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
- 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

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

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

41 **1. Introduction**

A growing concern of environmental quality has been a major driver for agroecosystems 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

46 (Rockström et al. 2009). As a response to these challenges, biochar application to 47 improve soil conditions and reduce mineral nitrogen (N) fertiliser use in agriculture has 48 been investigated. However, there are still considerable knowledge gaps in some areas 49 as highlighted by Sakrabani et al. (2017) and Tammeorg et al. (2017). 50 Biochar is a carbon-rich by-product of pyrolysis at low oxygen concentrations 51 (Lehmann et al., 2006). The chemical and physical composition of biochar is highly 52 variable, depending on the type of feedstock, pyrolysis conditions, namely the 53 temperature, and postproduction handling. Biochars have complex porous structures 54 with large surface areas, an affinity for charged particles, an ability to increase the soil 55 water holding capacity (WHC) (Ulyett et al., 2014), and to retain nitrate-N (NO₃-N) 56 (Kammann et al., 2015) and ammonia-N (NH₃-N) (Taghizadeh-Toosi et al., 2012). 57 Thereby biochar affects nutrient cycling and organic matter decomposition (Biederman 58 and Harpole, 2013) with both environmental and agronomical implications. Most of the 59 biochar products investigated in biogeochemical research are produced at high 60 temperatures (>500 °C) providing highly recalcitrant and decay-resistant products 61 (Sakrabani et al., 2017). Contrary low temperature biochars (~300 °C) have not 62 received the same attention, in spite of the fact that they have higher bioavailability and 63 provide carbon and other nutrients to the microbial community and thereby potentially 64 can increase mineralization rates and nutrient supply to plant roots and soil 65 microorganisms (Kumar et al. 2013). To fill this knowledge gap we have investigated 66 the effect of a commercial alkaline low-temperature (280°C) biochar, made from 67 conifer wood chips, on soil microbial processes indicative of changes in soil nutrient 68 cycling in soils from an organic vegetable farm using composed manure as N fertiliser 69 and a conventional vegetable farm using urea as N source. To compare the impact of a 70 low temperature biochar addition in these two systems commonly used biological

Page 4 of 38

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).

77 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).

84 The biochar used was produced from conifer wood chips in a fast pyrolysis 85 process by the Polish company "Fluid Spółka Akcyjna". The temperature of pyrolysis 86 ramped up at 10°C/min and had a residence time of 10 min after reaching the 280°C 87 maximum temperature. Soils, biochar and composted FYM provided by farm B, were 88 fully characterized. Methods used are described in Sparks et al., (1996): pH was 89 measured by a glass electrode using a 1:2.5 (material : water) ratio; TKN was 90 determined by Kjeldahl method after sample digestion; N_{min} (N-NH₄⁺ + N-NO₃⁻) was 91 determined by molecular absorption spectrophotometry using a segmented flow auto-92 analyzer, after extraction with 2*M* KCl at a 1:10 (soil:water) ratio; available P and K 93 concentrations were determined through Egner-Riehm procedure; total P and K 94 concentration determined by Ammonium Vanadate method (Póvoas and Barral, 1992).

95 The same biochar treatments were applied to both soils and to all experiments 96 (Table 1). Biochar was mixed with both soils, previously air dried at room temperature 97 and sieved in a 2 mm mesh, to achieve even distribution. The rate of biochar applied 98 (5% and 10%) was similar to those used in other studies (Jeffery et al., 2011; Paz-99 Ferreiro et al., 2012; Ameloot et al., 2014; Ouyang et al., 2014a). Urea and composted 100 FYM were added to the soils A and B respectively, with and without biochar addition, 101 in accordance to their previous management, at rates equivalent to an application of 170 102 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

105 Batches of 300 g of each treatment mixture (Table 1) were placed into round

106 polyethylene containers (500 cm³) and incubated aerobically in an Aqua Tag incubation

107 chamber at $24 \pm 2^{\circ}$ C for 60 days (D60). Enough batches were prepared to allow

108 destructive sampling in triplicates every 15 days, including at day 0 (D0). The mixtures

109 were maintained at 60% soil water holding capacity (WHC) by monitoring the weight

110 of the soil filled containers every two days and correcting with distilled water whenever

111 needed. WHC was determined (Póvoas and Barral, 1992), in triplicates, by saturating

112 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.

114 2.1.1 Soil pH

115 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,

117 stirred for one hour prior measurement using a Thermo Electron Corporation

118 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)
- 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
- buffered solution (pH 6; 4 mL), toluene (0.25 mL) and *p*-nitrophenyl- β -*d*-
- 129 glucopyranoside (1 ml) for 1 hour at 37°C. 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),
- toluene (0.25 mL) and sodium p-nitrophenyl phosphate (p-NPP) (1 mL) at 37°C 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 μ L) were added to the wells in the microtiter deep-well plate containing the soil mixtures (approximately 0.5 g to fill the wells), and a total of 36 replicates per sampling date × 4 (3C sources + water)) were generated. The detection plate was read at 600 nm in a microplate reader before the beginning of the incubation and after 6 h of incubation at 24°C ± 2°C. Data was normalized for time zero, to eliminate differences in colour between wells due to uneven gel density.

150 2.1.4 Mineral Nitrogen

151 The mineral N (NH₄⁺-N and NO₃⁻-N) content was determined by segmented flow

152 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

155 *et al.* (2005). Prior to analysis, KCl extracts were stored in the fridge until the next day.

156 2.2 The impact of biochar rate on NH₃ emissions

157 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 NH₃ emissions were measured, until the moisture content had dropped

160 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 NH₃ 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. NH₃ 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 $p \le 0.05$, 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 ($p \le 0.05$).

- 187 Additionally, the WHC increased with increasing biochar application rate. Biochar
- 188 presented a high adsorption capacity due to its specific porosity (0.008 cm³.g⁻¹ total
- pores and 0.0007 cm³.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

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 ($p \le 0.05$) (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

209than conventionally managed soils, both at D0 and D60 (Table 3), as expected.210Additions of biochar to the conventionally farmed soil A raised the organic matter211contents relative the control ($p \le 0.05$) by about 58% and 73% for the 5% and 10%212biochar treatments respectively, and remained practically constant over the 60-day213incubation 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%

Page 10 of 38

biochar treatments. This increase had declined to 33% and 46% for 5% and 10%

216 biochar treatments respectively by day 60.

217 3.1 Enzyme activity

218 The three soil enzymes investigated behaved differently throughout the experimental 219 period (Table 4). Dehydrogenase activity was higher in conventionally managed soil 220 treatments (soil A) compared to organically managed soil treatments (soil B) at the start 221 of the experiment (D0). Conventionally managed treatments (see Table 1) ranged from 222 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 223 224 without compost (B, BC). All treatments showed a decreasing trend over time. Biochar 225 treatments tended to show lower dehydrogenase activity than the controls ($p \le 0.05$). 226 This difference was more pronounced at the beginning (D0) of the incubation period, 227 especially for the higher rate of biochar ($p \le 0.05$), with the exception of the treatments 228 where biochar was also mixed with compost (C5 and C10, at setting date D0). After 60 229 days, dehydrogenase activity had declined substantially in all treatments. The two 230 treatments where some dehydrogenase activity was still detected at the end of the 231 experiment were B (control) and BC (compost) treatments. Their rates were 232 significantly larger ($p \le 0.05$) compared to the remaining treatments. β -glucosidase activity > 1 µmol *p*-NP g⁻¹ h⁻¹ was only found in the controls of 233 234 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 235 236 the conventionally managed control soil (A) followed by similar activities of 5.29±0.97 and $4.88\pm1.42 \text{ }\mu\text{mol }p\text{-NP }\text{g}^{-1}\text{ }h^{-1}\text{ for BC and B, respectively, at the beginning of the$ 237 238 experiment (D0). At D30, β -glucosidase activities significantly decreased for A and B

soil treatments in general, but increased for BC to 7.30±0.44 μ mol *p*-NP g⁻¹ h⁻¹ (p \leq 239 0.05); and at D60 their activities were reduced to $< 1 \text{ }\mu\text{mol }p\text{-NP }g^{-1} \text{ }h^{-1}$. 240 241 For soils amended with biochar or urea, β -glucosidase activity remained below 1 μ mol p-NP g⁻¹ h⁻¹ throughout the measurement period. After 60 days, no significant 242 243 difference ($p \le 0.05$) 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 ($p \le 0.05$) than the other 247 treatments but not amongst themselves. Phosphatase activities were >6.6 μ mol *p*-NPP g⁻¹ h⁻¹ for the conventionally 248 249 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 ($p \le 0.05$). 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 ($p \le 0.05$).

257 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

Page 12 of 38

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 \pm 0.48 µg CO ₂ -C g ⁻¹ h ⁻¹ (10%)
268	biochar+urea U10) to 7.42 \pm 0.52 µg CO ₂ -C g ⁻¹ h ⁻¹ (5% biochar A5) in conventionally
269	managed soil A, and from 4.36 \pm 1.05 µg CO ₂ -C g ⁻¹ h ⁻¹ (10% biochar+compost C10) to
270	$6.50\pm1.84 \ \mu g \ CO_2$ -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 CO2-C g^{-1} h^{-1} (10\%
275	biochar+urea, U10, D30) and 16.76 μg CO2-C g ⁻¹ h ⁻¹ (5% biochar, D0) in
276	conventionally managed soil A5, and from 4.57 μg CO2-C g^{-1} h^{-1} (10\%
277	biochar+compost C10, D45) to 15.22 μ g 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

- 288 significantly different between BC and B10 treatments, and between C5 and C10 (p \leq
- 289 0.05). N-acetyl glucosamine induced respiration rates did not differ ($p \le 0.05$) between
- BC, C5 and U10, between B and U10, and between C5 and C10.
- 291 3.3 Soil Mineral Nitrogen

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

294 NH₄⁺-N concentrations were higher at the beginning of the experiment (D0) for 295 both conventionally (A) and organically (B) managed treatments (Figure 3a), ranging 296 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 297 between $16.63\pm0.25 \text{ mg kg}^{-1}$ (B) and $18.20\pm0.08 \text{ mg kg}^{-1}$ (B10) in the latter. NH₄⁺-N 298 concentrations declined after 15 days (D15) in all treatments and were below the 299 detection limit for most of the remaining study period (D30 and D45) which is why data 300 for D30 and D45 are not shown in the graphs. For both farm management systems, NH4⁺-N concentrations decreased with time. This decline was greater for the 301 302 conventionally managed treatments, particularly the urea treatments, as they produced 303 very large NH₄⁺-N concentrations at the start of the incubation period. At D0 biochar 304 amendments significantly reduced NH₄⁺-N concentrations in the A and AU treatments, 305 whereas after 60 days, biochar addition to soils did not significantly affect NH₄⁺-N 306 concentrations ($p \le 0.05$) regardless of the application rate, but the presence of urea did. 307 Conversely, organically managed treatments with and without compost plus 5% of 308 biochar (B5 and C5) did not differ throughout the entire incubation period ($p \le 0.05$), 309 whereas the higher rate of applied biochar with and without compost varied (D0 and 310 D60).

311	NO3 ⁻ -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 NO3N concentrations compared
319	to the biochar treatments (B5=C5; B10=C10) ($p \le 0.05$). 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 ($p \le 0.05$).

326 3.4 Ammonia emission

327 In general, larger fluctuations of NH₃ 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

- increased with time, from 22°C at the start (data not shown) to 25°C after 10 days.
- 331 Largest NH₃ concentrations were measured from the soil A with urea addition only
- 332 (AU, 1.03 ± 0.59 NH₃ mg kg⁻¹) at day 2, decreasing in total 55% after 10 days. The effect
- 333 of biochar on NH₃ concentrations in conventionally managed treatments varied
- throughout the experiment. The treatments with the higher rate of biochar (A10 and

335 U10) had smaller NH₃ concentrations compared to the treatments with the lower 336 biochar rate (A5 and U5) at days 2, 6 and 8, while the opposite occurred on day 4. 337 However, lower NH₃ concentrations were in general measured with the higher rate of 338 biochar for all sampling dates, except the final date (D10). 339 A differing range in biochar effects on NH₃ emissions was also observed for 340 organically managed treatments (Figure 4). After two days, the treatments with the 341 lower biochar rate (B5 and C5) tended to release larger amounts of NH₃ compared to 342 the treatments with 10% biochar (B10 and C10). After 10 days, no difference was 343 observed between the treatments with both compost and biochar (C5 and C10) and 344 between conventionally managed control and treatments with biochar and organically 345 managed controls and treatments with just biochar (A, A5, A10, B, BC, B5 and B10).

346 **4. Discussion**

347 Discrepancies in the biogeochemical response to biochar amendments to soils are

348 frequently reported and demonstrate that there are no universal responses to biochar use

349 (Kolb et al., 2009; Prayogo et al., 2014). Different behaviours may result from

350 variations in biochar types (e.g. feedstock, pyrolysis conditions), application rates, soil

351 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.*,

Page 16 of 38

359 2017; Tammeorg et al., 2017). The magnitude of this effect in our study depended on 360 the biochar application rate. The larger biochar application rate of 10% supplied more 361 organic carbon and thereby increased the WHC to a greater extent than the 5% rate. It is 362 interesting to observe that biochar additions increased the WHC in the conventionally 363 farmed low organic matter soil by 46%, but in the organically farmed soil with a higher 364 organic matter only by 28%. Equally the soil organic matter content increased when 365 biochar was added, and this increase was slightly lower with the larger biochar 366 application rate of 10%. The stable percentage of SOM in the conventionally managed 367 soil compared to a decline in SOM in the organically managed soils, suggests larger 368 mineralization rates. This difference did not translate into the impact of biochar on soil 369 enzyme activities or microbial respiration rates.

370 Changes in soil pH after biochar additions has been shown to increase the soil 371 pH for a range of biochar products (Ouyang et al.2014a). The rate of increase may be 372 very different, depending on the product or soil conditions. In our study the soil pH 373 slightly increased when biochar was added to the conventionally managed soils by 0.3 374 pH units. Similar small rates of increase to those (0.1 - 0.2 pH units) were also reported 375 by Anderson et al. (2011) for a perennial grassland treated with woody biochar in New 376 Zealand. But the opposite was the case for the organically managed soils, for which the 377 pH decreased by 0.2. This may happen in the short term due to the microbial 378 decomposition of easily mineralizable small organic molecules, that produce CO₂, 379 organic acids and initial ammonia, what decrease soil pH. Soil B had a higher content of 380 SOM and thus more mineralizable compounds. 381 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 dehvdrogenase 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

Page 18 of 38

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

ones, reducing their availability and thus inhibiting enzyme activity. Even though

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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 Wevers *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 NH₄⁺-N concentrations were lower in the biochar treatments 449 compared to the controls whilst NO₃-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

Page 20 of 38

459 treatments had a higher NH₄⁺-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 NH₄⁺ 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 NO₃-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^{-1}

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, NO₃⁻-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 NH₄⁺-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).

484	NH ₃ volatilization is promoted by high soil pH, temperature and low soil
485	moisture content, provided there is a suitable N source, such as NH_4^+ -N or urea
486	(Taghizadeh-Toosi et al., 2012). These conditions were met by some of our treatments,
487	particularly AU. In the organically managed soil treatments NH ₃ volatilization was
488	almost constant during the 10-day incubation period, whereas in the conventionally
489	managed soils the urea treatments produced considerably higher losses. However, when
490	comparing the controls (A and AU) with the biochar treatments (A5, A10, U5 and U10),
491	it can be seen that biochar, in general, caused a decrease in NH ₃ emissions, which may
492	be attributable to NH_3 hydrolysis to NH_4^+ followed by adsorption to biochar,
493	immobilization or nitrification, as suggested by Mandal et al. (2016). Taghizadeh-Toosi
494	et al. (2012) also found that biochar could adsorb NH_3 and significantly decrease its
495	volatilization from ruminant urine.

496 **5.** Conclusions

497 Our short-term 60 day experiments suggest that different previous farm managements 498 (conventional vs. organic) as well as different fertilisation practices (mineral vs. 499 organic) should be considered when adding biochar, as these variables affect biochar 500 impacts in soil. The impact of biochar produced at low temperatures on soil biological 501 processes, such as enzymatic and microbial activities, is similar to those obtained at 502 high temperature, thus proving that there is no need to increase the energy expenditure 503 to produce biochar, to obtain a good product. The benefit of low temperature biochar 504 production is the lower energy requirements, while improving water holding capacity of 505 the soil, and in some cases increasing microbial respiration. This in turn, can increase 506 SOM mineralization in the short term.

507 Biochar addition significantly decreased dehydrogenase and β-glucosidase 508 activities in both conventionally and organically managed soils. However, this effect 509 was more pronounced in the conventionally managed soil and when urea was added. 510 The largest phosphatase activity was observed in treatments with biochar addition, 511 especially for the organically managed soil treatments. This is most likely due to a 512 greater release and availability of organic phosphorus. Biochar decreased NH₄⁺-N 513 content in the conventionally managed soil and a progressive increase in NO₃⁻-N, while 514 in the organically managed soil, biochar had no effect on NH₄⁺-N concentration, but 515 promoted a decrease in NO₃-N, that happened probably through denitrification. 516 Volatilization of NH₃ was higher in urea treatments than in treatments with compost, 517 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
687 688	Table 2. Physical and chemical properties of the biochar, composted farmyard manure 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) ($n=3\times4$). For
695	each sampling date, significant differences ($p \le 0.05$) 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) ($n=3\times4$). For
698	each sampling date, significant differences ($p \le 0.05$) are indicated by different letters.
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Table 1. Treatments applied to the conventionally managed soil A and to the organically

709	managed	soil	B.
10)	managea	5011	υ.

Treatments of soil A		Treatments of soil B			
A	Soil A control (no fertilizer added)	В	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		

Table 2. Physical and chemical properties of the biochar, composted farmyard manure

and soils used in the incubation experiments.

Parameter	Biochar	Composted	Conventionally	Organically
		FYM	managed soil A	managed soil B
рН	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

719 **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

721 replicates measured at the beginning (D0) and end (D60) of the 60-day incubation

722 experiment.

Treatmen ts	WHC (%)]	рН	SOM (%)			
Sampling date (days)	D0	D0 D60		D0	D60		
		Conventionall	y farmed soil A				
Α	27 ± 2.3 c	7.20 ± 0.2 c	7.21 ± 0.2 c	$1.40 \pm 0.1 \text{ c}$	1.37 ± 0.2 c		
A5	$32 \pm 1.8 \text{ b}$	7.46 ± 0.3 a	7.53 ± 0.3 a	$2.21\pm0.2\;b$	$2.24\pm0.2~b$		
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	$28 \pm 5.1 \text{ c}$	7.19 ± 0.4 bc	7.20 ± 0.3 c	$1.43 \pm 0.08 \text{ c}$	1.41 ± 0.2 c		
U5	33 ± 2.3 ab	$7.38\pm0.4\ b$	$7.44\pm0.2\;b$	2.23± 0.2 b	$2.28\pm0.3\ b$		
U10	42 ± 5.8 a	$7.42 \pm 0.2 \text{ b}$ $7.43 \pm 0.3 \text{ b}$		2.43 ± 0.3 a	2.42 ± 0.2 a		
	Organically farmed soil B						
В	$44 \pm 4.3 \text{ b}$	7.43 ± 0.1 a	7.50 ± 0.2 a	$3.83 \pm 0.2 \text{ d}$	$4.07 \pm 0.2 \text{ d}$		
B5	50 ± 1.8 a	$7.34\pm0.2\ b$	7.33 ± 0.3 c	$6.12\pm0.3~b$	$5.41\pm0.3\ b$		
B10	52 ± 3.3 a	$7.26\pm0.1\ b$	$7.43\pm0.3\ b$	6.39 ± 0.4 a	$5.94\pm0.3\ b$		
BC	48 ± 2.4 b	7.47 ± 0.4 a	7.51 ± 0.2 a	4.15 ± 0.2 c	$4.04\pm0.2~d$		
C5	47± 1.7 b	7.47 ± 0.1 a	7.51 ± 0.2 a	$6.13 \pm 0.2 \text{ b}$	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 ($p \le 0.05$) 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

the average and standard deviations (n=3) at D0, D30, D60 = 0, 30, 60 days after setup.

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		Dehydrogenase			β-glucosidase			Phosphatase		
Treatments		(µg TPF g ⁻¹ ł	I ⁻¹)	(μ	(µmol <i>p</i> -NP g ⁻¹ h ⁻¹)			(µmol <i>p</i> -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 ($p \le 0.05$) are indicated by different letters within each732row. Note that the statistical analysis was performed separately for conventionally managed soil A and

organically managed soil B.

Page 37 of 38









 $\blacksquare B \blacksquare B5 \blacksquare B10 \blacksquare BC \blacksquare C5 \blacksquare C10$





■B ■B5 ■B10 ■BC ■C5 ■C10















■A ■A5 ■A10 ■AU ■U5 ■U10



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Cordovil, Cláudia M d S

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