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

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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 280ºC, 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 (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 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 ^{O}C) providing highly recalcitrant and decay-resistant products (Sakrabani et al., 2017). Contrary low temperature biochars (~300 ^{O}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^{O}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 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 (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 NH_3 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) (38º53'52.0''N 9º25'12.2''W; 38º53'23.3''N 9º22'57.1''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 10ºC/min and had a residence time of 10 min after reaching the 280ºC 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} ($N\text{-}NH_4^+ + N\text{-}NO_3^-$) was determined by molecular absorption spectrophotometry using a segmented flow auto-analyzer, after extraction with 2*M* 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 cm^3) and incubated aerobically in an Aqua Tag incubation chamber at 24 ± 2ºC 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.

119 *2.1.2 Enzyme activity*

120 Enzyme activities were determined for all the treatments mixtures at the beginning of
121 the incubation experiment (D0), after 30 (D30) and 60 days (D60). For dehydrogenase
122 activity, soil samples (3 g) from each destructive triplicate were mixed with 0.1% (w/v)
123 triphenyltetrazolium chloride in Tris-buffer (0.1*M*; pH 7.6; 3 mL) and incubated at 25°C
124 for 16 h, followed by the quantification of the triphenylformazan (TPF) formed by
125 spectrophotometry (546 nm) as described by Tabatabai (1997). β-glucosidase activity
126 was obtained through the determination by spectrophotometry (400nm) of the *p*-
127 nitrophenol (*p*-NP) released after the incubation of the soil samples (1 g) with a
128 buffered solution (pH 6; 4 mL), toluene (0.25 mL) and *p*-nitrophenyl-β-*d*-
129 glucopyranoside (1 ml) for 1 hour at 37ºC. Soil phosphatase activity was assayed by
130 colorimetric estimation of the *p*-nitrophenol released by spectrophotometry (400nm)
131 after the soil samples (1 g) were incubated with a buffered solution (pH 6.5; 4 mL),
132 toluene (0.25 mL) and sodium p-nitrophenyl phosphate (*p*-NPP) (1 mL) at 37ºC for 1
133 hour (Tabatabai and Bremner, 1969). The spectrophotometer used was a segmented
134 flow analyser from Skalar.

135 *2.1.3 Soil microbial respiration*

136 The physiological profiles of the microbial communities (CLPP) were determined at the
137 beginning (D0) and every 15 days (D15, D30, D45, D60) using the MicroResp method
138 (Campbell *et al.,* 2003), which is a colorimetric detection (with cresol red in the
139 detection plate) to measure soil respiration in the presence of three different C sources.
140 Three carbon substrates (D-glucose, citric acid and N-acetyl-D-glucosamine) were
141 prepared at 1% (m/v) in deionized water to determine substrate-induced respiration
142 (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 24ºC ± 2ºC. 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 (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 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}; 5×5×2 cm) soaked in 7 ml of phosphoric acid (0.5M) and then fixed to acrylic plates (7×7×0.3 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 × 3 plastic containers supported by four plastic rods to fully cover the container. This procedure was the one that proved

167 to be more efficient among several combinations tested (Alves *et al*., 2011). To ensure
168 that there was no contamination between containers they were arranged randomly and
169 spaced 30 cm apart from each other. Foams were collected every two days and washed
170 with 200 ml of deionized water on a Buckner funnel attached to a vacuum pump. NH_3
171 was then determined using the segmented-flow analyser detailed above.

172 ***2.3 Statistical analysis***

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

181 **3. Results**

182 The characterization of biochar, composted farmyard manure and soils A and B are
183 shown in table 2. An addition of biochar to both conventionally and organically
184 managed soils increased the WHC of the soils (Table 3). The WHC in the treatments
185 with organically managed soil was higher than in the conventionally managed soil
186 treatments when comparing the same biochar treatments and controls ($p \leq 0.05$).
187 Additionally, the WHC increased with increasing biochar application rate. Biochar
188 presented a high adsorption capacity due to its specific porosity (0.008 $cm^3.g^{-1}$ total
189 pores and 0.0007 $cm^3.g^{-1}$ micro pores <20 Å). For the conventionally managed soil A,
190 this increase relative to the control was 18% and 22% for the 5% and 10% biochar

191 application rate, respectively. The increase in WHC for the organically managed soil B
192 was lower with only ~14% for the 5% biochar application rate and 18% for the 10%
193 rate, relative to the control. Contrary, compost addition did not affect the WHC (Table
194 3).

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

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

208 The organically managed soil had considerable larger organic matter contents
209 than conventionally managed soils, both at D0 and D60 (Table 3), as expected.
210 Additions of biochar to the conventionally farmed soil A raised the organic matter
211 contents relative the control ($p \leq 0.05$) by about 58% and 73% for the 5% and 10%
212 biochar treatments respectively, and remained practically constant over the 60-day
213 incubation period. Contrary, in the organically managed soils the increase in SOC at the
214 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 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 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 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 β-glucosidase activity of 15.88±1.79 μmol *p*-NP g^{-1} 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 μmol *p*-NP g^{-1} 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

239 soil treatments in general, but increased for BC to 7.30±0.44 µmol *p*-NP g^{-1} h^{-1} ($p \leq$
240 0.05); and at D60 their activities were reduced to < 1 µmol *p*-NP g^{-1} h^{-1}.

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

248 Phosphatase activities were >6.6 µmol *p*-NPP g^{-1} h^{-1} for the conventionally
249 managed treatments with urea and biochar (U5, U10) and for the two organically
250 managed controls (B, BC) at D0 (Table 4). For the remaining treatments phosphatase
251 activities only ranged between 0.42±0.03 and 1.06±0.12 µmol *p*-NPP g^{-1} h^{-1} and were
252 not significantly different from each other ($p \leq 0.05$). After 30 days of incubation,
253 phosphatase activity had increased for all treatments. Largest increases, 8 to 39 fold,
254 were observed for the treatments that had the low phosphatase activities at D0. By day
255 60 (D60), all treatments had declined significantly to an average rate of 0.47 µmol *p*-
256 NPP g^{-1} h^{-1}, with no significant difference between treatments ($p \leq 0.05$).

257 ***3.2 Soil Microbial Respiration Rates***

258 Figure 1 and Figure 2 show soil respiration rates from the controls (water only) and
259 those induced by addition of three different carbon substrates: glucose, citric acid and
260 N-acetyl glucosamine for the conventionally (A) and organically (B) managed soils
261 respectively. In general, largest soil respiration rates were measured at the beginning of
262 the incubation period and declined in a similar manner during the first 15 days for all

263 treatments. Thereafter, treatments remained constant until the end of the incubation
264 period, with exception of the conventionally managed soils (A) on the last measurement
265 date (D60). For these soil treatments, respiration rates had increased slightly in all
266 treatments between D45 and D60.

267 At D0, control respiration rates ranged from 4.05±0.48 μg CO_2-C g^{-1} h^{-1} (10%
268 biochar+urea U10) to 7.42±0.52 μg CO_2-C g^{-1} h^{-1} (5% biochar A5) in conventionally
269 managed soil A, and from 4.36±1.05 μg CO_2-C g^{-1} h^{-1} (10% biochar+compost C10) to
270 6.50±1.84 μg CO_2-C g^{-1} h^{-1} (5% biochar B5) in organically managed soil B (Figures 1a,
271 2a). Glucose (Figures 1b, 2b) and N-acetyl glucosamine (Figures 1d, 2d) induced
272 respiration rates were similar to the control respiration rates (Figures 1a, 2a). Citric acid
273 induced respiration rates (Figures 1c, 2c), on the other hand, were more than twice as
274 large compared to the control and ranged between 4.18 μg CO_2-C g^{-1} h^{-1} (10%
275 biochar+urea, U10, D30) and 16.76 μg CO_2-C g^{-1} h^{-1} (5% biochar, D0) in
276 conventionally managed soil A5, and from 4.57 μg CO_2-C g^{-1} h^{-1} (10%
277 biochar+compost C10, D45) to 15.22 μg CO_2-C g^{-1} h^{-1} (5% biochar B5, D0) in
278 organically managed soil B.

279 For both the conventionally (A) and organically (B) managed soils, respiration
280 rates in the 5% biochar application rate treatments, were significantly different from the
281 remaining treatments ($p \leq 0.05$) throughout the incubation period for the control (water
282 only) and the three selected carbon substrates (Figures 1, 2). Significant relationships
283 specific to each sampling date were as follows. At D0 control respiration rates did not
284 differ ($p \leq 0.05$) between U5 and U10 treatments, and also between C5 and C10, AU
285 and A10, and BC and B10. These relationships were also found for glucose (AU=A10;
286 U5=U10), citric acid (BC=B10) and for N-acetyl glucosamine (AU=A10) induced
287 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).

NH_4^+-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±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).

311 NO_3^--N concentrations increased in all conventionally managed treatments
312 during the first 45 days, (Figure 3b), and declined at the end of the incubation
313 experiment (D60). At all sampling dates, NO_3^--N concentrations were significantly
314 larger when biochar was added. The impact of biochar was the largest at D45, when A5
315 and A10 treatments were around 83% and 82% larger than the control (A), and U5 and
316 U10 were 85% and 87% higher than the urea control (AU). Organically managed
317 treatments, however, revealed a different behaviour with time. Initially (D0), the non-
318 biochar treatments (B and BC) had significantly larger NO_3^--N concentrations compared
319 to the biochar treatments (B5=C5; B10=C10) ($p \leq 0.05$). However, after 45 days, the
320 NO_3^--N concentrations in B10 treatment were 54.9% lower than the control (B),
321 whereas the concentrations in B5 were 23.4% higher. Conversely, the concentrations of
322 NO_3^--N in both biochar and compost treatments (C5 and C10) were 17% and 56.3%
323 lower when compared to BC, respectively. Even though no difference was found
324 between B10 and C10 at days 30 and 45, after the 60-day incubation period all biochar
325 treatments significantly differed ($p \leq 0.05$).

326 ***3.4 Ammonia emission***

327 In general, larger fluctuations of NH_3 concentrations were observed throughout the 10-
328 day incubation period for the conventionally managed soil (A) compared to organically
329 managed soil (B) treatments (Figure 4). During the incubation period, air temperature
330 increased with time, from 22ºC at the start (data not shown) to 25ºC after 10 days.
331 Largest NH_3 concentrations were measured from the soil A with urea addition only
332 (AU, 1.03±0.59 NH_3 mg kg^{-1}) at day 2, decreasing in total 55% after 10 days. The effect
333 of biochar on NH_3 concentrations in conventionally managed treatments varied
334 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 CO_2, 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

384 facilitates soil organic matter oxidation (Makoi *et al.,* 2008). In our study
385 dehydrogenase activities declined with time and were much reduced in the presence of
386 biochar. The enzyme activity could have increased right after the biochar addition, but
387 the first sampling was at day 30 which may have masqueraded earlier effects. Similar
388 trends were observed by Ouyang *et al.* (2014a), investigating the impact of biochar
389 dehydrogenase activity in a loamy soil, and also after applying sewage sludge derived
390 biochar to a forest soil (Paz-Ferreiro *et al.* 2012). The latter authors implied that the
391 high heavy metal concentration in the sewage derived biomass may have been
392 responsible for the reduction in dehydrogenase activity.
393 Similar to dehydrogenase, also β-glucosidase activity in biochar treatments was
394 significantly lower during the entire experimental period when compared to the
395 controls, as also reported previously (Paz-Ferreiro *et al.,* 2012). This decrease may be
396 partially due to the fact that the optimum pH for β-glucosidase activity is in general
397 acidic and the pH of the soils in our study were neutral to alkaline, and on average
398 slightly increased during the incubation period, in some cases significantly. Contrary
399 Ventura *et al.* (2014) reported that the addition of an alkaline wood biochar to an apple
400 orchard did not effect β-glucosidase activity. Also in our study, β-glucosidase behaviour
401 in the conventionally managed control soil treated with urea (AU) was significantly
402 higher than in biochar treatments (A5, A10) at the beginning (D0) and at the end (D60)
403 of this study. The effect of biochar on β-glucosidase activity in our study is
404 inconclusive, and needs further research.
405 The lower dehydrogenase and β-glucosidase activities in the biochar treatments
406 may be caused by the condensed aromatic structures and physically resistant to
407 degradation of the wood-based biochar in contrast to manure-based biochars (Ouyang *et*
408 *al.,* 2014b). Indeed, woody biochar tends to adsorb more substrate than manure based

409 ones, reducing their availability and thus inhibiting enzyme activity. Even though
410 biochar increases the soil absorption capacity and stabilizes soil-enzyme interactions in
411 some cases (Sun *et al.,* 2014), further long-term studies need to verify this, especially as
412 woody biochar tends to exhibit beneficial effects on soil microbial abundance much
413 later (> 60 days) (Gul *et al.,* 2015) than manure based biochar.
414 Phosphatase activity is associated with the demand for P by microorganisms and
415 plants (Piotrowska-Długosz, 2014) and is inversely proportional to plant available P
416 (Amador *et al.,* 1997; Sinsabaugh *et al.,* 1993). In our study phosphatase activity was
417 largest after the 30-day incubation period, unlike the dehydrogenase and β-glucosidase
418 activities which were largest at the start of the incubation period and declined thereafter.
419 In general, microbial biomass increases after biochar application (Liu *et al.* 2016)
420 because biochar may promote nutrient cycling in soil. This includes phosphorus (P)
421 mobilization by stimulation of the soil microbial activity; and the response is strongly
422 dependent on soil type (Deb *et al.* 2016). The reason for the decline in phosphatase
423 activity between day 30 and day 60 may be a decline in substrate availability. Addition
424 of biochar increased phosphatase activity, especially when urea and biochar (U5, U10)
425 were added. Our results are in line with the observation that phosphatase activity is
426 mainly promoted by a low inorganic phosphorus content or by an increase in organic
427 matter and hence organic P (Nannipieri *et al.,* 2011). Bell *et al.* (2006) also observed
428 increases in phosphatase activity after manure application which may be explained by
429 enhanced P mineralization.
430 Contrasting microbial responses to biochar addition can be found in the
431 literature (Kolb *et al.,* 2009; Zimmerman *et al.,* 2011; Dempster *et al.,* 2012).
432 Differences in physiochemical properties of the soil and biochar products are an
433 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

459 treatments had a higher NH_4^+-N content at the start of the experiment, especially in urea
460 treatments and in non-biochar treatments, but declined considerably afterwards,
461 possibly followed by nitrification or NH_4^+ adsorption to biochar, clay particles or other
462 types of organic matter. The extent of NH_4^+-N decline was greater in the conventionally
463 managed treatments (A), presumably attributable to the larger initial N content in
464 relation to the organically managed ones (B) (Kelly *et al.,* 2015).

465 Ippolito *et al.* (2014) described progressive NO_3^--N increases in biochar
466 amended soils during 12 months for all biochar application rates used. This means that
467 mineral N release is slower, and there is less nitrate losses. However, the largest
468 increase occurred with the lower biochar application rate. Although on a much shorter
469 time scale, the results from the present study also showed a continuous increase in NO_3^-
470 -N concentrations in biochar treatments during the first 45 days, especially in the
471 conventional farming treatments, declining in the last 15 days of incubation, while non-
472 biochar treatments showed almost a constant behaviour. Additionally, in the
473 conventionally managed soil treatments, NO_3^--N concentrations were in general higher
474 in the treatments with the higher biochar application rate. Conversely, the organically
475 managed soil treatments showed lower concentrations of NO_3^--N with the higher rate of
476 biochar. These results relate to those of Prayogo *et al.* (2014), who found NH_4^+-N levels
477 became reduced between day 30 and day 90, suggesting net immobilization. Other
478 authors (Rondon *et al.,* 2007; Deenik *et al.,* 2010) observed a decrease in soil mineral N
479 (N immobilization) in the presence of low-temperature biochars with high volatile
480 matter content and high C/N ratio, supporting thus our findings in the later incubation
481 stage. Once the available C is exhausted, the immobilized N may be remineralised,
482 therefore supporting biochar's potential ability to act as a slow-release fertiliser
483 (Kammann *et al.,* 2015).

NH_3 volatilization is promoted by high soil pH, temperature and low soil moisture content, provided there is a suitable N source, such as 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 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 NH_3 emissions, which may be attributable to NH_3 hydrolysis to 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 NH_3 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.

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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=3×4). 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=3×4). For
698 each sampling date, significant differences ($p \leq 0.05$) are indicated by different letters.
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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

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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. Nkj_ Kjeldahl nitrogen
717
718

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

Treatments	**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**

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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.
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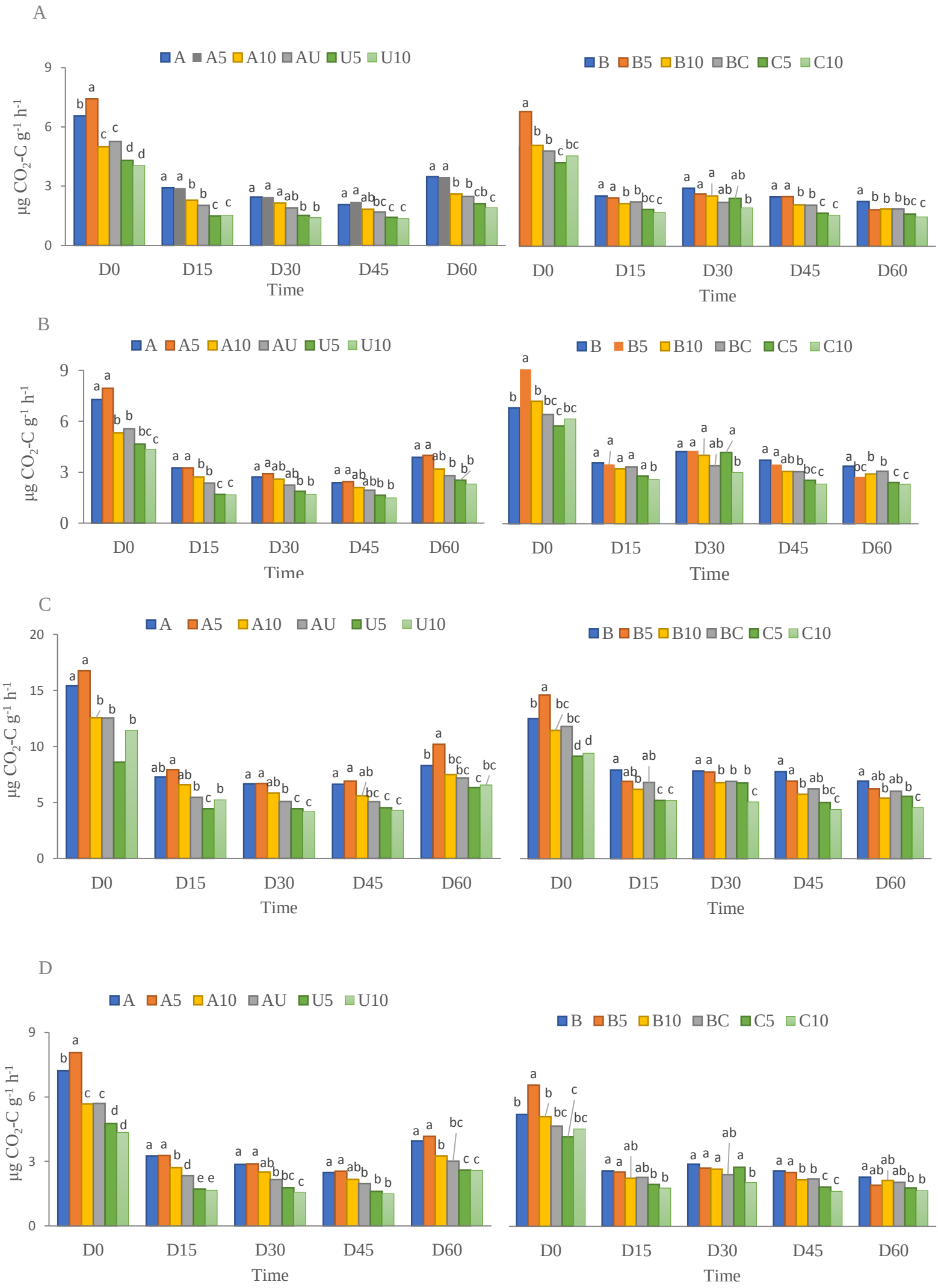
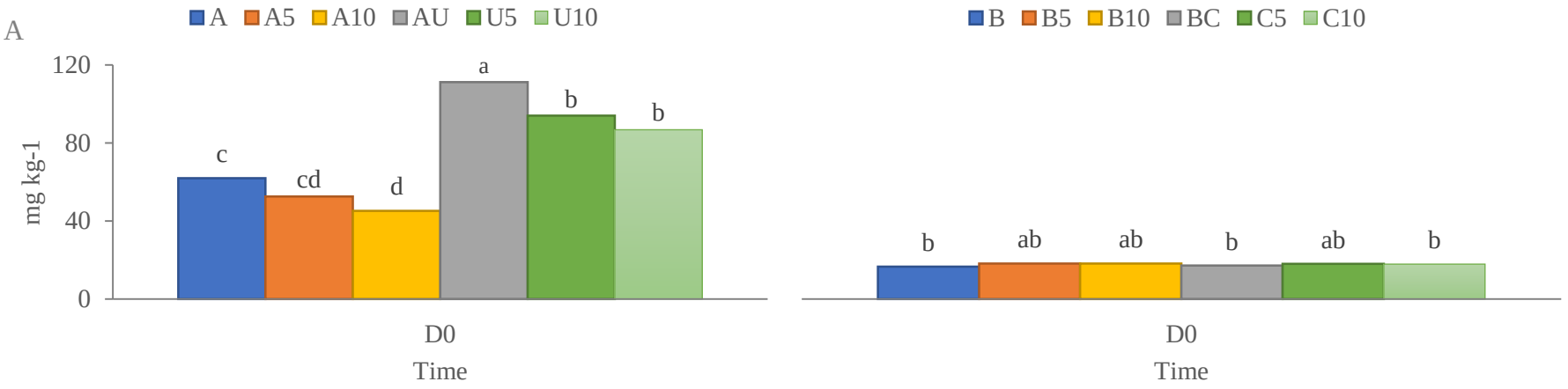
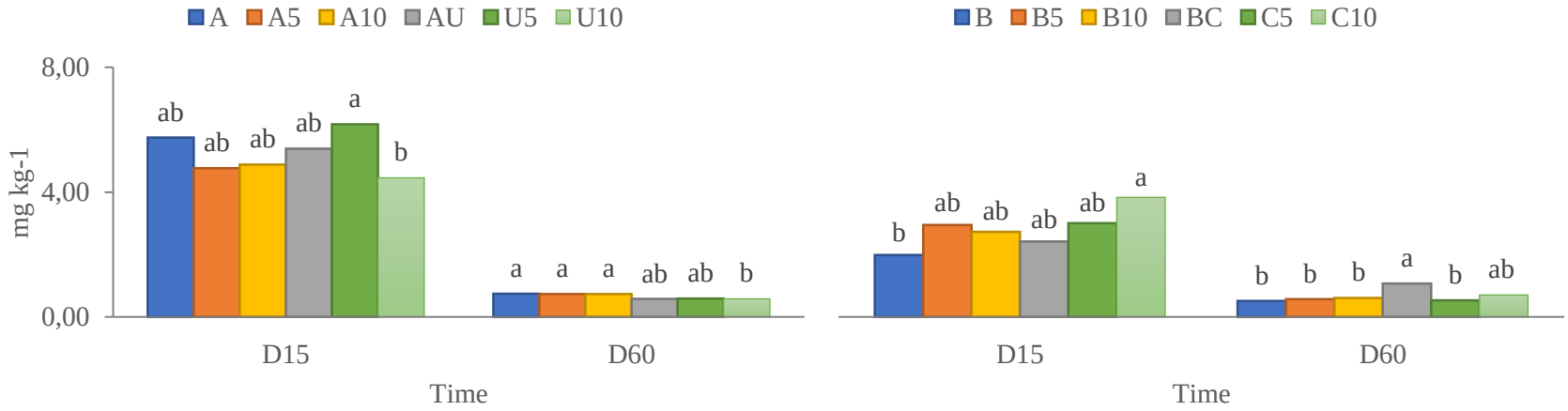
Treatments	Dehydrogenase (µg TPF g^{-1} h^{-1})			β-glucosidase (µmol *p*-NP g^{-1} h^{-1})			Phosphatase (µmol *p*-NPP g^{-1} h^{-1})		
Sampling date	D0	D30	D60	D0	D30	D60	D0	D30	D60
A	3.16a*	0.91a	0.05b	15.88a	7.98a	0.15ab	0.86c	5.99c	0.56a
A5	0.82b	0.18c	0.03b	0.27c	0.12cd	0.09b	0.73c	9.46b	0.49a
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.

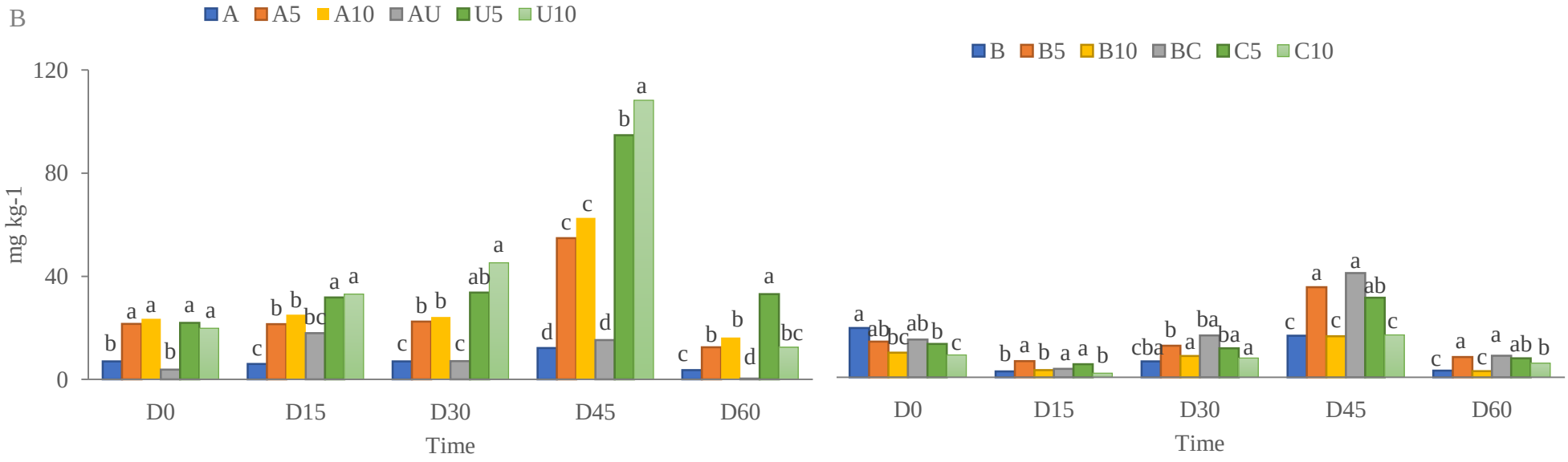

A
A A5 A10 AU U5 U10
B B5 B10 BC C5 C10
µg CO2-C g-1 h-1
D0 D15 D30 D45 D60
Time
B
C
D

A


A A5 A10 AU U5 U10
120
80
40
0
mg kg-1
c cd d a b b
D0
Time
B B5 B10 BC C5 C10
b ab ab b ab b
D0
Time


A A5 A10 AU U5 U10
8,00
4,00
0,00
mg kg-1
ab ab ab ab a b
a a a ab ab b
D15
D60
Time
B B5 B10 BC C5 C10
b ab ab ab ab a
b b b a b ab
D15
D60
Time

B


A A5 A10 AU U5 U10
120
80
40
0
mg kg-1
b a a b a a
c b b bc a a
c b b c ab a
d c c d b a
c b b d a bc
D0
D15
D30
D45
D60
Time
B B5 B10 BC C5 C10
a ab bc ab b c
b a b a a b
cba b a ba ba a
c a c a ab c
c a c a ab b
D0
D15
D30
D45
D60
Time

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

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