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Can biochar reduce soil greenhouse gas emissions from a Miscanthus bioenergy crop?

Running title: Biochar and Miscanthus soil GHG emissions

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1 Abstract

Energy production from bioenergy crops may significantly reduce greenhouse gas (GHG) emissions through substitution of fossil fuels. Biochar amendment to soil may further decrease the net climate forcing of bioenergy crop production, however this has not yet been assessed under field conditions. Significant suppression of soil nitrous oxide (N_2O) and carbon dioxide (CO_2) emissions following biochar amendment has been demonstrated in short-term laboratory incubations by a number of authors, yet evidence from long-term field trials has been contradictory. This study investigated whether biochar amendment could suppress soil GHG emissions under field and controlled conditions in a *Miscanthus X Giganteus* crop and whether suppression would be sustained during the first two years following amendment.

In the field, biochar amendment suppressed soil CO_2 emissions by 33% and annual net soil CO_2 equivalent (eq.) emissions (CO_2, N_2O and methane, CH_4) by 37% over two years. In the laboratory, under controlled temperature and equalised gravimetric water content, biochar amendment suppressed soil CO_2 emissions by 53% and net soil CO_2 eq. emissions by 55%. Soil N_2O emissions were not significantly suppressed with biochar amendment, although they were generally low. Soil CH_4 fluxes were below minimum detectable limits in both experiments.

These findings demonstrate that biochar amendment has the potential to suppress net soil CO_2 eq. emissions in bioenergy crop systems for up to two years after addition, primarily through reduced CO_2 emissions. Suppression of soil CO_2 emissions may be due to a combined effect of reduced enzymatic activity, the increased carbon-use efficiency from the co-location of soil microbes, soil organic matter and nutrients and the precipitation of CO_2

43 onto the biochar surface. We conclude that hardwood biochar has the potential to improve the
44 GHG balance of bioenergy crops through reductions in net soil CO_2 eq. emissions.

45

2 Introduction

The EU has a target for 20% of all energy to come from renewable sources by 2020 (The European Commission 2009). Bioenergy combustion currently makes up 2% of primary energy generation in the UK and is expected to increase to 8 - 11% of the UK's primary energy to help meet this 2020 target (Committee on Climate Change 2011; The Department of Energy and Climate Change 2012). The sustainability and greenhouse gas (GHG) balance of first-generation bioenergy crops has received considerable attention and criticism in the literature (Crutzen *et al.* 2007; Searchinger *et al.* 2008; Smeets *et al.* 2009; Whitaker *et al.* 2010). Second-generation bioenergy crop production is typically responsible for lower GHG emissions over its life cycle than first-generation bioenergy crops due to less intensive management practices (Hillier *et al.* 2009; Rowe *et al.* 2011). Nevertheless, methods to improve the sustainability of all bioenergy crop-types are being considered (Gopalakrishnan *et al.* 2009; Thornley *et al.* 2009).

One of the most promising biomass energy crops in the UK in terms of environmental sustainability is Miscanthus (*Miscanthus x Giganteus*) (Rowe *et al*., 2009; Whitaker *et al.* 2010). This crop is a perennial rhizomatous C_4 grass that is planted on approximately 13,500 ha of UK cropland (Don *et al.* 2012). Miscanthus requires minimal soil preparation and common management practices involve adding a relatively small amount of nitrogen (N), if any, during the first few years to benefit rhizome development. It is generally known that high yields are maintained after this period (Lewandowski *et al.* 2000; Rowe *et al.* 2009), although recent work suggests that additional N inputs in the fourth year could improve yields by 40% (Wang *et al.* 2012).

Biochar is a carbon (C)-rich substance produced from biomass and applied to soils. It is being promoted as a climate change mitigation tool as it has the potential to increase soil C

sequestration and reduce soil GHG emissions when applied as a soil amendment (Woolf *et al.* 2010). For this reason, combining bioenergy cultivation with biochar application to improve the GHG balance of bioenergy crops is an attractive proposition. Biochar is created by heating biomass in a low-oxygen environment (a process called pyrolysis, typically heated to between 350 and 600 °C). One option for biochar production is to produce it concurrently with energy (Laird *et al.* 2009).

Several life cycle assessments (LCAs) demonstrated that producing energy and biochar concurrently from biomass and subsequently applying the biochar to arable crop soil resulted in greater carbon abatement than producing energy alone from biomass or fossil fuel energy production (Gaunt & Lehmann 2008; Roberts *et al.* 2010; Hammond *et al.* 2011). Carbon abatement primarily consisted of increased soil stable carbon content (40 - 66%) and offsetting fossil fuel energy (14 - 48%). The remainder was attributed to indirect effects of biochar on the soil, such as increased fertiliser use efficiency, reduced soil GHG emissions and increased soil organic carbon (SOC) stocks. According to one LCA study, a 30% increase in SOC following biochar amendment would reduce net GHG emissions from small-scale bioenergy/biochar production by up to 60% (Hammond *et al.* 2011). Suppressed soil N_2O emissions of 25 – 50% contribute only 1.2 – 4.0% of the total emission reduction following biochar amendment (Roberts *et al.* 2010; Hammond *et al.* 2011). However, this figure may be an underestimate; one study on first generation biofuels has suggested that the conversion factor of newly-fixed N to N_2O production may be 3 – 5% as opposed to the default conversion factor from agricultural lands of 1% used by the Intergovernmental Panel on Climate Change (Crutzen *et al.* 2007).

It is important to fully understand the mechanisms by which biochar amendment to soil may affect soil C and N cycling in order to estimate soil GHG fluxes from such systems. Carbon dioxide (CO_2) emissions from soil organic matter (SOM) result from the mineralisation of

resident soil C and are strongly affected by soil temperature, the form and lability of soil C and soil moisture conditions (Rustad *et al.* 2000; Cook & Orchard 2008). Nitrous oxide (N_2O) from soil is produced via three primary pathways, nitrification, nitrifier denitrification and denitrification (Khalil *et al.* 2004; Wrage *et al.* 2005; Gillam *et al.* 2008). Nitrification is dominant under aerobic conditions, whereas under increasingly anaerobic conditions (e.g. at high water filled pore space, WFPS, > 70%), denitrification is the dominant pathway (Bateman & Baggs 2005). Nitrous oxide production is also constrained by temperature, inorganic-N content, pH and the form and concentration of labile C (Hofstra & Bouwman 2005).

We have found from previous work that soil CH_4 fluxes are negligible from this Miscanthus site (Case *et al.* 2012). Methane fluxes are mediated by processes known as CH_4 oxidation under aerobic and methanogenesis under anaerobic conditions, and are primarily affected by temperature, substrate availability and the form and content of organic matter (Castro *et al.* 1995; Le Mer & Roger 2001).

There is evidence to suggest that a co-benefit of biochar amendment is a reduction in soil CO_2 emissions (Lehmann *et al.* 2011), however there are few long-term studies available to support this. Those that exist are contradictory, with increased, decreased and variable effects observed (Kuzyakov *et al.* 2009; Major *et al.* 2009; Zimmerman *et al.* 2011). It is known that fresh biochar addition may add a large amount of labile C to the soil, therefore increasing soil CO_2 emissions. However, this is likely to be a short-term effect (Zimmerman *et al.* 2011). In the longer term, biochar is hypothesised to increase recalcitrant soil C and may even increase soil microbial biomass by agglomeration of SOM and nutrients onto the biochar surface (Lehmann *et al.* 2011). It is not yet clear whether this will lead to decreased or increased native soil C mineralisation in the long term (Lehmann *et al.* 2011; Spokas 2012). Biochar amendment may also reduce the activity of multiple C-mineralising enzymes, therefore

reducing soil CO_2 emissions (Jin 2010), although this has not yet been confirmed in a published study (Bailey *et al.* 2011).

Biochar is also hypothesised to have suppressive effects on soil N_2O emissions. This has been observed in short-term laboratory studies (Spokas & Reicosky 2009; Singh *et al.* 2010; Case *et al.* 2012), but has yet to be demonstrated in a long-term field study (e.g. Jones *et al.* 2012). Several studies have demonstrated that biochar amendment can modify soil physical properties, particularly by increasing the water holding capacity (WHC) and decreasing the bulk density (BD) of soil, leading to a reduced WFPS of soil with biochar amendment and therefore lower soil N_2O emissions (Van Zwieten *et al.* 2010; Karhu *et al.* 2011; Case *et al.* 2012). Also, in low inorganic-N soils, fresh biochar may immobilise significant amounts of inorganic-N, limiting the substrate available to soil nitrifiers and denitrifiers for N_2O production (Clough & Condron 2010; Taghizadeh-Toosi *et al.* 2011). Biochar amendment may also affect enzyme activity relevant to N_2O production (Anderson *et al.* 2011).

The authors have shown previously that biochar amendment significantly suppressed soil N_2O emissions from Miscanthus soils incubated under standardised conditions in short-term experiments (four months), but had no effect on soil CO_2 emissions (Case *et al.* 2012). The aims of this study were to investigate whether biochar amendment would significantly reduce soil GHG emissions from a Miscanthus crop under field conditions and over the long-term (up to two years from biochar amendment) and to determine the effect of biochar amendment on net soil CO_2 equivalent (eq.) emissions from Miscanthus soils.

To address these aims, we monitored GHG emissions from biochar-amended and un-amended soils in the field for two years. Given that changes in temperature and moisture over time will affect biochar-amended soils differently from un-amended soil, due to higher WHC (Case et al. 2012) and differing thermal properties (Genesio *et al.* 2012; Meyer *et al.* 2012),

144 we also investigated GHG fluxes from biochar-amended soils under standardised
145 environmental conditions (10 – 14 months after amendment). This was done to control for
146 environmental factors known to influence C and N cycling in soils (Reichstein *et al.* 2000;
147 Dobbie & Smith 2001; Cook & Orchard 2008). We hypothesised that under field and
148 standardised conditions, biochar amendment would suppress soil CO_2 and N_2O emissions and
149 net soil CO_2 eq. emissions. We also hypothesised that soil CH_4 fluxes would be too low to
150 detect any significant differences with biochar amendment.

151

3 Materials and Methods

3.1 Biochar and field site description

The biochar used in this study was the same as that used in Case *et al.* (2012). Briefly, biochar was produced from thinnings of hardwood trees (oak, cherry and ash, Bodfari Charcoal, UK). The feedstock was heated in a ring kiln, first to 180 °C to allow the release of volatile gases, and then to approximately 400 °C for 24 hours. The biochar was subsequently 'chipped' to achieve a post-production size of up to 15 mm. The biochar had a total C content of 72.3 ± 1.5 % (n = 3), a total N content of 0.71 ± 0.01 % (n = 3), an extractable NH_4^+ and NO_3^- content below detectable limits (< 1 mg kg^{-1} NH_4^+-N and < 1.3 mg kg^{-1} NO_3^--N, n = 3), a pH of 9.25 ± 0.04 (n = 4), a gravimetric moisture content (GMC) of 3.1 ± 0.4 % and a cation exchange capacity of 145 $cmol^+$ kg^{-1} (n = 1, analysed by ICP-OES). Further biochar properties are available in the supporting material of Case *et al.* (2012).

The field site used for this study was a Miscanthus plantation close to Lincoln, Lincolnshire, UK. Prior to Miscanthus planting in 2006, the field had followed a rotation of one year oilseed rape, three years wheat. The crop was planted at a density of 10,000 rhizomes ha^{-1} without N fertilisation during or subsequent to establishment (Drewer *et al.* 2012). The soil was a dense, compacted sandy loam with 53 % sand, 32 % silt and 15 % clay, a BD of 1.51 ± 0.02 g cm^{-3} (n = 10), chemical properties of which are shown in Fig. 1 (May 2010 control). The crop received no N fertiliser before or during the field experiment.

3.2 Effects of biochar on GHG fluxes in the field

Five random sampling blocks were established within the Miscanthus field in May 2010. In each of these blocks, three circular plots of 2 m diameter were created, at least 5 m apart, in between the Miscanthus shoots to prevent rhizome damage. In each block, one plot was an

175 un-mixed 'control' plot. Litter was removed from the remaining ten plots and the soil was
176 mixed to 10 cm depth using hand tools. Biochar was applied to the second plot at a rate of 49
177 t ha^{-1} and mixed into the top 0 - 10 cm using hand tools (amended), while the remaining plot
178 was also mixed to 10 cm but had no biochar applied (un-amended). Litter was then evenly re-
179 applied. To monitor soil GHG emissions from the field plots, PVC chamber collars were
180 permanently installed in the centre of each plot and pushed into the soil to a depth of 2 cm.
181 The chambers had an average height of 16 cm from the soil surface, an internal diameter of
182 39 cm and a headspace volume of 19 l. At the start of gas measurements, the chambers were
183 covered with a metal lid and connected to the chamber with metal bulldog clips. The lid
184 contained a central septum for gas collection and a plastic tube connected to a partially-filled,
185 open Tedlar bag (DuPont, USA) in order to equilibrate the chamber atmosphere with air
186 pressure changes outside of the chamber (Nakano *et al.* 2004). Headspace atmospheric
187 samples (10 ml, 0.05% of the total chamber headspace volume) were taken at 0, 10, 20 and
188 30 minutes following enclosure and injected into 3 ml gas-tight sample vials (Labco, UK)
189 using the static chamber method (Livingston & Hutchinson 1995).

190 Soil temperature was monitored in each plot with a Tiny Tag temperature logger with integral
191 stab probe (Gemini Data Loggers, UK) and volumetric soil moisture content (VMC, 0 – 6 cm
192 depth) was measured using a hand-held ML2x Theta Probe (Delta T Devices, UK). The
193 probes were calibrated by creating a linear calibration of measured VMCs from un-amended
194 and amended soil at a range of known GMCs (from 15 – 35%, supporting information).
195 Volumetric moisture contents were converted into GMC using soil BD measurements from
196 May 2012 (Fig. 1). Further environmental conditions at the field site (air temperature,
197 rainfall, Fig. 2) were obtained through the British Atmospheric Data Centre, using data from
198 a Met Office weather station situated 2 km away from the field site (Natural Environment
199 Research Council 2012; The Met Office 2012).

Soil samples were taken to 10 cm depth. Before biochar amendment to the field plots in May 2010, soil samples were taken from the five control plots. In March 2011, three soil samples were taken from each of the five un-amended and amended field plots and in May 2012 one soil sample was taken from each of the control, un-amended and amended plots. Soil samples were analysed for soil pH, extractable NH_4^+ and NO_3^-, total C and N, GMC and BD. All were frozen at - 20 °C for up to four weeks until analysis apart from for GMC and BD, for which analysis was conducted immediately. Water filled pore space was calculated from the GMC at each time point and the BD of the soil from May 2012 (two years after amendment), using a particle density of 2.65 g cm^{-3} (Ohlinger 1995).

3.3 Effect of biochar on GHG fluxes under controlled conditions 10 - 14 months after amendment

In order to assess the effects of biochar on soil GHG fluxes, soil cores were collected from the field plots in March 2011, ten months after biochar application. Two intact soil cores were taken from each of the five amended and un-amended plots following the same procedure described in Case *et al.* (2012). PVC pipes (W 102 mm, H 215 mm) were inserted into the soil as deep as possible using hand tools (150 – 180 mm) and excavated from the surrounding soil. The soil cores were stored at 4 °C for 40 days following collection, then placed at 16 °C (mean soil temperature of the field site June - September 2009) in the dark for three days before gas sampling to allow any initial flush of soil CO_2 emissions induced by warming to pass (Reichstein *et al.* 2000). Soil cores were maintained at field moist conditions (23 % GMC) for the duration of the experiment. The chosen soil GMC was based on the mean monthly soil VMC measured directly at the site over one year (Feb 2009 to Feb 2010). Surplus water was allowed to drain into a removable container on the base of the core, which was airtight when connected to the rest of the apparatus.

To analyse soil GHG fluxes, headspace gas samples were taken (10 ml, 1% of the chamber headspace volume of 0.9 l) and injected into 3 ml sample vials (Labco, USA) using the unvented static enclosure method (Livingston & Hutchinson 1995). The headspace atmosphere was sampled at 0, 20, 40 and 60 minutes following enclosure. Details regarding headspace design are available in Case *et al.* (2012). Gas samples were taken from all soil cores at seven time points, at day 4, 17, 31, 46, 67, 116 and 120. After the final gas sampling, the soil cores were stored at 4 °C and soil samples were collected within four days (10 cm depth). Soil samples were homogenised and analysed for soil pH, extractable NH_4^+, NO_3^-, total C and N. Soil samples were frozen at – 20 °C for up to four weeks until analysis.

3.4 Soil chemical and physical analyses

Soil pH was determined using deionised water (soil/biochar:H_2O, 1:2.5 w:v), using a Kent-Taylor combination pH electrode (Asea Brown Boveri, Switzerland) (Emmett *et al.* 2008). Soil NH_4^+ and NO_3^- were extracted using 0.8 M (6%) potassium chloride (KCl), and analysed on a Seal AQ2 discrete analyser (Bran and Luebbe, UK) using discrete colorimetric procedures (Maynard & Kalra 1993). Total C and N content of 0.1 g oven-dried soil (from a 5 g sample ground and sieved to < 2 mm) was analysed on a LECO Truspec total CN analyser (LECO, USA) with an oven temperature of 950 °C (Sollins *et al.* 1999). Gravimetric moisture content and BD were conducted according to standard methods (Ohlinger 1995; Emmett *et al.* 2008) and soil WFPS derived from these values as described in Section 3.2.

3.5 Headspace gas analyses

Two different gas chromatograph (GC) systems were used to analyse headspace GHG concentrations. For the first year of the field experiment, CO_2 and CH_4 concentrations were analysed on a PerkinElmer Autosystem GC (PerkinElmer, USA) fitted with two flame ionization detectors (FID) operating at 130 (FID alone) and 300 °C (FID with methaniser)

248 respectively. Nitrous oxide concentrations were analysed on a PerkinElmer Autosystem XL
249 GC using an electron capture detector (ECD) operating at 360 °C. Both GCs contained a
250 stainless steel Porapak Q 50 - 80 mesh column (length 2 m, outer diameter 3.17 mm),
251 maintained at 100 °C and 60 °C for the CO_2/CH_4 and N_2O GCs respectively. For the second
252 year of the field experiment and the laboratory experiment, concentrations of N_2O, CO_2 and
253 CH_4 were analysed on a PerkinElmer Autosystem XL GC. The GC was fitted with an FID
254 with methaniser operating at 300 °C and an ECD operating at 360 °C. The same column was
255 used for this GC as described above, maintained at 60 °C.

256 Results were calibrated against certified gas standards (Air Products, UK). The minimum
257 detection limits (MDLs) of the GC systems were calculated based on chamber deployment
258 time, number of samples taken per hour and the analytical precision of the instrument (co-
259 efficient of variation %) following Parkin & Venterea (2010). The MDLs were 6.7 CO_2-C mg
260 m^{-2} h^{-1}, 8.0 μg CH_4-C m^{-2} $h^{-1}$and 12.4 μg N_2O-N m^{-2} h^{-1} for the field experiment and 3.7 mg
261 CO_2-C m^{-2} h^{-1}, 4.4 μg CH_4-C m^{-2} h^{-1} and 8.6 μg N_2O-N m^{-2} h^{-1} for the laboratory experiment.
262 Headspace gas fluxes were calculated from the linear flux of CO_2, N_2O or CH_4 concentration
263 in the chamber headspace following enclosure according to the approach of Holland *et al.*
264 (1999). We used the linear accumulation of headspace CO_2 concentrations to eliminate vials
265 from analysis that had their air-tightness compromised during sampling or subsequent
266 storage. We found that CH_4 fluxes from the soil were below the MDL of the GC throughout
267 both experiments, and N_2O fluxes were below the MDL except for the first gas sampling time
268 point in the field (June 2010). Regardless of whether fluxes were below the MDL or not, we
269 used them in subsequent analysis (Sjögersten & Wookey 2002; McNamara *et al.* 2008).

270 Nitrous oxide and CH_4 fluxes were converted into net soil CO_2 eq. emissions using the global
271 warming potential over a 100 year period of 298 (N_2O) and 25 (CH_4) given by Solomon *et al.*
272 (2007). Net soil CO_2 eq. emissions per year (kg CO_{2eq} ha^{-1} yr^{-1}) were derived by calculating

273 the mean daily GHG flux of the un-amended and amended treatments over the two-year time
274 period, and multiplying this value by 365 days. Laboratory experiment conditions were
275 representative only of field conditions in summer. Therefore, to compare net soil CO_2 eq.
276 emissions from the field and laboratory experiment, we converted fluxes into kg CO_{2eq} ha^{-1}
277 $summer^{-1}$, where 'summer' was defined as the length of the summer months (92 days, the
278 number of days in June, July and August).

279 **3.6 Statistical analyses**

280 Statistical analyses were conducted using R version 2.15.2 (The R Project 2012). Data
281 exploration was conducted following the procedure in Zuur *et al.* (2010a). Linear mixed-
282 effects models were run using NLME package version 3.1-105, with GHG fluxes, GMC or
283 WFPS as the response variable and 'plot' or 'soil core' as the random factor for the field and
284 laboratory experiments respectively. The models were refined taking into account
285 independent variable heterogeneity and correlation, and validated following the guidance
286 provided in Zuur *et al.* (2010b).

287 T-test comparisons were used for chemical and physical soil properties and the comparison of
288 soil N_2O fluxes from un-amended and amended plots at the first time point in the field.
289 Levene's test was initially used to determine whether there was a significant difference in
290 response variable variance for the un-amended and amended soil. If a significant difference
291 was found ($p < 0.05$), we used Welch's t-test for unequal variances; otherwise an unpaired,
292 two-sample t-test was used.

293

4 Results

4.1 Effects of biochar on soil GHG fluxes in the field

Over the two year measurement period, soil CO_2 emissions were significantly lower with biochar amendment ($p < 0.05$, Table 1). Mean soil CO_2 emissions in the un-amended plots were 43.2 ± 5.5 compared with 28.8 ± 3.4 mg CO_2-C m^{-2} h^{-1} in the amended plots, a suppression of 33% (Fig. 2, n = 37). At times of lower soil temperature, soil CO_2 fluxes were low ($p < 0.001$, Table 1); in winter and spring of 2011 and 2012, both un-amended and amended plots emitted less than 20 mg CO_2-C m^{-2} h^{-1} (Fig. 2).

Soil N_2O emissions were 216.4 ± 80.8 in un-amended soil compared with 41.8 ± 24.1 μg N_2O-N m^{-2} h^{-1} at the first time point in the field (June 2010, Fig. 2, n = 5). Although soil N_2O emissions were lower in biochar-amended soils, at the first time point, this result was not significant (two-sample t-test, t = 2.2, df = 8.0, $p > 0.05$). Nitrous oxide fluxes were very much lower thereafter, with a mean of 0.4 ± 1.9 and 1.8 ± 2.0 N_2O-N μg m^{-2} h^{-1} (n = 33, Fig. 2) for the un-amended and amended treatments respectively. Soil CH_4 fluxes were below MDL throughout the experiment, with an overall average of -1.2 ± 3.6 and 5.2 ± 4.4 CH_4-C μg m^{-2} h^{-1} respectively for the un-amended and amended treatments (n = 37).

Net soil CO_2 eq. emissions were reduced by 37% with biochar amendment (averaged over 2 years, Table 2). In un-amended soils, 8% of net soil CO_2 eq. emissions came from N_2O emissions while for the amended plots, 3% came from N_2O emissions (Table 2). High N_2O emissions contributed disproportionately to net soil CO_2 eq. emissions in June 2010 compared to the other months of the measurement period, contributing 26% of net soil CO_2 eq. emissions for un-amended soil compared with 11% for amended soil (Table 2). When this time point was removed from the dataset (June 2010), the contribution of N_2O fluxes to net

317 soil CO_2 eq. emissions over two years reduced to 0.1 and 0.9% in un-amended and amended
318 soil respectively (Table 2). In the summer of 2010 and 2011, biochar amendment to soil
319 suppressed net soil CO_2 eq. emissions by 55% and 41% respectively (Table 2).

320 Monitoring of soil physical properties for two years revealed that biochar amendment did not
321 significantly affect soil GMC (Fig. 2, Table 1). Soil GMC in both treatments was higher at
322 times of lower soil temperature ($p < 0.001$, Table 1). Biochar amendment significantly
323 decreased soil BD. For example, 24 months after amendment (May 2012) BD was reduced
324 from 1.62 ± 0.07 g cm^{-3} to 1.35 ± 0.07 g cm^{-3} ($n = 5$, $p < 0.05$, Fig. 1, Table 3). Soil WFPS
325 over the two years was reduced with biochar amendment ($p < 0.05$, Fig. 2, Table 1).

326 Biochar amendment significantly affected soil chemical properties. Ten months after
327 amendment (March 2011), biochar-amended soils had significantly higher total C content,
328 CN ratio and pH relative to un-amended soils ($p < 0.001$, $p < 0.001$, $p < 0.01$, Fig. 1, Table 3,
329 $n = 15$). Soil total N, NH_4^+ and NO_3^- contents were not significantly affected by biochar
330 amendment at any time point ($p > 0.05$, Fig. 1, Table 3, $n = 15$).

331 **4.2 Effects of biochar on soil GHG fluxes under controlled conditions**

332 During a four-month laboratory incubation under controlled environmental conditions (10
333 months after biochar amendment to the field), biochar amendment had significant effects on
334 soil GHG emissions. Averaging over the 120 days, biochar amendment significantly
335 decreased soil CO_2 emissions by 53%, from 30.2 ± 2.1 to 14.1 ± 1.5 mg CO_2-C m^{-2} h^{-1} ($p <$
336 0.001, Table 4, Fig. 3, $n = 41$). Carbon dioxide emissions also decreased significantly with
337 time in biochar-amended and un-amended soils ($p < 0.001$, Table 4). Biochar amendment had
338 no significant effect on soil N_2O fluxes ($p > 0.05$, Table 3). Nitrous oxide emissions from soil
339 cores were generally low, on average 20.3 ± 6.4 compared to 5.8 ± 1.4 N_2O-N μg m^{-2} h^{-1} in
340 the un-amended and amended soil cores respectively (Fig. 3, $n = 41$). Methane fluxes from

341 soil cores were similarly low, on average 0.3 ± 1.1 compared to 1.8 ± 1.3 CH_4-C μg m^{-2} h^{-1} in
342 the un-amended and amended soil cores respectively (n = 41). Biochar amendment reduced
343 net soil CO_2 eq. emissions by 55% (Table 2). Nitrous oxide fluxes contributed 8% and 5% to
344 net soil CO_2 eq. emissions for the un-amended and amended soils respectively over the whole
345 experiment (Table 2). Biochar amendment had no significant effect on soil chemical
346 properties (Fig. 4, Table 5, n = 5).

347

5 Discussion

Suppression of soil GHG emissions from Miscanthus soils due to biochar amendment has been shown previously in short-term experiments by the authors, conducted under controlled-environment conditions (Case *et al.* 2012). The aim of this present study was to investigate whether the suppressive effect of biochar amendment would be detected under field conditions over a longer time period of two years. In addition, to control for environmental factors known to influence C and N cycling in soils, we monitored GHG fluxes from field-amended soil under controlled "summer" conditions (constant temperature and GMC). We have demonstrated that biochar amendment may have the potential to reduce net soil CO_2 eq. emissions from a Miscanthus crop soil. Over 2 years in the field, soil CO_2 emissions were suppressed by 33% on average and net soil CO_2 eq. emissions were 37% lower with biochar amendment. In the summer, biochar amendment reduced net soil CO_2 eq. emissions in the field by 55 and 41% in 2010 and 2011 respectively. In a four-month laboratory incubation under controlled "summer" conditions the effect was similar; net soil CO_2 eq. emissions were reduced by an average of 55%.

In the few long-term studies published (almost all in non-bioenergy crops), biochar amendment has been shown to suppress or have negligible effects on soil CO_2 emissions, with a few notable exceptions (Wardle *et al.* 2008; Major *et al.* 2009; Spokas 2012). There are several theories to explain why biochar amendment to soil may decrease soil CO_2 emissions. It has been hypothesised that biochar may increase microbial biomass in soil by the complexation of SOM with biochar particles and yet simultaneously induce 'negative priming' of native soil carbon mineralisation (Liang *et al.* 2010; Woolf & Lehmann 2012). The agglomeration of SOC on the biochar surface may result in a co-location of substrate, nutrients and micro-organisms and therefore promote greater C-use efficiency by the

372 microbial community (Lehmann *et al.* 2011). Also, biochar amendment may reduce the
373 activity of carbohydrate-mineralising enzymes such as glucosidase and cellobiosidase and
374 increase the activity of others such as alkaline phosphatase (Jin 2010). However, the effect of
375 biochar on soil enzyme activity is reported to be highly variable due to reactions between at
376 least one type of biochar (switchgrass) and the target substrate (Bailey *et al.* 2011).

377 Abiotic reactions may also contribute to the suppression of soil CO_2 emissions. Soil-derived
378 CO_2 may precipitate onto the biochar surface as carbonates, aided by the high pH of the
379 biochar and high content of alkaline metals (Joseph *et al.* 2010; Lehmann *et al.* 2011). The
380 biochar used in this study had a high pH and relatively high content of alkaline metals
381 compared to other biochars (supporting information, Case *et al.* (2012)) and may therefore
382 have caused significant precipitation onto the biochar surface. We conclude that a
383 combination of the biotic and abiotic mechanisms mentioned above may explain the
384 suppression of soil CO_2 emissions observed during this study.

385 It has been shown in forest ecosystems that low soil inorganic-N content may limit soil C
386 mineralisation and resulting soil respiration (Norby *et al.* 2010). The Miscanthus soil in our
387 study was initially very low in inorganic-N and this was unaffected by biochar amendment,
388 indicating that biochar did not increase soil inorganic-N immobilisation. This is contrary to
389 published data from other studies (van Zwieten *et al.* 2010; Dempster *et al.* 2012; Case *et al.*
390 2012). Based on this finding, we cannot explain lower soil CO_2 emissions by an effect of
391 biochar amendment on N immobilisation.

392 Soil CO_2 emissions consist of both soil and root respiration (Sulzman *et al.* 2005). It is
393 possible that biochar additions in the field may have affected the growth of Miscanthus above
394 and below ground, feeding back into effects on root respiration. Whilst we did not directly
395 measure the yield of the Miscanthus shoots surrounding the field plots, we did not observe

396 any difference in shoot height from visual observation. Although the 2 m diameter field plots
397 were placed entirely in between the Miscanthus where no shoots were growing, it is certain
398 that the root system of the Miscanthus was present underneath the plots. Soil CO_2 emissions
399 from control (un-mixed) plots in the field were not significantly different from un-amended
400 (mixed) plots over the course of the two-year field study (data not shown), indicating that
401 mixing the soil did not significantly affect root activity or growth.

402 Biochar amendment could reduce root respiration either by reducing root activity or growth,
403 or by killing existing roots. In the laboratory using soil collected 10 months after biochar
404 amendment, we observed suppression of soil CO_2 emissions with biochar amendment despite
405 the absence of live roots, indicating that differences in live root activity could not explain the
406 suppression of soil CO_2 emissions. It is possible that biochar amendment may have
407 significantly reduced root growth and/or increased root necromass underneath the plots in the
408 10 months following amendment. However, we are not aware of any specific mechanism to
409 explain why biochar would reduce root growth or kill roots apart from increased nutrient
410 limitation, which was not an issue in our study (Lehmann *et al.* 2011), or the presence of
411 toxic substances on the biochar itself, which we have shown in a previous study not to be the
412 case with this biochar (Case *et al.* 2012). The evidence therefore suggests that biochar
413 amendment did not significantly affect root growth or activity in this study.

414 Soil CO_2 emissions in the field were unexpectedly low in May 2011 and May 2012 compared
415 to other months of relatively high soil temperature (Fig. 2). Low soil CO_2 emissions of
416 similar magnitude were observed on the same day at the field site (Bottoms, Robertson, pers.
417 comm.). This may be explained by the fact that our May samplings occurred less than one
418 month following the annual Miscanthus harvest, a time when there is likely to be minimal
419 contribution from plant/root respiration as plant shoots have not yet emerged from the soil.

In both the field and the laboratory experiment, soil WFPS was lower with biochar amendment. However, as soil WFPS with biochar amendment was closer to the ideal range for soil CO_2 emissions (above 60%), we conclude that the physical effects of biochar amendment on the soil do not explain the suppression of soil CO_2 emissions (Linn & Doran 1984). Biochar amendment increased soil pH 10 months after amendment. However, as pH levels were close to seven in both the un-amended and amended soils and were not significantly different 14 or 24 months after amendment, we cannot say conclusively that increased pH due to biochar amendment can explain lower soil CO_2 emissions.

Our observations of reduced soil CO_2 emissions following biochar addition are particularly relevant within the context of the overall GHG balance of bioenergy crops. If lower soil CO_2 emissions were to continue into the long-term, there would be a relative increase in SOC in amended compared to un-amended soil. The authors of one LCA study concluded that if there is no change in SOC stocks following biochar amendment then biochar production gives only a small carbon abatement benefit compared to gasification, whereas an increase in SOC makes pyrolysis look favourable in terms of carbon abatement (Hammond *et al.* 2011). According to their sensitivity analysis, if a finding of a suppression of soil CO_2 emissions of 30% were continued into the future within a small-scale biochar-production system, net GHG emissions from the system could be reduced by up to 60%. However, two years is too short a time to say with confidence whether this will be the case in the Miscanthus system that we have investigated as a part of this study.

In the field, soil N_2O emissions one month after amendment (June 2010) were high in the un-amended soils, and whilst N_2O emissions from biochar-amended plots were lower, the suppression was not significant. Soil N_2O fluxes were low in all treatments thereafter from September 2010 to May 2012 and in laboratory-incubated soils. Soil N_2O fluxes are highly variable temporally and a large proportion of emissions occur in ‘bursts’ following wetting or

N-fertilisation events, which increase soil denitrifier activity (Dobbie & Smith 2001; Sänger *et al.* 2010). High soil N_2O emissions at this field site in June 2010 have been corroborated by other researchers and may be explained by rainfall on the sampling day (Bottoms 2012, Fig. 2). With the exception of the June 2010 sampling, the timing of gas sampling did not occur shortly following topsoil saturation from a rain event, therefore denitrifier activity was not stimulated.

We found that soil N_2O emissions were highly variable and were a relatively minor component of net soil CO_2 eq. emissions, which is in agreement with other published data from the same field site (Drewer *et al.* 2012).

Considering only un-amended field plots, soil N_2O emissions contributed only 8% to net soil CO_2 eq. emissions on an annual basis, compared to 2% from Drewer *et al.* (2012).We found that N_2O production during the summer season were larger; in the field in 2010, 1.75 ± 0.65 g N_2O m^{-2} $summer^{-1}$ was emitted from un-amended soil and 0.02 ± 0.02 g N_2O m^{-2} $summer^{-1}$ in 2011, while Drewer *et al.* (2012) found that overall N_2O production to be 0.014 g N_2O m^{-2} $summer^{-1}$. In the laboratory, we found that N_2O fluxes were 0.16 g N_2O m^{-2} $summer^{-1}$ in un-amended soil. In this present study, we used a similar gas sampling technique to that of Drewer *et al.* (2012). We cannot explain why soil N_2O fluxes in our study were higher than that of Drewer *et al.* (2012). Nevertheless, we conclude that soil N_2O emissions are a relatively minor component of net soil CO_2 eq. emissions from Miscanthus soil. To support this further, LCAs of biochar/bioenergy production reported that suppression of soil N_2O emissions following biochar amendment was a relatively minor constituent of potential climate forcing, even in arable crop systems (Roberts *et al.* 2010; Hammond *et al.* 2011).

We return to the central question that underlies this study: can biochar reduce net soil CO_2 eq. emissions from a Miscanthus energy crop? Assuming that Miscanthus crops are managed

469 with minimal inorganic-N addition and that hardwood-derived biochar produced by slow-
470 pyrolysis is applied to the soil in significant quantities (~ 50 t ha^{-1}), we conclude that biochar
471 amendment may have the potential to reduce net soil CO_2 eq. emissions from Miscanthus
472 soils through the reduction of soil CO_2 emissions. This is particularly relevant when
473 considering the overall GHG balance of bioenergy/biochar production, where reduced soil
474 CO_2 emissions over the long term and the resulting increase in SOM content has been
475 identified as one of the most significant factor influencing the sustainability of combined
476 bioenergy/biochar production (Hammond *et al.* 2011).

477 Future research should consider that the effect of biochar amendment on climate abatement in
478 Miscanthus crop systems may be different to that of biochar in arable systems, particularly
479 when taking into account the low nutrient status of Miscanthus crop soil. A key research
480 priority should be to investigate the effects of biochar amendment on the overall GHG
481 balance of bioenergy/biochar production systems on a range of soil types in order to assess
482 the global warming potential of the Miscanthus system with and without biochar amendment.
483 We have observed suppression of soil CO_2 emissions with biochar amendment, however, use
484 of eddy covariance techniques would enable the effects of biochar amendment on net
485 ecosystem exchange to be estimated, providing additional information on the effects of
486 biochar on C exchange within the crop/soil and atmosphere. Also, the mechanisms
487 underlying the suppression of soil CO_2 emissions should be further investigated over the long
488 term, such as the effect of biochar on the activity of CO_2-producing soil enzymes, the
489 increased carbon-use efficiency from the co-location of soil microbes, soil organic matter and
490 nutrients and the precipitation of soil-derived CO_2 onto the biochar surface as carbonates.

491

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501

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685 7 Supporting information

686 The supporting information file contains two figures, S5 and S6, which show the calibration
687 lines used to convert field-experiment soil volumetric moisture content into gravimetric
688 moisture content in un-amended and amended soil respectively.

8 Tables

Table 1: Variables affecting carbon dioxide (CO_2) fluxes, soil gravimetric moisture content (GMC) and Water Filled Pore Space (WFPS) in Miscanthus field plots, either un-amended or amended with biochar, over two years of seasonal measurements. Data outputs presented are those from refined linear mixed-effects models using plot as the random factor and accounting for independent variable heterogeneity where necessary following the procedure in Zuur *et al.,* (2010). n = 5. Symbols indicate p-value significance of the term: - = not present in refined model, * = p < 0.05, ** = p < 0.01,*** = p < 0.001. Refer to Fig. 2 for the data underlying these statistical outputs.

Response variable	**Independent variable**							
	Biochar		**WFPS**		**Soil**		**Biochar * Soil**	
	t	**p**	**t**	**p**	**t**	**p**	**t**	**p**
Soil N_2O emissions	-1.52	ns	-1.01	ns	-0.14	ns	0.36	ns
Soil CO_2 emissions	2.29	*	-	-	10.25	***	-4.06	***
Soil CH_4 emissions	-	-	-	-	-	-	-	-
Total CO_2 equivalent emissions	2.50	*	-	-	9.45	***	-3.66	***
GMC	-2.06	ns	-	-	-5.85	***	1.77	ns
WFPS	-3.15	*	-	-	-3.38	**	1.70	.

696 Table 2: The effect of biochar amendment on net soil CO_2 equivalent emissions from field plots or soil cores placed under controlled
697 environmental conditions. Mean CO_2 equivalent emissions were calculated from the mean soil GHG emissions sampled during the period
698 specified by the 'Sample dates included' column, and mean CO_2 equivalent production was calculated by multiplying this value by the number
699 of days specified by the column 'Time Period'. The time period 'Year' indicates 365 days, while 'Summer' indicates 92 days (the number of
700 days in June, July and August). The sample date 'Lab incubation' indicates that gas sampling data was used from the whole 120-day laboratory
701 incubation (Fig. 3). Data indicate mean, SE indicates ± standard error, n = 5.

Experiment	Time period	Sample dates included	Biochar treatment	Mean CO_2 equivalent emissions (net soil $CO_{2eq.}$ µg m^{-2} h^{-1})	SE	Mean CO_2 equivalent production over time period (net soil $CO_{2eq.}$ t ha^{-1} time $period^{-1}$)	SE	Number of samples in calculations
Field	Year	2010-2012	Un-amended	172.2	23.5	15.0	2.4	37
	Year	2010-2012	Amended	108.9	13.0	9.5	1.3	37
Field	Year (without first measurement)	2010-2012	Un-amended	137.3	20.0	12.0	1.8	33
	Year (without first measurement)	2010-2012	Amended	100.8	13.8	8.8	1.3	32
Field	Summer	2010/2011	Un-amended	289.4	43.1	6.4	1.2	10
	Summer	2010/2011	Amended	138.3	16.1	3.1	0.5	9
Field	Summer	2010	Un-amended	395.1	51.5	8.7	1.9	5
	Summer	2010	Amended	175.9	16.3	3.9	0.7	4
Field	Summer	2011	Un-amended	183.6	11.2	4.1	0.3	5
	Summer	2011	Amended	108.2	16.2	2.4	0.4	5
Laboratory	Summer	Lab incubation	Un-amended	120.2	9.7	2.7	0.2	45
	Summer	Lab incubation	Amended	54.6	6.0	1.2	0.1	41

702

703 Table 3: The effect of biochar amendment on physico-chemical properties of soils sampled 10 months (March 2011, also day 0 of laboratory
704 experiment) and 24 months (May 2012) after biochar addition to field plots (0 – 10 cm depth). Variability between the two groups was
705 determined with Levene's test, the resulting outputs in the table are either from two-sample t-tests for equal variance (Levene's test $p > 0.05$), or
706 Welch's t-test for unequal variance (Levene's test $p < 0.05$). n = 14 for un-amended, n = 15 for amended samples (3 replicates per plot). Symbols
707 indicate the p-value significance of the term: ns = not significant, * = < 0.05, ** = < 0.01, *** = < 0.001. Refer to Fig. 1 for the data underlying
708 these statistical outputs.

Response variable	10 months after amendment			24 months after amendment		
	t	df	p	t	df	p
Total C	-4.20	18.7	***	-1.46	8.0	ns
Total N	1.78	26.0	ns	-1.38	8.0	ns
CN ratio	-4.86	18.7	***	-1.59	4.1	ns
NH_4^+	-0.73	8.0	ns	-0.73	8.0	ns
NO_3^-	0.04	27.0	ns	-1.42	8.0	ns
pH	-2.81	27.0	**	0.26	8.0	ns
Bulk density	- 4.01	18	***	2.31	8.0	*

709

710 Table 4: The effect of biochar amendment and incubation time on greenhouse gas fluxes from soil cores incubated under controlled
711 environmental conditions. 'Time' represents the number of days from the start of the laboratory experiment. Data outputs presented are those
712 from refined linear mixed-effects models using plot as the random factor and accounting for independent variable heterogeneity where necessary
713 following the procedure in Zuur *et al.* (2010). Symbols indicate the p-value significance of the term: - = not present in refined model, ns = not
714 significant, * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$. Refer to Fig. 3 for the data underlying these statistical outputs.

Response variable	Independent variable					
	Biochar		Time		Biochar * Time	
	t	p	t	p	t	p
Soil N_2O emissions	0.86	ns	-	ns	-	ns
Soil CO_2 emissions	2.83	*	-	***	-	-
Soil CH_4 emissions	-	-	-	-	-	-
Total CO_2 equivalent emissions	2.68	*	-	**	-	-

715

716 Table 5: The effect of biochar amendment on soil chemical properties (0 - 10 cm) at the end of a four-month laboratory incubation. Variability
717 between the two groups was determined with Levene's test, the resulting outputs in the table are either from two-sample t- tests for equal
718 variance (Levene's test $p > 0.05$), or Welch's t-test for unequal variance (Levene's test $p < 0.05$). Symbols indicate the p-value significance of
719 the term: ns = not significant. Refer to Fig. 4 for the data underlying these statistical outputs.

Response variable	t	df	p
Total C	- 1.48	8.0	ns
Total N	- 1.45	8.0	ns
CN ratio	- 1.25	8.0	ns
NH_4^+	1.17	8.0	ns
NO_3^-	1.76	8.0	ns
pH	- 0.50	8.0	ns

9 Figure legends

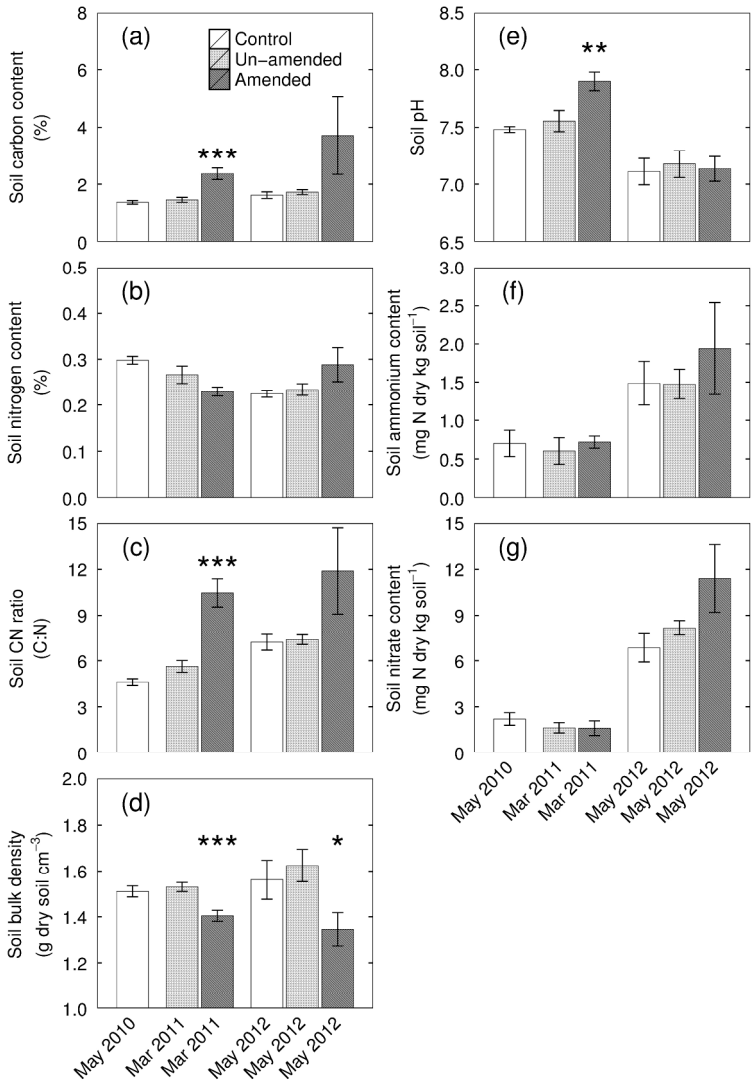
Fig. 1. The effect of biochar amendment on physico-chemical properties of soil (0 – 10 cm depth) taken from un-mixed control plots in May 2010 (n = 5), and from un-amended and amended plots 10 months (March 2011, n = 15, 3 replicates per plot) and 24 months after biochar addition in (May 2012, n = 5): soil (a) total C content (%); (b) total N content (%); (c) CN ratio; (d) pH; (e) ammonium content; (f) nitrate content and (g) bulk density. Bar plots represent mean ± standard error (n = 5). Annotations above bars indicate significant difference between un-amended and amended soil cores at the same time point: ** = $p < 0.01$, *** = $p < 0.001$. Statistical model outputs underlying these results are presented in Table 3.

Fig. 2. The effect of biochar amendment on soil fluxes of (a) N_2O and (b) CO_2 from Miscanthus field plots (June 2010 - May 2012), and environmental conditions (c-e) over the same period: (c) soil temperature and daily maximum air temperature (^oC); (d) soil gravimetric moisture content (%) and cumulative daily rainfall (mm day^{-1}); and (e) soil water-filled pore space (%). Arrow indicates time of soil core collection for the laboratory incubation (30th March 2011). The horizontal dotted line in graph (a) indicates 0. The † symbol indicates missing probe values due to the soil being too dry to analyse (replaced with assumed 18 % volumetric moisture content for both treatments). Data points represent mean ± standard error (n = 5). Statistical model outputs underlying these results are presented in Table 1.

Fig. 3. The effect of biochar amendment on soil fluxes of (a) N_2O, (b) CO_2 and (c) the controlled WFPS of Miscanthus soil cores incubated in the laboratory. Soil cores were collected from field plots 10 months after biochar addition (30th March 2011). Data points

represent mean ± standard error (n = 5). Statistical model outputs underlying these results are presented in Table 4.

Fig. 4. The effect of biochar amendment on physico-chemical properties of soil cores (0 – 10 cm depth) taken from un-amended and amended cores at the end of the four-month laboratory experiment (n = 5): soil (a) total C content (%); (b) total N content (%); (c) CN ratio; (d) pH; (e) ammonium content; and (f) nitrate content. Bars represent mean ± standard error (n = 5). Statistical model outputs underlying these results are presented in Table 5. Pre-laboratory experiment chemical and physical data are presented in Fig. 1 (March 2011).



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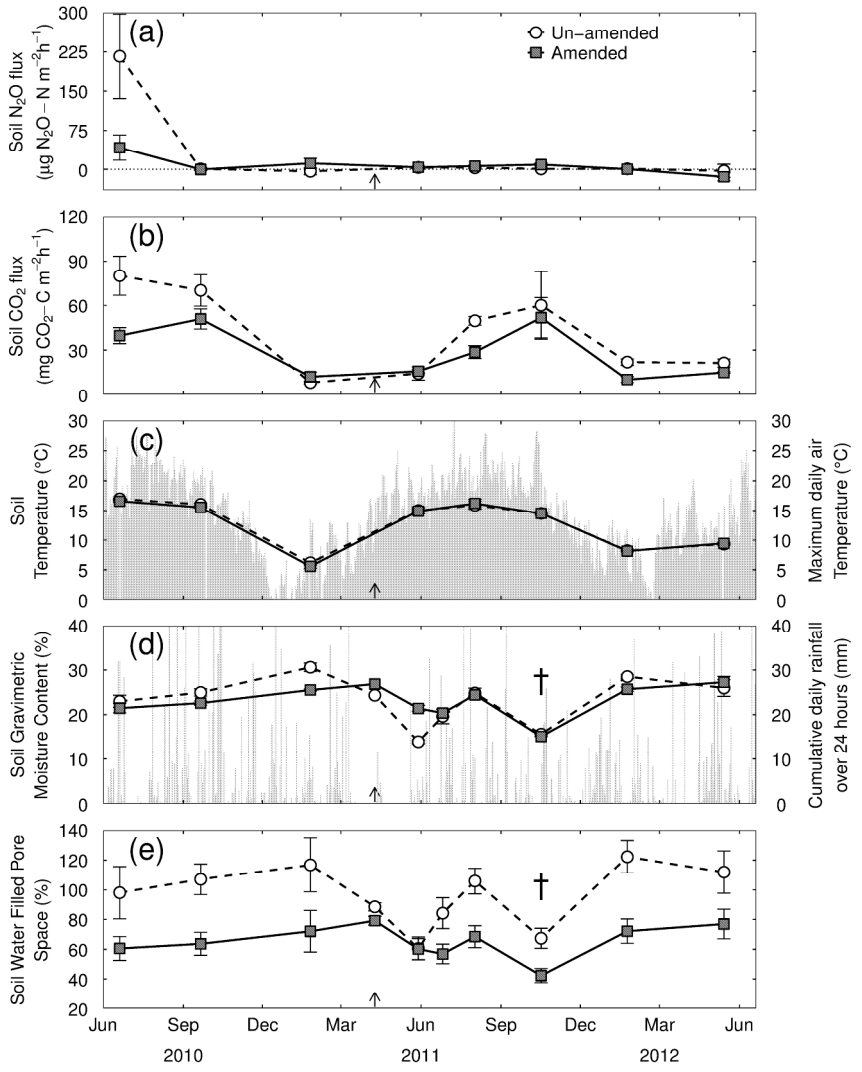

Fig. 2. The effect of biochar amendment on soil fluxes of (a) N2O and (b) CO2 from Miscanthus field plots (June 2010 - May 2012), and environmental conditions (c-e) over the same period: (c) soil temperature and daily maximum air temperature (oC); (d) soil gravimetric moisture content (%) and cumulative daily rainfall (mm day-1); and (e) soil water-filled pore space (%). Arrow indicates time of soil core collection for the laboratory incubation (30th March 2011). The horizontal dotted line in graph (a) indicates 0. The † symbol indicates missing probe values due to the soil being too dry to analyse (replaced with assumed 18 % volumetric moisture content for both treatments). Data points represent mean ± standard error (n = 5). Statistical model outputs underlying these results are presented in Table 1.
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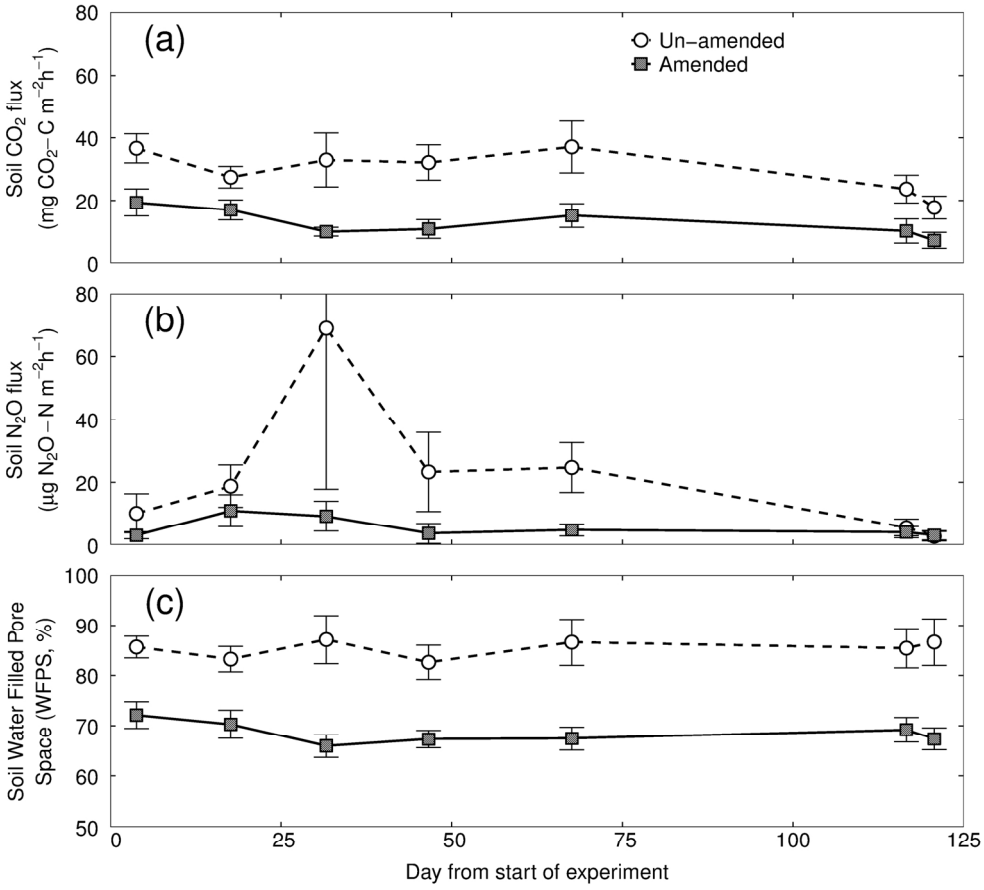

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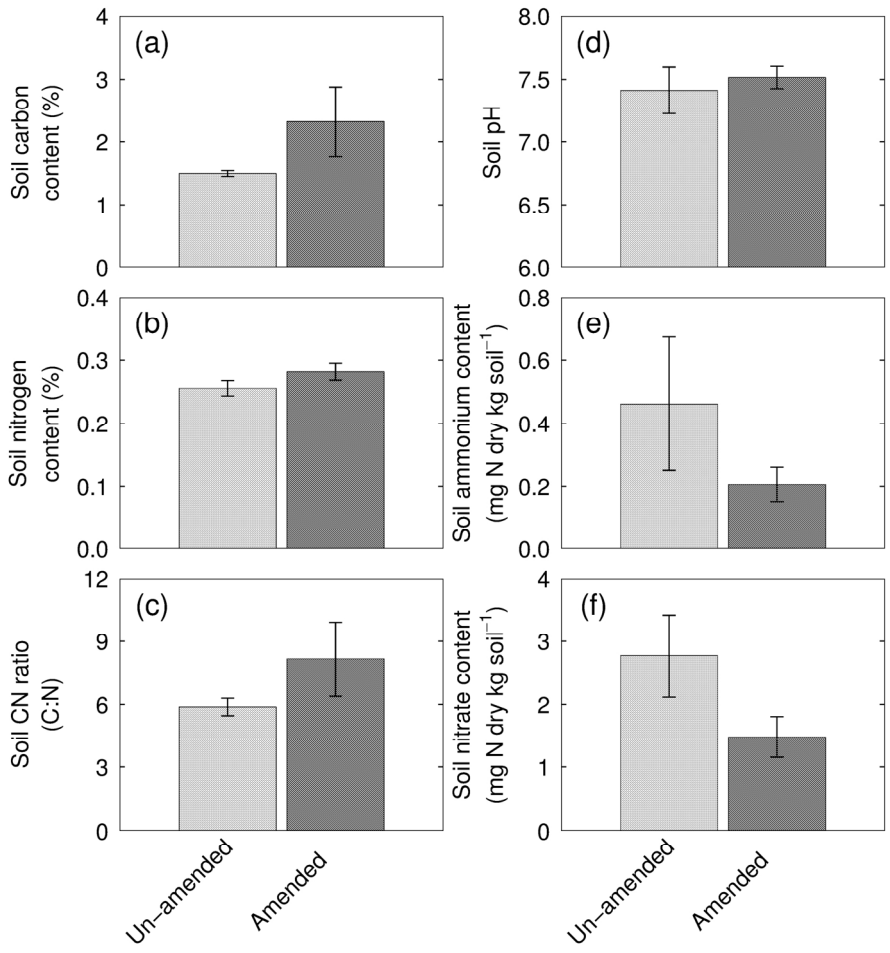

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