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Soil Biology and Biochemistry

DOI:
10.1016/j.soilbio.2017.03.012

Published: 01/07/2017

Peer reviewed version

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

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ABSTRACT

Wood represents the single most important source of renewable energy worldwide and depending on the mechanism of energy production can lead to the production of by-products with vastly different properties (i.e. wood ash (WA) from incineration and biochar (BC) from pyrolysis). These are typically applied to land, however, a critical comparison of their impact on soil quality and carbon (C) cycling is lacking. To address this, we generated biochar (450°C) and wood ash (870°C) from the same mixed hardwood feedstock and added it to an agricultural grassland at comparable rates under both laboratory and field conditions (10 t ha^{-1} and 571 kg ha^{-1} for BC and WA, respectively). We hypothesized that alkaline, nutrient-rich wood ash would stimulate microbial activity, resulting in the loss of soil organic matter (SOM), while biochar which is recalcitrant to microbial attack would promote the stabilization of native SOM. The effects on the soil microbial community and soil C and N cycling were determined over 1 year. Overall, biochar promoted soil quality by enhancing nutrient availability (P and K), moisture retention and increasing soil C content. However, it was also associated with an increase in below-ground CO_2 loss. As plant productivity was unaffected and laboratory incubations of biochar with ^{14}C-labeled SOM showed no indication of priming, we deduce that this CO_2 originates from the biochar itself. This is supported by the lack of effect of biochar on soil N cycling, microbial biomass and community structure. Wood ash had almost no effect on either soil quality or vegetation quality (yield and foliar nutrient content) under field conditions but did induce negative SOM priming under both laboratory and field conditions. We conclude that when applied at field-relevant rates, neither amendment had a detrimental effect on native SOM cycling. While wood ash promotes the retention of native SOM, biochar may be a better strategy for enhancing SOM levels because of its intrinsic recalcitrant character, however, this needs to be offset against the reduced amount of energy derived from pyrolysis in comparison to incineration.

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

1. Introduction

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

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

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

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

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

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

2. Materials and methods

2.1. Biochar and wood ash production

Biochar was made by pyrolyzing (450°C, 48 h) the mechanically chipped trunks and large branches of *Fraxinus excelsior* L., *Fagus sylvatica* L. and *Quercus robur* L. (BioRegional HomeGrown®; BioRegional Charcoal Company Ltd, Wallington, Surrey, UK). Complete incineration of 10 t of this wood-based biochar at 870 °C, yielded 571 kg of wood ash. Biochar was milled to a homogenous powder, and both materials were sieved to <5 mm before use. Physical and chemical properties of the biochar and wood ash soil amendments are given in Table 1. Total elemental analysis was performed with a S2 Picofox TXRF Spectrometer (Bruker Corp., Billerica, MA). Specific surface area was determined by the BET (Brunauer-Emmett-Teller) N_2 adsorption method using a TriStar 3020 analyzer (Micromeritics Inc., Norcross, GA). Cation exchange capacity (CEC) was determined according to the method of Sumner and Miller (1996).

2.2. Field site

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

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

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

2.3. Soil quality analysis

Soil samples were taken fortnightly for the first four months, then monthly for eight months. Five random topsoil samples (0-10 cm) were removed from each plot using a core sampler, bulked and transported to the laboratory within 2 h of sampling and stored in gas permeable plastic bags at 4°C until required. Within 24 h of collection, soil samples were sieved to pass 2 mm, extracted and stored in the freezer at -20°C. All extractions followed the same protocol: soil samples of 5 g were shaken for 30 min at 200 rev min^{-1} using either 1 M KCl, 0.5 M K_2SO_4 or 0.5 M acetic acid (1:5 w/v), centrifuged (3220 *g*) for 10 min and filtered (Whatman No. 42), the samples were subsequently stored for analysis at -20°C in polypropylene vials (MISR/SAC, 1985; Jones and Willett, 2006).

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

and SOM determined by loss-on-ignition at 500°C (16 h), both wt %. Exchangeable K and plant-available P were extracted using 0.5 M acetic acid (1:5 w/v) and the filtered extracts analyzed using a Model 410 Flame photometer (Sherwood Scientific, Cambridge, UK) for K and colorimetrically for P (Murphy and Riley, 1962). Dissolved organic carbon (DOC) and extractable organic nitrogen (EON) were extracted using 0.5 M K_2SO_4 (1:5 w/v) and determined using a Multi N/C 2100S (Analytik-Jena AG, Jena, Germany). Total C and N were analyzed on dry samples using a TruSpec® CN analyzer (Leco Corp., St Joseph, MI). Available N was extracted using 1 M KCl (1:5 w/v) and colorimetric analysis of NO_3^- using the vanadate method of Miranda et al. (2001) and NH_4^+ using the Na-salicylate-hypochlorite procedure of Mulvaney (1996). Free amino acids were extracted using 1 M KCl (1:5 w/v) and determined using a Varian Cary Eclipse Fluorescence Spectrophotometer (Jones et al., 2002). Potential net N mineralization was estimated on a monthly basis using the anaerobic incubation method of Keeney (1982).

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

2.4. Plant-soil respiratory CO_2 flux in the field

CO_2 flux measurements were carried out *in-situ*, using an automated LI-8150 multiplexer automated CO_2 flux system (LI-COR Inc., Lincoln, NE). Dark chambers (LI-COR LI-8100-104), 20.3 cm in diameter, were delimited by polyvinyl chloride (PVC) collars that were permanently inserted *ca.* 5 cm into the soil from the start of the field trial, with one chamber in each plot. Soil CO_2 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 CO_2 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 CO_2 flux immediately after field application of the amendments.
190
191 *2.5. ^{14}C-SOM mineralization in the laboratory*
192 A short-term (50 d) incubation experiment was carried out with 10 mm sieved ^{14}C-
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 ^{14}C-labelled SOM had been labelled 5 years previously with ^{14}C-labelled glucose. Briefly, a
196 dilute solution (5 l) of ^{14}C-uniformly labelled glucose (< 1 nM; 12.8 MBq l^{-1}; PerkinElmer,
197 UK) was dispensed uniformly across replicate 1 m^2 plots. Five years after label incorporation
198 into the plant-soil system the ^{14}C 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 cm^2)
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 $^{14}CO_2$ 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 $^{14}CO_2$ collected as $NaH^{14}CO_3$ 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 $^{14}CO_2$ evolved collected in Oxosol
215 scintillation fluid (National Diagnostics Ltd, Hessle, 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 µg 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 ± 38 µg 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

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

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

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

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

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

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 CO_2 loss from the field*

287 The soil amendment effect on soil CO_2 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 CO_2 flux, with the biochar plots resulting in a significantly higher
290 soil CO_2 flux than the control treatment, which displayed a significantly higher soil CO_2 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 CO_2 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 CO_2 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 ^{14}C-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 ^{14}C-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 $^{14}CO_2$ 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 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

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

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

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

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

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

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

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

4.3. Soil amendment-induced priming of SOC

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

community. All the evidence therefore suggests that the increase in CO_2 is due to the progressive breakdown of biochar by microbial processes. This is consistent with Jones et al. (2011b) who showed that the water soluble component of this biochar was highly susceptible to microbial breakdown.

Conversely, both the laboratory and field incubation studies provided strong evidence for the negative priming of native SOM in the presence of wood ash. The reduction observed in the field (ca. 5%) was less than observed in the laboratory (ca. 28%), however, the field measurements also include plant-derived respiration and CO_2 originating from below the soil layer containing the wood ash. Both of these would effectively dilute the negative priming effect observed in the field. It should be noted that the measurement windows were different between the laboratory and field experiments. The laboratory incubations examined the early impact of wood ash amendments while the field measurements looked at the later effects. The laboratory incubations provided strong evidence that wood ash resulted in a persistent negative impact on SOM turnover, suggesting that the field observations were probably not due to changes in plant growth and metabolism. Further, we clearly show that wood ash has no significant impact on the partitioning of glucose-derived C within the soil microbial biomass (i.e. substrate C use efficiency), or the turnover of the biomass itself (Fig. 3). This is consistent with small overall changes in soil microbial community structure determined with PLFAs (Table 4). The exact mechanism for this negative priming therefore remains unknown but appears to be unrelated to macronutrient bioavailability or its heavy metal content which is low (Table 1, Table S2). It could be that the CEC and specific surface area of the wood ash chemically stabilizes SOM, however, this requires further investigation. In addition, in the presence of water, wood ash can recrystallize and form concrete which could physically protect SOM (Aamr-Daya et al., 2008; Illikainen et al., 2014).

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

could be much more important. Given that the total below-ground CO_2 flux at the field site is 15.1 t C ha^{-1} y^{-1} (J.F. Farrar, unpublished), then based on our estimates, negative priming could account for a net storage of 0.76 t C ha^{-1} y^{-1}. Cleary, this is much less than the instantaneous C benefit derived from biochar even at low dose rates (<10 t ha^{-1}).

5. Conclusions

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

Acknowledgements

This work was undertaken using funds provided by the Welsh European Funding Office under the SEREN Programme. We thank Mark Hughes and Llinos Hughes (Bangor University) for assistance with the establishment and maintenance of field plots. We also acknowledge

436 Prof. Joann Whalen and two anonymous reviewers for their constructive comments which
437 improved this manuscript.
438

439 **References**

440 Ameloot, N., Sleutel, S., Case, S.D.C., Alberti, G., McNamara, N.P., Zavalloni, C., Vervisch,
441 B., Vedove, G. delle, De Neve, S., 2014. C mineralization and microbial activity in four
442 biochar field experiments several years after incorporation. Soil Biology & Biochemistry
443 78, 195-203.
444 Aamr-Daya, E., Langlet, T., Benazzouk, A., Queneudec, M., 2008. Feasibility study of
445 lightweight cement composite containing flax by-product particles: Physico-mechanical
446 properties. Cement & Concrete Composites 30, 957-963.
447 Ameloot, N., De Neve, S., Jegajeevagan, K., Yildiz, G., Buchan, D., Funkuin, Y.N., Prins, W.,
448 Bouckaert, L., Sleutel, S., 2013. Short-term CO_2 and N_2O emissions and microbial
449 properties of biochar amended sandy loam soils. Soil Biology & Biochemistry 57, 401-
450 410.
451 Anders, E., Watzinger, A., Rempt, F., Kitzler, B., Wimmer, B., Zehetner, F., Stahr, K.,
452 Zechmeister-Boltenstern, S., Soja, G., 2013. Biochar affects the structure rather than the
453 total biomass of microbial communities in temperate soils. Agricultural and Food
454 Science 22, 404-423.
455 Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving
456 agricultural benefits from biochar application to temperate soils: a review. Plant and Soil
457 337, 1-18.
458 Bartelt-Ryser, J., Joshi, J., Schmid, B., Brandl, H., Balser, T., 2005, Soil feedbacks of plant
459 diversity on soil microbial communities and subsequent plant growth. Perspectives in
460 Plant Ecology Evolution and Systematics 7, 27-49.

461 Bass, A.M., Bird, M.I., Kay, G., Muirhead, B., 2016. Soil properties, greenhouse gas emissions
462 and crop yield under compost, biochar and co-composted biochar in two tropical
463 agronomic systems. Science of the Total Environment 550, 459-470.
464 Biederman, L.A., Harpole, W.S., 2012. Biochar and its effects on plant productivity and
465 nutrient cycling: a meta-analysis. Global Change Biology Bioenergy 5, 202-214.
466 Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and
467 their dependence on soil microbial biomass and community structure: Critical review.
468 Biology and Fertility of Soils 45, 115-131.
469 Campbell, C. A., Lafond, G.P., Vandenbygaart, A. J., Zentner, R.P., Lemke, R., May, W.E.,
470 Holzapfel, C.B., 2011. Effect of crop rotation, fertilizer and tillage management on spring
471 wheat grain yield and N and P content in a thin Black Chernozem: A long-term study.
472 Canadian Journal of Soil Science 91, 467-483.
473 Cantrell, K.B., Hunt, P.G., Uchimiya, M., Novak, J.M., Ro, K.S., 2012. Impact of pyrolysis
474 temperature and manure source on physicochemical characteristics of biochar.
475 Bioresource Techology 107, 419-428.
476 Castaldi, S., Riondino, M., Baronti, S., Esposito, F.R., Marzaioli, R., Rutigliano, F. A., Vaccari,
477 F.P., Miglietta, F., 2011. Impact of biochar application to a Mediterranean wheat crop on
478 soil microbial activity and greenhouse gas fluxes. Chemosphere 85, 1464-1471.
479 Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values
480 of greenwaste biochar as a soil amendment. Australian Journal of Soil Research 45, 629-
481 634.
482 Cross, A., Sohi, S.P., 2011. The priming potential of biochar products in relation to labile
483 carbon contents and soil organic matter status. Soil Biology & Biochemistry 43, 2127-
484 2134.

485 Dai, Z., Zhang, X., Tang, C., Muhammad, N., Wua, J., Brookes, P.C., Xu, J., 2017. Potential
486 role of biochars in decreasing soil acidification - A critical review. Science of the Total
487 Environment 581/582, 601-611.
488 DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., Holben, W.E., 2006. Wildfire-produced
489 charcoal directly influences nitrogen cycling in ponderosa pine forests. Soil Science
490 Society of America Journal 70, 448-453.
491 Demeyer, A., Voundi Nkana, J., Verloo, M., 2001. Characteristics of wood ash and influence
492 on soil properties and nutrient uptake: an overview. Bioresource Technology 77, 287-
493 295.
494 Evans, A., Strezov, V., Evans, T.J., 2009. Assessment of sustainability indicators for renewable
495 energy technologies. Renewable & Sustainable Energy Reviews 13, 1082-1088.
496 FAO, 2016. Wood Energy. Rome, Italy.
497 Farrar, J., Boddy, E., Hill, P.W., Jones, D.L., 2012. Discrete functional pools of soil organic
498 matter in a UK grassland soil are differentially affected by temperature and priming. Soil
499 Biology & Biochemistry 49, 52-60.
500 Griffiths, R., Thomson, B.C., James, P., Bell, T., Bailey, M., Whiteley. A.S., 2011. The
501 bacterial biogeography of British soils. Environmental Microbiology 13, 1642-54.
502 Gul, S., Whalen, J.K., 2016. Biochemical cycling of nitrogen and phosphorus in biochar-
503 amended soils. Soil Biology & Biochemistry 103, 1-15.
504 HMSO, 2014. Waste exemption: U10 spreading waste to benefit agricultural land. Her
505 Majesty's Stationery Office, London, UK.
506 Illikainen, M., Tanskanen, P., Kinnunen, P., Korkko, M., Peltosaari, O., Wigren, V.,
507 Osterbacka, J., Talling, B., Niinimaki, J., 2014. Reactivity and self-hardening of fly ash
508 from the fluidized bed combustion of wood and peat. Fuel 135, 69-75.

509 Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of
510 the effects of biochar application to soils on crop productivity using meta-analysis.
511 Agriculture, Ecosystems & Environment 144, 175-187.
512 Jones, D.L., Willett, V.B., 2006. Experimental evaluation of methods to quantify dissolved
513 organic nitrogen (DON) and dissolved organic carbon (DOC) in soil. Soil Biology &
514 Biochemistry 38, 991-999.
515 Jones, D.L., Owen, A.G., Farrar, J.F., 2002. Simple method to enable the high resolution
516 determination of total free amino acids in soil solutions and soil extracts. Soil Biology &
517 Biochemistry 34, 1893-1902.
518 Jones, D.L., Edwards-Jones, G., Murphy, D.V., 2011a. Biochar mediated alterations in
519 herbicide breakdown and leaching in soil. Soil Biology & Biochemistry 43, 804-813.
520 Jones, D.L., Murphy, D.V., Khalid, M., Ahmad, W., Edwards-Jones, G., DeLuca, T.H., 2011b.
521 Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically
522 mediated. Soil Biology & Biochemistry 43, 1723-1731.
523 Jones, D.L., Rousk, J., Edwards-Jones, G., DeLuca, T.H., Murphy, D.V., 2012. Biochar-
524 mediated changes in soil quality and plant growth in a three year field trial. Soil Biology
525 & Biochemistry 45, 113-124.
526 Jones, D.L., Quilliam, R.S., 2014. Metal contaminated biochar and wood ash negatively affect
527 plant growth and soil quality after land application. Journal of Hazardous Materials 276,
528 362-370.
529 Kemmitt, S.J., Wright, D., Goulding, K.W.T., Jones, D.L., 2006. pH regulation of carbon and
530 nitrogen dynamics in two agricultural soils. Soil Biology & Biochemistry 38, 898-911.
531 Keeney, D. R., 1982. Nitrogen-availability indexes. In Methods of soil analysis. Part 2. 2nd Ed
532 711-730. Page, A.L., Miller, R.H., Keeney, R.R. eds. American Society of Agronomy,
533 Madison, WI, USA.

534 Krejsl, J.A., Scanlon, T.M., 1996. Evaluation of beneficial use of wood-fired boiler ash on oat
535 and bean growth. Journal of Environmental Quality 25, 950-954.
536 Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon
537 decomposition and incorporation into soil microbial biomass estimated by ^{14}C labeling.
538 Soil Biology & Biochemistry 41, 210-219.
539 Kuzyakov, Y., 2010. Priming effects: Interactions between living and dead organic matter. Soil
540 Biology & Biochemistry 42, 1363-1371.
541 Lehmann, J., 2007. A handful of carbon. Nature 447, 143-144.
542 Lehmann, J., Joseph, S., 2009. Biochar for Environmental Management: An Introduction.
543 Earthscan, London, UK.
544 LI-COR (2014) Capturing and processing soil GHG Fluxes using the LI-8100A. Application
545 Note 138. LI-COR Biosciences, Lincoln, NE, USA.
546 Liu, X.Y., Zhang, A.F., Ji, C.Y., Joseph, S., Bian, R.J., Li, L.Q., Pan, G.X., Paz-Ferreiro, J.,
547 2013. Biochar's effect on crop productivity and the dependence on experimental
548 conditions-a meta-analysis of literature data. Plant and Soil 373, 583-594.
549 Lucchini, P., Quilliam, R.S., Deluca, T.H., Vamerali, T., Jones, D.L., 2014. Increased
550 bioavailability of metals in two contrasting agricultural soils treated with waste wood-
551 derived biochar and ash. Environmental Science and Pollution Research 21, 3230-3240.
552 Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 2011. Short-term soil priming
553 effects and the mineralisation of biochar following its incorporation to soils of different
554 pH. Soil Biology & Biochemistry 43, 2304-2314.
555 Lychuk, T.E., Izaurralde, R.C., Hill, R.L., McGill,W.B.,Williams, J.R., 2014. Biochar as a
556 global change adaptation: predicting biochar impacts on crop productivity and soil
557 quality for a tropical soil with the Environmental Policy Integrated Climate (EPIC)
558 model. Mitigation and Adaptation Strategies for Global Change 20, 1437-1458.

559 Marculescu, C., 2012. Comparative analysis on waste to energy conversion chains using
560 thermal-chemical processes. Energy Procedia 18, 604-611.
561 Marks, E.A.N., Alcañiz, J.M., Domene, X., 2014. Unintended effects of biochars on short-term
562 plant growth in a calcareous soil. Plant and Soil 385, 87-105.
563 McDermitt, D., Xu, L., Gracia, R., Madsen, R., Anderson, D., 2005. On equalizing pressure in
564 a soil respiration chamber with pressure in the ambient air under windy conditions,
565 Geophysical Research Abstracts, 7, 05841.
566 Merino, A., Omil, B., Fonturbel, M.T., Vega, J.A., Balboa, M.A., 2016. Reclamation of
567 intensively managed soils in temperate regions by addition of wood bottom ash
568 containing charcoal: SOM composition and microbial functional diversity. Applied Soil
569 Ecology 100, 195-206.
570 Miranda, K.M., Espey, M.G., Wink, D.A., 2001. A rapid, simple spectrophotometric method
571 for simultaneous detection of nitrate and nitrite. Nitric Oxide 5, 62-71.
572 MISR/SAC, 1985. Advisory soil analysis and interpretation. The Macaulay Institute for Soil
573 Research and Scottish Agricultural Colleges Bulletin 1, 1-13.
574 Mulvaney, R.L., Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N.,
575 Tabatabai, M.A., Johnston, C.T., Sumner, M.E., 1996. Nitrogen - inorganic forms.
576 Methods of soil analysis. Part 3-chemical methods. 1123-1184. Page, A.L., Miller, R. H.,
577 and Keeney, R. R. eds. American Society of Agronomy, Madison, WI, USA.
578 Murphy, J., Riley, J.P., 1962. A modified single solution method for the determination of
579 phosphate in natural waters. Analytica Chimica Acta 27, 31-36.
580 Quilliam, R.S., Marsden, K.A., Gertler, C., Rousk, J., DeLuca, T.H., Jones, D.L., 2012.
581 Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are
582 influenced by time since application and reapplication rate. Agriculture, Ecosystems &
583 Environment 158, 192-199.

584 Quilliam, R.S., Glanville, H.C., Wade, S.C., Jones, D.L., 2013a. Life in the 'charosphere' Does
585 biochar in agricultural soil provide a significant habitat for microorganisms? Soil Biology
586 & Biochemistry 65, 287-293.
587 Quilliam, R.S., DeLuca, T.H., Jones, D.L., 2013b. Biochar application rate reduces root
588 nodulation in clover but increases nitrogenase activity in nodules. Plant and Soil 366, 83-
589 92.
590 R Development Core Team, 2011. R: A language and environment for statistical computing.
591 R Foundation for Statistical Computing, Vienna, Austria.
592 Saarnio, S., Heimonen, K., Kettunen, R., 2013. Biochar addition indirectly affects N_2O
593 emissions via soil moisture and plant N uptake. Soil Biology & Biochemistry 58, 99-106.
594 Singh, B., Singh, B.P., Cowie, A.L., 2010. Characterisation and evaluation of biochars for their
595 application as a soil amendment. Australian Journal of Soil Research 48, 516-525.
596 Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration.
597 Soil Biology & Biochemistry 42, 2345-2347.
598 Subedi, R., Kammann, C., Pelissetti, S., Taupe, N., Bertora, C., Monaco, S., Grignani, C., 2015.
599 Does soil amended with biochar and hydrochar reduce ammonia emissions following the
600 application of pig slurry? European Journal of Soil Science 66, 1044-1053.
601 Subedi, R., Taupe, N., Ikoyi, I., Bertora, C., Zavattaro, L., Schmalenberger, A., Leahy, J.J.,
602 Grignani, C., 2016. Chemically and biologically-mediated fertilizing value of manure-
603 derived biochar. Science of the Total Environment 550, 924-933.
604 Sumner, M.E., Miller, W.P., 1996. Cation exchange capacity and exchange coefficients, In
605 Sparks, D.L. (ed.) Methods of Soil Analysis. Part 3. Chemical methods. SSSA Book
606 Series 5, Soil Science Society of America and American Society of Agronomy, Madison,
607 WI, USA.

van Laer, T., de Smedt, P., Ronsse, F., Ruysschaert, G., Boeckx, P.,Verstraete, W., Buysse, J., Lavrysen, L.J., 2015. Legal constraints and opportunities for biochar: a case analysis of EU law. Global Change Biology Bioenergy 7, 14-24.

Ventura, M., Alberti, G., Viger, M., Jenkins, J.R., Girardin, C., Baronti, S., Zaldei, A., Taylor, G., Rumpel, C., Miglietta, F., Tonon, G., 2014. Biochar mineralization and priming effect on SOM decomposition in two European short rotation coppices. Global Change Biology Bioenergy 7, 1150-1160.

Wardle, D. A, Nilsson, M.-C., Zackrisson, O., 2008. Fire-derived charcoal causes loss of forest humus. Science. 320, 629-629.

Watzinger, A., Feichtmair, S., Kitzler, B., Zehetner, F., Kloss, S., Wimmer, B., Zechmeister-Boltenstern, S., Soja, G., 2014. Soil microbial communities responded to biochar application in temperate soils and slowly metabolized ^{13}C-labelled biochar as revealed by ^{13}C PLFA analyses: results from a short-term incubation and pot experiment. European Journal of Soil Science 65, 40-51.

Yu, X.Y., Ying, G.G., Kookana, R.S., 2009. Reduced plant uptake of pesticides with biochar additions to soil. Chemosphere 76, 665-671.

Zimmerman, A.R., 2010. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). Environmental Science & Technology 44, 1295-1301.

Zimmerman, A.R., Gao, B., Ahn, M.-Y., 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biology & Biochemistry 43, 1169-1179.

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 ± SEM.

633 **Fig. 2** Diurnal variation of soil CO_2 flux with soil temperature under the biochar, wood ash and
634 control treatments. Temperature averaged for each cycle (n = 12), soil CO_2 flux averaged for
635 each block (n = 3)

636 **Fig. 3** Mineralization of (a) ^{14}C-labelled native SOM and (b) ^{14}C-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 $^{14}CO_2$ evolution ± SEM (n
639 = 4 for biochar and wood ash and n = 8 for control).