845-852.
 557 doi:10.1016/j.soilbio.2010.12.023
- 558 Deb, D., Kloft, M., Lassig, J., Walsh, S., 2016. Variable effects of biochar and P
 559 solubilizing microbes on crop productivity in different soil conditions. *Agroecology and*
 560 *Sust Food Syst*, 40, 145–168. <http://dx.doi.org/10.1080/21683565.2015.1118001>
- 561 Deenik, J.L., McClellan, T., Uehara, G., Antal, M.J., Campbell, S., 2010. Charcoal
 562 volatile matter content influences plant growth and soil nitrogen transformations. *Soil Sci*
 563 *Soc Am J*, 74, 1259–1270.
- 564 Degens, B.P., Harris, J.A., 1997. Development of a physiological approach to measuring
 565 the catabolic diversity of soil microbial communities. *Soil Biol Biochem*, 19, 1309-1320.
- 566 Dempster, D.N., Gleeson, D.B., Solaiman, Z.M., Jones, D.L., Murphy, D.V., 2012.
 567 Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar
 568 addition to a coarse textured soil. *Plant Soil*, 354, 311-324.
- 569 EC. Council directive 91/676/EEC concerning the protection of waters against pollution
 570 caused by nitrates from agricultural sources.
 571 http://ec.europa.eu/environment/water/water-nitrates/index_en.html. Accessed
 572 November 2017.

- 573 Gul, S., Whalen, J.K., Thomas, B.W., Sachdeva, V., 2015. Physico-chemical properties
574 and microbial responses in biochar amended soils: Mechanisms and future directions.
575 *Agric, Ecosyst Environ*, 206, 46-59.
- 576 Gul, S., Whalen, J.K., 2016. Biochemical cycling of nitrogen and phosphorus in biochar-
577 amended soils. *Soil Biol Biochem*, 103, 1-15.
- 578 Ippolito, J., Stromberger, M., Lentz, R., Dungan, R., 2014. Hardwood biochar influences
579 calcareous soil physicochemical and microbiological status. *J Environ Qual*, 43, 681-689.
- 580 Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative
581 review of the effects of biochar application to soils on crop productivity using meta-
582 analysis. *Agric, Ecosyst Environ*, 144, 175-187.
- 583 Kammann, C., Schmidt, H-P, Messerschmidt N, Linsel S, Steffens D, Müller C, Koyro,
584 H.W., Conte, P., Joseph, S., 2015. Plant growth improvement mediated by nitrate capture
585 in co-composted biochar. *Scientific Reports*, 5, 11080.
- 586 Kelly, C.N., Calderón, F.C., Acosta-Martínez, V., Mikha, M.M., Benjamin, J.,
587 Rutherford, D.W., Rostad, C., 2015. Switchgrass biochar effects on plant biomass and
588 microbial dynamics in two soils from different regions. *Pedosphere*, 25, 329-342.
- 589 Kolb, S.E., Fermanich, K.J., Dornbush, M.E., 2009. Effect of charcoal quantity on
590 microbial biomass and activity in temperate soils. *Soil Sci Soc Am J*, 73, 1173–1181.
- 591 Kumar, S., Masto, R.E., Ram, L.C., Sarkar, P., George, J., Selvi, V.A., 2013. Biochar
592 preparation from *Parthenium hysterophorus* and its potential use in soil application. *Ecol*
593 *Engineering*, 55, 67-72.

- 594 Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial
595 ecosystems – a review. *Mitig Adapt Strategies Global Change*, 11, 395-419.
- 596 Liu, S., Zhang, Y., Zong, Y., Hu, Z., Wu, S., Zhou, J., Jin, Y., Zou, J., 2016. Response of
597 soil carbon dioxide fluxes, soil organic carbon and microbial biomass carbon to biochar
598 amendment: a meta-analysis, *Global Change Biol Bioenergy*, 8, 392–406.
599 <http://dx.doi.org/10.1111/gcbb.12265>
- 600 Makoi, J., Ndakidemi, P., 2008. Selected Soil Enzymes: Examples of their potential 407
601 roles in the ecosystem. *African J Biotechnol*, 7, 181-191.
- 602 Mandal, S., Thangarajan, R., Bolan, N.S., Sarkar, B., Khan, N., Ok, Y.S., Naidu, R., 2016.
603 Biochar-induced concomitant decrease in ammonia volatilization and increase in nitrogen
604 use efficiency by wheat. *Chemosphere*, 142, 120-127.
- 605 Marousek J, Vochozka M, Plachy J, Zak J., 2017. Glory and misery of biochar. *Clean*
606 *Techn Environ Pol*, 19, 311–317. DOI 10.1007/s10098-016-1284-y
- 607 Nannipieri, P., Giagnoni, L., Landi, L., Renella, G., 2011. Role of Phosphatase Enzymes
608 in Soil. In: Phosphorus in Action, *Soil Biol*, 26, 215-243.
- 609 Ouyang, L., Zhang, R., 2013. Effects of biochars derived from different feedstocks and
610 pyrolysis temperatures on soil physical and hydraulic properties. *J Soils Sedim*, 13, 1561–
611 1572.
- 612 Ouyang, L., Tang, Q., Yu, L., Zhang, R., 2014a. Effects of amendment of different
613 biochars on soil enzyme activities related to carbon mineralization. *Soil Res*, 52, 706:716.
- 614 Ouyang, L., Yu, L., Zhang, R., 2014b. Effects of amendment of different biochars on soil
615 carbon mineralization and sequestration. *Soil Res*, 52, 46-54.

- 616 Paz-Ferreiro, J., Gascó, G., Gutiérrez, B., Méndez, A., 2012. Soil biochemical activities
617 and the geometric mean of enzyme activities after application of sewage sludge and
618 sewage sludge biochar to soil. *Biol Fertil Soils*, 48, 511-517.
- 619 Piotrowska-Długosz, A., 2014. Enzymes in Soil Fertility. In: Enzymes in Agricultural
620 Sciences, OMICS Group, pp 44-79.
- 621 Póvoas, I. e Barral, M.F., 1992. Métodos de Análise de Solos. Lisboa, Instituto de
622 Investigação Científica Tropical, Ministério do Planeamento e da Administração do
623 Território, 61 p. Comunicações do IICT, Série de Ciências Agrárias, Nº 10.
- 624 Prayogo, C., Jones, J.E., Baeyens, J., Bending, G.D., 2014. Impact of biochar on
625 mineralization of C and N from soil and willow litter and its relationship with microbial
626 community biomass and structure. *Biol Fertil Soils*, 50, 695-702.
- 627 R Core Team. 2013. R: A language and environment for statistical computing. R
628 Foundation for Statistical Computing, Vienna, Austria. URL <http://www.R-project.org/>.
- 629 Rockström, J., W. Steffen, K. Noone, Å. Persson, F. S. Chapin, III, E. Lambin, T. M.
630 Lenton, M. Scheffer, C. Folke, H. Schellnhuber, B. Nykvist, C. A. De Wit, T. Hughes, S.
631 van der Leeuw, H. Rodhe, S. Sörlin, P. K. Snyder, R. Costanza, U. Svedin, M.
632 Falkenmark, L. Karlberg, R. W. Corell, V. J. Fabry, J. Hansen, B. Walker, D. Liverman,
633 K. Richardson, P. Crutzen, and J. Foley, 2009. Planetary boundaries:exploring the safe
634 operating space for humanity. *Ecology and Society*, 14 (2), 32.
- 635 Rondon, M.A., Lehmann, J., Ramirez, J., Hurtado, M., 2007. Biological nitrogen fixation
636 by common beans (*Phaseolus vulgaris* L.) increases with bio-char additions. *Biol Fertil*
637 *Soils*, 43, 699–708.

- 638 Sakrabani, R., Kern, J., Mankasingh, U., Zavalloni, C., Zanchettin, G., Bastos, A.,
 639 Tammeorg, P., Jeffery, S., Glaser, B., Verheijen, F., 2017. Representativeness of
 640 European biochar research: part II – pot and laboratory studies. *J Environ Engineer*
 641 *Landscape Manage.*, 25, 152-159. doi 10.3846/16486897.2017.1331167.
- 642 Sinsabaugh, R.L., Antibus, R.K., Linkins, A.E., McClaugherty, C.A., Rayburn, L.,
 643 Repert, D., Weiland, T., 1993. Wood decomposition: nitrogen and phosphorus dynamics
 644 in relation to extracellular enzyme activity. *Ecol*, 74, 1586-1593.
- 645 Sokal, R.R., Rohlf, F.J., 1995. Biometry: The Principles and Practice of Statistics in
 646 Biological Research. 3rd edition. W.H. Freeman and Company, New York, pp 442-446.
- 647 Spokas, K.A., Cantrell, K.B., Novak, J.M., Archer, D.A., Ippolito, J.A., Collins, H.P.,
 648 Boateng, A.A., Lima, I.M., Lamb, M.C., McAloon, A.J., Lentz, R.D., Nichols, K.A.,
 649 2012. Biochar: a synthesis of its agronomic impact beyond carbon sequestration. *J*
 650 *Environ Qual*, 41, 973–989.
- 651 Sun, Z., Bruun, E.W., Arthur, E., Jonge, L.W., Moldrup, P., Hauggaard-Nielsen, H.,
 652 Elsgaard, L., 2014. Effect of biochar on aerobic processes, enzyme activity, and crop
 653 yields in two sandy loam soils. *Biol Fertil Soils*, 50, 1087-1097.
- 654 Tabatabai, M.A., Bremner, J.M., 1969. Use of p-nitrophenylphosphate for assay of 425
 655 soil phosphatase activity. *Soil Biol Biochem*, 1, 301-307.
- 656 Taghizadeh-Toosi, A., Clough, T.J., Condon, L.M., Sherlock, R.R., Anderson, C.R.,
 657 Craigie, R.A., 2011. Biochar incorporation into pasture soil suppresses in situ nitrous
 658 oxide emissions from ruminant urine patches. *J Environ Qual*, 40, 468-476.

- 659 Taghizadeh-Toosi, A., Clough, T.J., Sherlock, R.R., Condon, L.M. A, 2012. Wood based
 660 low temperature biochar captures NH₃-N generated from ruminant urine-N, retaining its
 661 bioavailability. *Plant Soil*, 353, 73-84.
- 662 Tammeorg et al., 2017. Biochars in soils: towards the required level of scientific
 663 understanding. *J Environ Engineer Landscape Manage*, ISSN 1648–6897 / eISSN 1822-
 664 4199.
- 665 Ulyett, J., Sakrabani, R., Hann, M., Kibblewhite, M., 2014. Impact of biochar addition on
 666 water retention, nitrification and carbon dioxide evolution from two sandy loam soils.
 667 *Eur J Soil Sci Special Issue on Biochar*, 65, 96-104.
- 668 Ventura, M., Zhang, C., Baldi, E., Fornasier, F.; Sorrenti, G., Panzacchi, P., Tonon, G.,
 669 2014. Effect of biochar addition on soil respiration partitioning and root dynamics in an
 670 apple orchard, *Eur J Soil Sci Special Issue on Biochar*, 65, 186–195.
 671 <http://dx.doi.org/10.1111/ejss.12095>
- 672 Verheijen, F., Jeffery, S., Bastos, A., van der Velde, M., Diafas, I., 2009. Biochar
 673 application to soils - a critical scientific review of effects on soil properties, processes and
 674 functions. EUR24099EN, Office for the Official Publications of the European
 675 Communities, Luxembourg, 149 pp.
- 676 Weyers, S.L., Gaskin, J., Liesch, A.M., Das, K.C., 2010. Earthworms, microbes and the
 677 release of c and n in biochar amended soil. In “Proceedings of the 2010 U.S. Biochar
 678 Initiative Conference, June 27-30, Ames, Iowa”.
- 679 WRB, 2006. World reference base for soil resources. World Soil Resources Reports No.
 680 103. FAO, Rome. ISBN 92-5-105511-4.

681 Zimmerman, A.R., Gao, B., Ahn, M.Y., 2011. Positive and negative carbon
682 mineralization priming effects among a variety of biochar-amended soils. *Soil Biol*
683 *Biochem*, 43, 1169-1179.

685 Table 1. Treatments applied to conventionally managed soil A and to the organically
686 managed soil B

687 Table 2. Physical and chemical properties of the biochar, composted farmyard manure
688 and soils used in the incubation experiments.

689 Table 3. Water holding capacity (WHC), pH and soil organic matter content (SOM) in
690 all the treatments (Table 1). The data shows the average and standard deviation of 3
691 replicates measured at the beginning (D0) and end (D6) of the 60-day incubation
692 experiment.

693 Figure 1. Soil A microbial respiration rates: additions of (a) control water; (b) glucose;
694 (c) citric acid and (d) N-acetyl glucosamine in all the treatments (table 1) ($n=3\times 4$). For
695 each sampling date, significant differences ($p \leq 0.05$) are indicated by different letters.

696 Figure 2. Soil B microbial respiration rates: additions of (a) control water; (b) glucose;
697 (c) citric acid and (d) N-acetyl glucosamine in all the treatments (table 1) ($n=3\times 4$). For
698 each sampling date, significant differences ($p \leq 0.05$) are indicated by different letters.

699

700

701

702

703

704

705

706

707

708 **Table 1.** Treatments applied to the conventionally managed soil A and to the organically
 709 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

710

711

712

713 **Table 2.** Physical and chemical properties of the biochar, composted farmyard manure
714 and soils used in the incubation experiments.

715

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 100g ⁻¹)	0.64	1.90	0.10	0.14
P (g 100g ⁻¹)	0.12	0.58	0.07	0.38
K (g 100g ⁻¹)	0.62	0.29	0.03	0.16
Ca (g 100g ⁻¹)	1.80	7.60	0.25	1.44
Mg (g 100g ⁻¹)	0.10	0.50	0.07	2.19

716 n.a. not applicable. N_{Kj}_ Kjeldahl nitrogen

717

718

Table 3. Water holding capacity (WHC), pH and soil organic matter content (SOM) in all the treatments (Table 1). The data shows the average and standard deviations of 3 replicates measured at the beginning (D0) and end (D60) of the 60-day incubation experiment.

Treatmen ts	WHC (%)	pH		SOM (%)	
Sampling date (days)	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

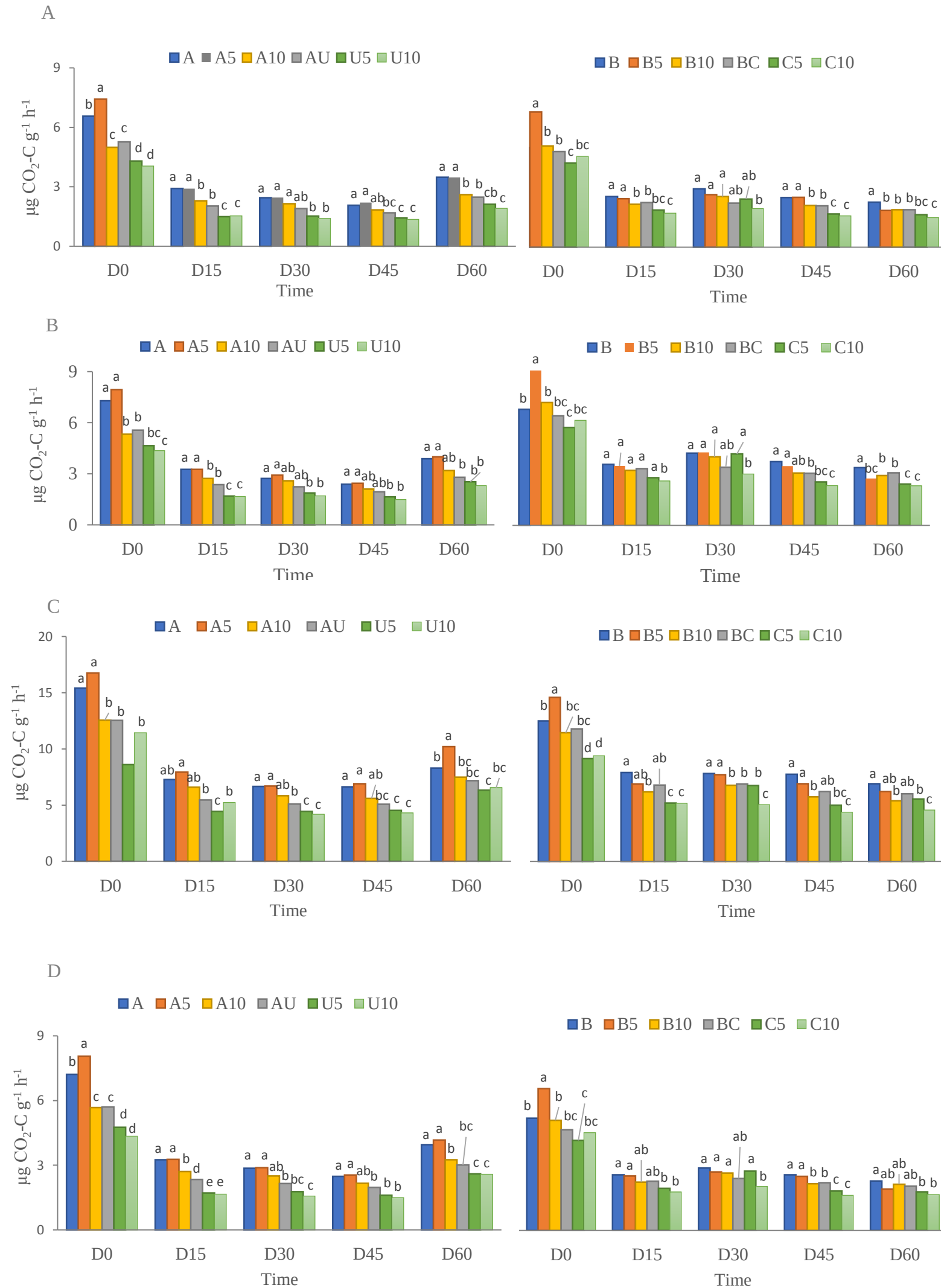
727

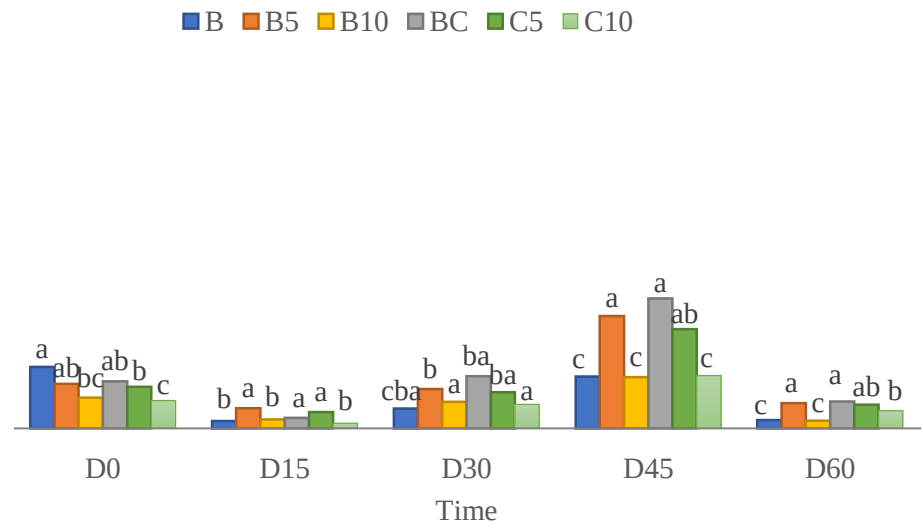
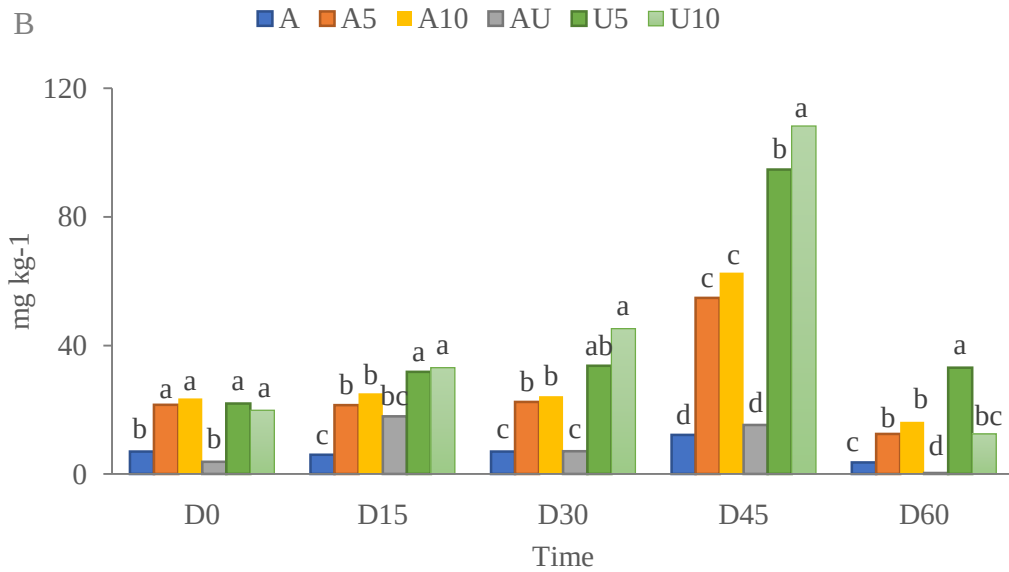
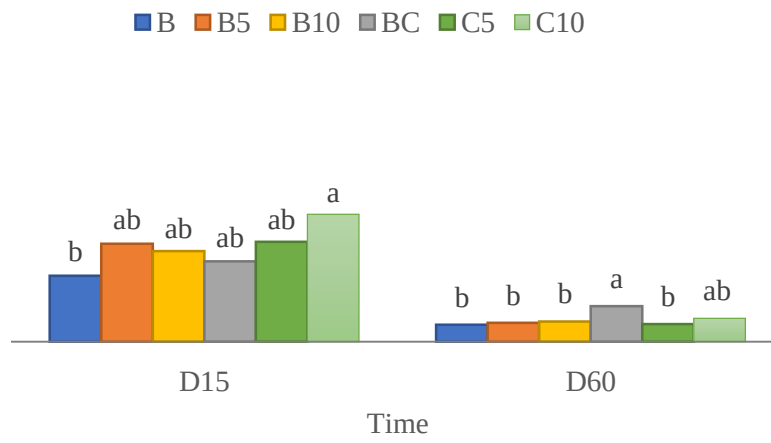
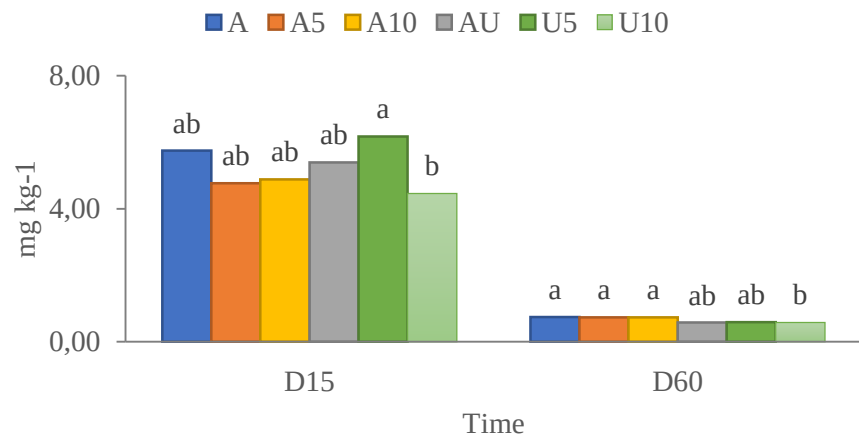
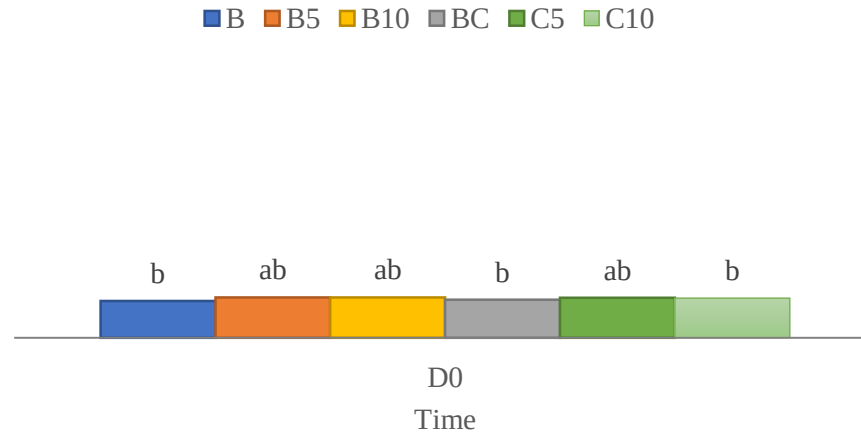
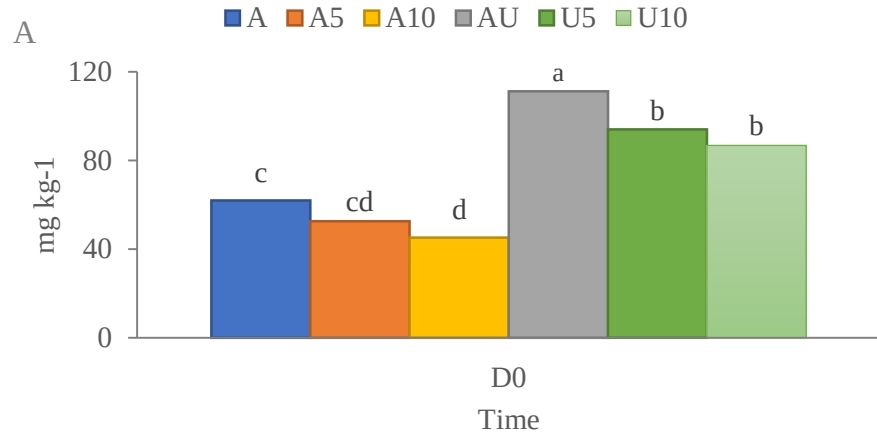
B.

728 **Table 4** Soils enzyme activities in all the treatments performed (Table 1). The data show
 729 the average and standard deviations (n=3) at D0, D30, D60 = 0, 30, 60 days after setup.
 730

Treatments	Dehydrogenase			β -glucosidase			Phosphatase		
	$(\mu\text{g TPF g}^{-1} \text{ h}^{-1})$			$(\mu\text{mol } p\text{-NP g}^{-1} \text{ h}^{-1})$			$(\mu\text{mol } p\text{-NPP g}^{-1} \text{ h}^{-1})$		
Sampling date	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	0.49a
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
B5	0.46b	0.19b	0.08c	0.59bc	0.79c	0.23bc	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	0.10bc	0.02c	0.51c	0.19cd	0.16c	0.52b	16.69a	0.37bc

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







DSPACE

<https://dspace.org/>

The impact of woody biochar on microbial processes in conventionally and organically managed arable soils

Cordovil, Cláudia M d S; Pinto, Renata; Silva, Beatriz; Sas-Paszt, Lidia; Sakrabani, Ruben ...

2019-05-24

Taylor and Francis

<https://doi.org/10.1080/00103624.2019.1614609>

Cordovil CM, Pinto R, Silva B, et al., (2019) The impact of woody biochar on microbial processes in conventionally and organically managed arable soils. Communications in Soil Science and Plant Analysis, Volume 50, Issue 12, 2019, pp. 1387-1402