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Jones, David; Hill, Paul; Chadwick, David

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1 **Critical comparison of the impact of biochar and wood ash on soil organic matter cycling and**
2 **grassland productivity**

3 Eleanor Y. Reed*, David R. Chadwick, Paul W. Hill, Davey L. Jones

4 *School of Environment, Natural Resources & Geography, Bangor University, Bangor,*
5 *Gwynedd, LL57 2UW, UK*

6

7

8 *Corresponding author: Eleanor Y. Reed

9 Email: ereed@wardell-armstrong.com

10 Tel: +44 (0)191 232 0943

11 Eleanor Y. Reed previously published under the name: Eleanor Y. Swain. Present address:
12 Wardell Armstrong LLP, 11 Waterloo Square, Newcastle upon Tyne, NE1 4DP, United
13 Kingdom

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24 **ABSTRACT**

25 Wood represents the single most important source of renewable energy worldwide and
26 depending on the mechanism of energy production can lead to the production of by-products
27 with vastly different properties (i.e. wood ash (WA) from incineration and biochar (BC) from
28 pyrolysis). These are typically applied to land, however, a critical comparison of their impact
29 on soil quality and carbon (C) cycling is lacking. To address this, we generated biochar (450°C)
30 and wood ash (870°C) from the same mixed hardwood feedstock and added it to an agricultural
31 grassland at comparable rates under both laboratory and field conditions (10 t ha⁻¹ and 571 kg
32 ha⁻¹ for BC and WA, respectively). We hypothesized that alkaline, nutrient-rich wood ash
33 would stimulate microbial activity, resulting in the loss of soil organic matter (SOM), while
34 biochar which is recalcitrant to microbial attack would promote the stabilization of native
35 SOM. The effects on the soil microbial community and soil C and N cycling were determined
36 over 1 year. Overall, biochar promoted soil quality by enhancing nutrient availability (P and
37 K), moisture retention and increasing soil C content. However, it was also associated with an
38 increase in below-ground CO₂ loss. As plant productivity was unaffected and laboratory
39 incubations of biochar with ¹⁴C-labeled SOM showed no indication of priming, we deduce that
40 this CO₂ originates from the biochar itself. This is supported by the lack of effect of biochar on
41 soil N cycling, microbial biomass and community structure. Wood ash had almost no effect on
42 either soil quality or vegetation quality (yield and foliar nutrient content) under field conditions
43 but did induce negative SOM priming under both laboratory and field conditions. We conclude
44 that when applied at field-relevant rates, neither amendment had a detrimental effect on native
45 SOM cycling. While wood ash promotes the retention of native SOM, biochar may be a better
46 strategy for enhancing SOM levels because of its intrinsic recalcitrant character, however, this
47 needs to be offset against the reduced amount of energy derived from pyrolysis in comparison
48 to incineration.

49 *Keywords:* black carbon; charcoal; life cycle assessment, nutrient cycling; PLFA.

50 **1. Introduction**

51 Wood is the most important global source of renewable energy, providing about 6% of the
52 global total primary energy supply (FAO, 2016). During energy production, the pyrolysis or
53 complete incineration of wood biomass results in the formation of biochar and ash respectively.
54 These by-products can be applied to agricultural soils as an organic amendment and/or a liming
55 agent to improve soil quality (Demeyer et al., 2001; Lehmann, 2007; Atkinson et al., 2010).
56 However, while wood ash has been used for many decades as a soil improver, legislation still
57 prevents the application of biochar to land in many countries (Van Laer et al., 2015). This is
58 due to the unintended risks and uncertainties surrounding its potential short- and long-term
59 impacts on agricultural productivity and environmental health (Marks et al., 2015; Subedi et
60 al., 2015). In addition, political decisions to adopt renewable energy technologies are
61 frequently made after a complete cradle-to-grave life cycle assessment (LCA) has been
62 undertaken (Evans et al., 2009). While pyrolysis yields less energy and has greater by-product
63 transport and processing costs than incineration, biochar application to agricultural land may
64 lead to a greater enhancement of soil quality and native soil organic matter (SOM) storage
65 (Lehmann and Joseph, 2009; Marculescu, 2012). Currently, however, no studies have been
66 undertaken to directly compare the impact of biochar and wood ash within the same soil system,
67 particularly under field conditions.

68 Current evidence on the impact of biochar and wood ash on soil functioning remains
69 contradictory with both positive and negative agronomic and environmental responses being
70 reported (Lychuk et al., 2014). These responses include changes in yields (Chan et al., 2007;
71 Bierderman and Harpole, 2012), altered C and nutrient dynamics (Singh et al., 2010; Gul and
72 Whalen, 2016), changes in soil greenhouse gas emissions (Bass et al., 2016) and reductions in
73 the efficacy of pesticides and herbicides (Yu et al., 2009; Jones et al., 2011a). The beneficial
74 properties of biochar have largely been attributed to its high surface area, surface charge density
75 and cation exchange capacity, intrinsic nutrient load (e.g. NPK and cations), low bulk density,

76 high porosity and high pH (Atkinson et al., 2010; Dai et al., 2016; Jones et al., 2012). However,
77 losses of C, N, sulfur (S) as well as acidic functional groups in biochar with increasing pyrolysis
78 temperature are unavoidable. In addition, biochar (particularly derived from manure, biosolids
79 or waste) increases the risk of heavy metal contamination as such elements become
80 concentrated with increasing pyrolysis temperature (Cantrell et al., 2012; Lucchini et al., 2014;
81 Subedi et al., 2016).

82 In contrast, the beneficial properties of wood ash have largely been linked to its high
83 alkalinity and nutrient load (Ca, Mg, P and K) (Demeyer et al., 2001). However, it is likely that
84 some of these properties will be short lived (e.g. nutrient and HCO_3^- release), and that over
85 time, the effect of these soil amendments will decrease as a consequence of both the movement
86 of the soil amendments in the soil profile, and the ongoing biogeochemical interactions with
87 the amendments (Quilliam et al., 2013ab).

88 In terms of LCA, one of the most important factors to be considered is whether biochar or
89 wood ash promotes the storage or release of C contained within native SOM. These changes
90 can be mediated through shifts in the size and activity of the soil microbial community, by
91 altering soil physical properties or by altering crop growth. In the case of biochar, many studies
92 have observed an immediate short-term elevation in CO_2 evolution after biochar amendment
93 (Smith et al., 2010; Zimmerman, 2010, 2011). This release of CO_2 may result from the biotic
94 consumption or abiotic release of some of the biochar components (Cross and Sohi, 2011; Jones
95 et al., 2011b), and/or the enhanced mineralization of native SOM (positive priming; Kuzyakov
96 et al., 2009; Jones et al., 2011b; Luo et al., 2011). However, studies have revealed both positive
97 and negative priming effects of biochar on native SOM, depending on the characteristics of the
98 biochar, soil type and the time after biochar application (Wardle et al., 2008; Cross and Sohi,
99 2011; Jones et al., 2011b; Biederman and Harpole, 2012; Ventura et al., 2014). In contrast, no
100 studies exist on the potential priming effect of SOM by wood ash, especially under field
101 conditions in agricultural soils (Merino et al., 2016).

102 The aim of this study was therefore to: (i) directly compare the effect of biochar and wood
103 ash on soil quality and crop productivity; and (ii) ascertain whether biochar or wood ash
104 induces SOM priming under both laboratory and field conditions. We hypothesized that
105 alkaline, nutrient-rich wood ash would stimulate microbial activity and induce positive priming
106 and the loss of SOM while biochar, which is resistant to microbial attack, would promote
107 stabilization of native SOM.

108

109 **2. Materials and methods**

110 *2.1. Biochar and wood ash production*

111 Biochar was made by pyrolyzing (450°C, 48 h) the mechanically chipped trunks and
112 large branches of *Fraxinus excelsior* L., *Fagus sylvatica* L. and *Quercus robur* L. (BioRegional
113 HomeGrown®; BioRegional Charcoal Company Ltd, Wallington, Surrey, UK). Complete
114 incineration of 10 t of this wood-based biochar at 870 °C, yielded 571 kg of wood ash. Biochar
115 was milled to a homogenous powder, and both materials were sieved to <5 mm before use.
116 Physical and chemical properties of the biochar and wood ash soil amendments are given in
117 Table 1. Total elemental analysis was performed with a S2 Picofox TXRF Spectrometer
118 (Bruker Corp., Billerica, MA). Specific surface area was determined by the BET (Brunauer-
119 Emmett-Teller) N₂ adsorption method using a TriStar 3020 analyzer (Micromeritics Inc.,
120 Norcross, GA). Cation exchange capacity (CEC) was determined according to the method of
121 Sumner and Miller (1996).

122

123 *2.2. Field site*

124 The field trial was established in September 2014 at Abergwyngregyn, Wales
125 (53°14'20"N, 4°00'47"W) on a flat field previously used for grass silage production (Fig. S1).
126 No herbicide sprays were used to desiccate the old sward prior to trial establishment. The soil
127 is a Eutric Cambisol sandy clay loam derived from freely-draining, mixed Ordovician glacial

128 till deposits. The experiment was designed as a randomized block with three treatments
129 (biochar, wood ash and control) and four replicate 3 m × 3 m plots. Biochar (BC) was spread
130 by hand on the soil surface at a rate of 10 t ha⁻¹, and wood ash (WA) at a rate of 571 kg ha⁻¹
131 (the quantity of ash produced by burning 10 t of biochar). All plots were then watered to
132 minimize dust losses. The treatments were subsequently mechanically harrowed into the
133 topsoil (0-10 cm Ah horizon) to ensure uniform mixing. The rate of wood ash amendment is
134 within the national limit for application to agricultural land (1 t ha⁻¹ y⁻¹; HMSO, 2014) while
135 the rate of biochar application was chosen based on likely rates of application by farmers.

136 In autumn 2014, a 2 year Italian Ryegrass (*Lolium multiflorum* L.) silage ley was sown
137 (Donke tet (50%), Gemini tetraploid (25%), Menbel (25%)) at a seed rate of 0.034 t ha⁻¹.
138 Following national policy (see on-line Supplementary Information), the plots received no
139 fertilizer or herbicide treatment throughout the experiment. Weather data recorded by an on-
140 site automated station for the experimental period is presented in Table S1.

141

142 2.3. Soil quality analysis

143 Soil samples were taken fortnightly for the first four months, then monthly for eight
144 months. Five random topsoil samples (0-10 cm) were removed from each plot using a core
145 sampler, bulked and transported to the laboratory within 2 h of sampling and stored in gas
146 permeable plastic bags at 4°C until required. Within 24 h of collection, soil samples were sieved
147 to pass 2 mm, extracted and stored in the freezer at -20°C. All extractions followed the same
148 protocol: soil samples of 5 g were shaken for 30 min at 200 rev min⁻¹ using either 1 M KCl,
149 0.5 M K₂SO₄ or 0.5 M acetic acid (1:5 w/v), centrifuged (3220 g) for 10 min and filtered
150 (Whatman No. 42), the samples were subsequently stored for analysis at -20°C in
151 polypropylene vials (MISR/SAC, 1985; Jones and Willett, 2006).

152 Soil pH and electrical conductivity (EC) were determined on field-moist soil (1:2 w/v
153 soil-to-distilled water). Soil moisture content (MC) was determined by drying at 105°C (24 h)

154 and SOM determined by loss-on-ignition at 500°C (16 h), both wt %. Exchangeable K and
155 plant-available P were extracted using 0.5 M acetic acid (1:5 w/v) and the filtered extracts
156 analyzed using a Model 410 Flame photometer (Sherwood Scientific, Cambridge, UK) for K
157 and colorimetrically for P (Murphy and Riley, 1962). Dissolved organic carbon (DOC) and
158 extractable organic nitrogen (EON) were extracted using 0.5 M K₂SO₄ (1:5 w/v) and
159 determined using a Multi N/C 2100S (Analytik-Jena AG, Jena, Germany). Total C and N were
160 analyzed on dry samples using a TruSpec[®] CN analyzer (Leco Corp., St Joseph, MI). Available
161 N was extracted using 1 M KCl (1:5 w/v) and colorimetric analysis of NO₃⁻ using the vanadate
162 method of Miranda et al. (2001) and NH₄⁺ using the Na-salicylate-hypochlorite procedure of
163 Mulvaney (1996). Free amino acids were extracted using 1 M KCl (1:5 w/v) and determined
164 using a Varian Cary Eclipse Fluorescence Spectrophotometer (Jones et al., 2002). Potential net
165 N mineralization was estimated on a monthly basis using the anaerobic incubation method of
166 Keeney (1982).

167 Microbial phospholipid fatty acids (PLFAs) tests were carried out to investigate the shift
168 in microbial community structure during the duration of the field trial as described in Bartelt-
169 Ryser et al. (2005). Individual PLFA concentrations were determined by GC-MS and
170 taxonomic groups ascribed using the Sherlock[®] PLFA Method and Tools Package (PLFAD1)
171 by Microbial ID Inc. (Newark, DE, USA). The results for each individual fatty acid were
172 expressed as a percentage of the total amount of fatty acids (mol%) found in a given sample.

173

174 *2.4. Plant-soil respiratory CO₂ flux in the field*

175 CO₂ flux measurements were carried out *in-situ*, using an automated LI-8150
176 multiplexer automated CO₂ flux system (LI-COR Inc., Lincoln, NE). Dark chambers (LI-COR
177 LI-8100-104), 20.3 cm in diameter, were delimited by polyvinyl chloride (PVC) collars that
178 were permanently inserted *ca.* 5 cm into the soil from the start of the field trial, with one
179 chamber in each plot. Soil CO₂ flux was measured continuously every 2 h in each plot using

180 an automated infrared gas analyzer (LI-COR LI-8100) connected to the multiplexer system. A
181 soil temperature thermistor (LI-COR 8150-203) was connected to each chamber to record soil
182 temperature in each plot. All calibration and system testing was undertaken according to LI-
183 COR (2014). In addition, the chambers contained a vent for pressure equilibration between the
184 closed chamber and the atmosphere (McDermitt et al., 2005). The soil CO₂ flux, soil
185 temperature and relative humidity (RH) of the air within the chambers, were measured for each
186 plot for 14 consecutive days in April 2015 to study diurnal variation in soils amended with
187 biochar and wood ash. These measurements were made in the growing season to capture the
188 combined response of both the plants and soil, however, we acknowledge that this may not
189 reflect the CO₂ flux immediately after field application of the amendments.

190

191 2.5. ¹⁴C-SOM mineralization in the laboratory

192 A short-term (50 d) incubation experiment was carried out with 10 mm sieved ¹⁴C-
193 labelled Eutric Cambisol soil (collected from next to the field experiment; 53°14'21"N,
194 4°00'56"W) sampled from 0-10 cm depth (Ah horizon) with roots and stones removed. The
195 ¹⁴C-labelled SOM had been labelled 5 years previously with ¹⁴C-labelled glucose. Briefly, a
196 dilute solution (5 l) of ¹⁴C-uniformly labelled glucose (< 1 nM; 12.8 MBq l⁻¹; PerkinElmer,
197 UK) was dispensed uniformly across replicate 1 m² plots. Five years after label incorporation
198 into the plant-soil system the ¹⁴C remaining within the soil was considered to contribute to the
199 quasi-stable SOM pool (Farrar et al., 2012).

200 After 17 d of pre-incubation (20 °C, 50% water filled pore space) to allow any sampling
201 and sieving effects to subside (Kemmitt et al., 2006), 0.8 l plastic flasks (surface area 30 cm²)
202 were filled with 168 g field moist soil (100 g DW). Corresponding directly to the field
203 application rates used above, the soil was amended with either biochar at a rate of 18 mg g⁻¹
204 soil, wood ash at a rate of 1 mg g⁻¹ soil or left unamended (control). Both treatments had four
205 replicates and the control ran with eight replicates to ensure an accurate baseline.

206 The ^{14}C produced by biodegradation of the ^{14}C -labelled SOM was captured by placing
207 a plastic scintillation vial containing 4.0 ml of 1 M NaOH inside the sealed plastic flask, on top
208 of the soil/treatment mixtures. The NaOH trap was replaced 14 times at increasing intervals
209 over a 50 d incubation period. The ^{14}C collected as $\text{NaH}^{14}\text{CO}_3$ in the NaOH was measured
210 by liquid scintillation counting in a Wallac 1404 scintillation counter (PerkinElmer Life
211 Sciences, Boston, MA) after mixing with HiSafe 3 scintillation fluid (Fisher Scientific,
212 Loughborough, UK). The initial ^{14}C content (prior to incubation) of the bulk soil was
213 determined after incineration of 0.1 g of sample within an OX400 Biological Sample Oxidizer
214 (RJ Harvey Instrument Corp., Hillsdale, NJ), with the ^{14}C evolved collected in Oxosol
215 scintillation fluid (National Diagnostics Ltd, Hesse, UK) and the ^{14}C content measured by
216 liquid scintillation counting as described above.

217 The incubation experiment was repeated, replacing the ^{14}C -labelled soil with unlabeled
218 soil from an adjacent plot, and labelling the microbial biomass 24 h before the start of the
219 incubation by adding ^{14}C -labelled glucose ($1.2 \mu\text{g C g}^{-1}$ DW soil) to each soil to produce an
220 activity similar to that of the first experiment (i.e. 1.03 Bq g^{-1} DW soil). This level of glucose
221 addition was sufficient to label the microbial biomass ($750 \pm 38 \mu\text{g C g}^{-1}$) but limit excessive
222 microbial growth. This additional experiment was designed to provide an indication of both
223 the real priming (loss of C from SOM) and apparent priming (loss of C from the microbial
224 biomass) effect of the substrates (Blagodatskaya and Kuzyakov, 2008; Kuzyakov, 2010).

225

226 *2.6. Plant yield and quality*

227 The grass sward was cut on three occasions; November 2014, March 2015 and May 2015.
228 At each grass cut, dry weight was determined after oven drying (80°C , 24 h). Foliar mineral
229 nutrient content (total P, Ca, Na and K) was determined after dry ashing (500°C , 16 h),
230 solubilization of the ash in 1 M HCl and determined as described above. Total tissue C and N
231 content were determined as described above.

232

233 2.7. Statistical analysis

234 To identify seasonal variations a repeated measures ANOVA was applied in the R
235 statistical environment (R Development Core Team, 2011), to all soil quality properties. Fixed
236 effects were sample time (time), treatment (tr) and time x treatment, with block (bl) treated as
237 a random effect. The fixed effects on soil parameters were determined. The analyses were
238 carried out using the aov function and residual normality was assessed using the qqnorm
239 function in R. Where necessary, data were square root transformed or ln transformed to achieve
240 normality. The combined data for year were analyzed first, and where interaction terms were
241 significant, further analyses were conducted at each level of the interacting factor. Differences
242 between significant main effect and interaction means were determined using Tukey's Honest
243 Significant Difference (HSD) tests, based on mixed-effects models using the glht function in
244 the multcomp package of R. Paired T tests were used to test for differences between biochar
245 and wood ash chemical properties.

246 Principal component analyses (PCA) using the proportion of microbial groups in the soil
247 were performed to compare the structure of the microbial community in the different treatments
248 and the respective initial soil samples were carried out using the PCA function in the
249 FactoMineR package in the R statistical environment. Statistical significance was assigned at
250 the $P < 0.05$ level.

251

252 3. Results

253 3.1. Chemical and physical properties of biochar and wood ash

254 The biochar displayed a significantly higher bulk density and lower CEC and specific
255 surface area than the wood ash ($P < 0.05$; Table 1). Consistent with previous work (Jones and
256 Quilliam, 2014; Lucchini et al., 2014), the EC and pH were significantly higher in the wood
257 ash relative to the biochar ($P < 0.05$). Complete incineration caused EON, NO_3^- and free amino

258 acids to become significantly more concentrated in the ash relative to the partially combusted
259 biochar ($P<0.05$), whilst total incineration caused a reduction of total C and N and available P
260 in the wood ash relative to the biochar ($P<0.05$; Table 1).

261

262 3.2. Effect of time and treatment on plant and soil properties

263 Overall, there was no significant treatment effect on the growth performance (dry matter
264 yield and plant height) or the cumulative nutrient uptake (N, P, K, Na and Ca) of the grass in
265 the first year after the application of either biochar or wood ash ($P>0.05$; data not shown).

266 The temporal dynamics of the measured soil quality parameters after biochar or wood
267 ash soil amendment are shown in Figure 1. Statistical analysis revealed that there was one
268 significant time and treatment interaction which showed that when the average soil moisture
269 content (MC) was greater than 20%, the biochar soil amendment displayed a significantly
270 higher MC, however, when the average soil moisture dropped below 20% (i.e. July 2015), there
271 was no treatment effect.

272 Biochar and wood ash amendment did not result in a significant change in EC,
273 exchangeable Na, soluble C and N (NO_3^- , NH_4^+ or amino acids) or rates of net N mineralization
274 ($P>0.05$; Fig. 1, Fig. S2). However, biochar significantly increased total soil C and SOM levels
275 relative to the wood ash and control treatments throughout each sample point of the trial
276 ($P<0.05$), and over the course of the year displayed increased concentrations of available P and
277 K relative to the control (Fig. 1, Fig. S3). Biochar and wood ash addition increased soil pH
278 ($P<0.001$) for the duration of the field trial, resulting in soils with a pH 0.3 units higher than
279 the control soil (Fig. 1).

280

281 *3.3. Effect of time and treatment on soil microbial communities*

282 While time of year significantly affected the size and composition of the soil microbial
283 community, neither biochar or wood ash amendment resulted in an appreciable change in these
284 soil properties (Table 2).

285

286 *3.4. Effect of biochar and wood ash addition on soil CO₂ loss from the field*

287 The soil amendment effect on soil CO₂ flux, soil temperature and relative humidity,
288 measured seven months after treatment application are shown in Table 3. Treatment had a
289 significant effect on the soil CO₂ flux, with the biochar plots resulting in a significantly higher
290 soil CO₂ flux than the control treatment, which displayed a significantly higher soil CO₂ flux
291 than the wood ash treatment ($P<0.001$; BC>C>WA; Table 3). The wood ash and control
292 treatments had an average soil CO₂ flux 10.6 and 5.0% lower than the biochar amended soil,
293 respectively. In all sites, a significant positive correlation was observed between soil CO₂ flux
294 and soil temperature ($r=0.770$).

295

296 *3.5. Effect of biochar and wood ash addition on SOM turnover in the laboratory*

297 The effect of biochar and wood ash on the mineralization of native ¹⁴C-SOM in
298 laboratory incubations is shown in Figure 3a. Overall, biochar displayed no significant priming
299 effect after 50 d, however, wood ash induced a negative priming response ($P<0.0001$; Fig. 3a).
300 The presence of wood ash significantly decreased the mineralization of the ¹⁴C-labeled native
301 soil by 28% over the 50 d incubation period relative to the control. The microbial community
302 was assessed at day 50, which revealed a significantly increased microbial biomass in the
303 biochar mesocosm relative to the control, whereas the wood ash resulted in a decreased
304 microbial biomass relative to the control (Table 4). Biochar stimulated the growth of putative
305 AM fungi and Gram-positive bacteria, whilst suppressing the growth of Eukaryotes, Fungi,

306 Anaerobes and Actinobacteria relative to the control and wood ash amended soils. The wood
307 ash treated soils revealed a suppressed growth of putative AM fungi relative to the control.

308 The effect of biochar and wood ash on microbial biomass turnover is shown in Figure 3b.
309 Despite an initial rapid rate of $^{14}\text{CO}_2$ release from the biochar amended soil in the first 10 d,
310 there was no overall effect of biochar or wood ash on the rate of microbial biomass
311 mineralization over the 50 d incubation period ($P=0.220$).

312

313 **4. Discussion**

314 *4.1. Vegetation responses to soil amendments and impacts on C cycling*

315 Our results suggest that biochar and wood ash applied prior to sward establishment had
316 no significant influence on plant growth or nutritional quality, compared to the non-amended
317 soil. This implies that neither amendment promoted above-ground C storage or led to greater
318 amounts of C entering the soil from leaf litter. Although we did not quantify rhizodeposition
319 or root/mycorrhizal turnover *in situ*, we have no evidence from the soil quality measurements
320 to suggest that these were strongly affected by either amendment.

321 The lack of growth response is consistent with previously studies using the same wood-
322 derived biochar (Jones et al., 2012; Quilliam et al., 2012). Recent meta-analyses of the impact
323 of biochar application on soil concluded, however, that applications of biochar to soil do on
324 average increase crop yields (Jeffery et al., 2011; Biederman and Harpole, 2012; Liu et al.,
325 2013). However, Jeffery et al. (2011) noted that crop responses are variable and dependent on
326 a multitude of factors, including, experimental set-up, soil properties, climatic conditions,
327 biochar properties, application rate and the interaction between biochar and fertilizers.
328 Typically, greatest positive yields arise from biochar applications of $>30 \text{ t ha}^{-1}$, an application
329 rate much greater than that applied in this study. Although high rates of biochar addition are
330 theoretically possible, the practicalities of obtaining sufficient quantities of biochar for large
331 field areas and the economic costs involved in production, processing and transport are likely

332 to prohibit such use on commercial farms. The doses used here are therefore more likely to be
333 representative of actual field use.

334 The wood ash results did not display the typical plant growth improvements associated
335 with ash addition to soil (Krejsl and Scanlon, 1996; Jones and Quilliam, 2014), however, the
336 dose rates used in this study were considerably lower than many previous studies. Our rates,
337 however, are just below the legal limits for wood ash application to agricultural land and were
338 therefore deemed to be more representative than previous studies. In addition, higher dose rates
339 would likely have resulted in excessive alkalization and heavy metal loading of the soil (Jones
340 and Quilliam, 2014).

341

342 *4.2. Soil quality responses to biochar and wood ash amendment and implications for C cycling*

343 Consistent with previous studies, the incorporation of biochar improved soil moisture
344 retention ($20\% < MC < 30\%$) (Jeffery et al., 2011; Saarnio et al., 2013). However, this effect was
345 not maintained once the soil moisture dropped below 20%. As the soil used here is freely
346 draining, SOM turnover is most negatively affected when the soil dries out in the summer
347 months. We therefore conclude that the slightly increased storage of water seen in the biochar
348 treatment was unlikely to greatly affect SOM turnover rates or plant productivity.

349 The addition of liming agents to acidic soils is known to increase pH and improve soil
350 quality due to increases in nitrification and plant productivity (Kemmitt et al., 2006; Jeffery et
351 al., 2011). The control soil used in this study, however, was close to the optimal pH for
352 grassland production (pH 6.2) and neither wood ash and biochar addition raised it excessively,
353 consistent with the application of realistic field doses. Soil pH is considered a key driver in the
354 regulating microbial community structure and rates of C cycling (Blagodatskaya and
355 Kuzyakov, 2008; Griffiths et al., 2011), with increases in pH generally enhancing microbial
356 activity. However, we did not observe, any *in situ* effect of the biochar or wood ash addition
357 on the amount of C stored in the soil microbial biomass, nor its turnover. The absence of

358 biochar effects on soil microbial biomass have previously been documented, and has been
359 attributed to soils already experiencing high rates of SOM mineralization and nitrification prior
360 to treatment (Castaldi et al., 2011; Anders et al., 2013; Ameloot et al., 2014; Watzinger et al.,
361 2014).

362 There was no significant treatment effect for any of the measured N parameters (total
363 N, EON, NH_4^+ , NO_3^- and net mineralization). These results suggest that biochar and wood ash
364 had no appreciable effect on SOM turnover and N cycling. This is evidenced by the lack of
365 change in crop productivity and foliar N content, which are highly responsive to N availability
366 (Campbell et al., 2011). It is also consistent with previous wood biochar studies in soils
367 expressing high rates of nitrification prior to biochar application (DeLuca et al., 2006; Jones et
368 al., 2012). This finding suggests that neither amendment can help offset the use of N fertilizers
369 (and the embedded C cost associated with their production) but are also unlikely to influence
370 rates of N_2O emissions.

371

372 *4.3. Soil amendment-induced priming of SOC*

373 The application of biochar increased the topsoil C content in the field by approximately
374 27% relative to the two other treatments (0-10 cm layer). This is consistent with the large
375 amount of C added in the biochar treatment (8.43 t C ha^{-1}) relative to that added in the wood
376 ash treatment (0.10 t C ha^{-1}). Whilst there was evidence of a small but measurable increase in
377 CO_2 efflux from the biochar plots in the field, this could be attributable to (i) increased plant
378 respiration, (ii) the biotically-mediated breakdown of the added biochar, (iii) abiotic release of
379 inorganic C contained in the biochar, or (iv) release of C from native SOM. As there was no
380 alteration in plant biomass yield we do not favor this explanation. In addition, the abiotic
381 release of C from biochar occurs quickly after introduction to soil and is not favored (Jones et
382 al., 2011b). Lastly, results from the 50 d incubation study suggest that biochar causes no ‘real’
383 priming of native SOM, nor ‘apparent’ priming from increased turnover of the soil microbial

384 community. All the evidence therefore suggests that the increase in CO₂ is due to the
385 progressive breakdown of biochar by microbial processes. This is consistent with Jones et al.
386 (2011b) who showed that the water soluble component of this biochar was highly susceptible
387 to microbial breakdown.

388 Conversely, both the laboratory and field incubation studies provided strong evidence
389 for the negative priming of native SOM in the presence of wood ash. The reduction observed
390 in the field (ca. 5%) was less than observed in the laboratory (ca. 28%), however, the field
391 measurements also include plant-derived respiration and CO₂ originating from below the soil
392 layer containing the wood ash. Both of these would effectively dilute the negative priming
393 effect observed in the field. It should be noted that the measurement windows were different
394 between the laboratory and field experiments. The laboratory incubations examined the early
395 impact of wood ash amendments while the field measurements looked at the later effects. The
396 laboratory incubations provided strong evidence that wood ash resulted in a persistent negative
397 impact on SOM turnover, suggesting that the field observations were probably not due to
398 changes in plant growth and metabolism. Further, we clearly show that wood ash has no
399 significant impact on the partitioning of glucose-derived C within the soil microbial biomass
400 (i.e. substrate C use efficiency), or the turnover of the biomass itself (Fig. 3). This is consistent
401 with small overall changes in soil microbial community structure determined with PLFAs
402 (Table 4). The exact mechanism for this negative priming therefore remains unknown but
403 appears to be unrelated to macronutrient bioavailability or its heavy metal content which is low
404 (Table 1, Table S2). It could be that the CEC and specific surface area of the wood ash
405 chemically stabilizes SOM, however, this requires further investigation. In addition, in the
406 presence of water, wood ash can recrystallize and form concrete which could physically protect
407 SOM (Aamr-Daya et al., 2008; Illikainen et al., 2014).

408 As wood ash contains only small amounts of C, its addition to soil only results in a small
409 increase in SOM (ca. +0.4%). However, its impact on repressing below-ground respiration

410 could be much more important. Given that the total below-ground CO₂ flux at the field site is
411 15.1 t C ha⁻¹ y⁻¹ (J.F. Farrar, unpublished), then based on our estimates, negative priming could
412 account for a net storage of 0.76 t C ha⁻¹ y⁻¹. Clearly, this is much less than the instantaneous C
413 benefit derived from biochar even at low dose rates (<10 t ha⁻¹).

414

415 **5. Conclusions**

416 Here we demonstrate that when realistic doses of biochar and wood ash are applied to an
417 inherently fertile grassland soil, both amendments result in no major changes in soil quality or
418 agronomic yield. A key finding was that wood ash repressed native SOM turnover while
419 biochar had no effect. Nevertheless, the retention of native soil organic C associated with wood
420 ash was low in comparison to the amount of C added in a single dose of biochar. However, this
421 needs to be balanced against the potential greater recovery of energy during the complete
422 incineration of the feedstock material. In addition, wood ash may have further benefits over
423 biochar as it easily pelletized and transported (facilitating land application) and is unlikely to
424 affect the efficacy of herbicides and pesticides applied to the soil. Most previous studies on
425 biochar have looked at its impact in comparison to an unamended control treatments or
426 conventional inorganic fertilizers. This study highlights the need for a greater comparison of
427 biochar to other organic wastes (e.g. compost) and products derived from energy production
428 (e.g. anaerobic digestate, wood ash). Ideally, these comparisons should be performed under
429 field conditions, at representative field application rates and also consider the socioeconomic
430 aspects of farm management.

431

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438

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629 **Figure legends**

630 **Fig. 1** Influence of soil amendment type, biochar (BC: solid line), wood ash (WA: dashed line)
631 and control (C: gray line) and time since application on soil quality parameters between
632 September 2014 and August 2015. Values are the mean of 4 replicates \pm SEM.

633 **Fig. 2** Diurnal variation of soil CO₂ flux with soil temperature under the biochar, wood ash and
634 control treatments. Temperature averaged for each cycle ($n = 12$), soil CO₂ flux averaged for
635 each block ($n = 3$)

636 **Fig. 3** Mineralization of (a) ¹⁴C-labelled native SOM and (b) ¹⁴C-labelled microbial biomass-
637 C in the presence and absence of the soil amendments biochar and wood ash. Experiments were
638 performed in the laboratory. Values represent cumulative means of ¹⁴CO₂ evolution \pm SEM (n
639 = 4 for biochar and wood ash and $n = 8$ for control).