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The priming potential of environmentally weathered pyrogenic carbon during land-use transition to biomass crop production

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Abstract

Since land-use change (LUC) to lignocellulosic biomass crops often causes a loss of soil organic carbon (SOC), at least in the short term, this study investigated the potential for pyrogenic carbon (PyC) to ameliorate this effect. Although negative priming has been observed in many studies, most of these are long-term incubation experiments which do not account for the interactions between environmentally weathered PyC and native SOC. Here, the aim was to assess the impact of environmentally weathered PyC on native SOC mineralization at different time points in LUC from arable crops to short rotation coppice (SRC) willow. At eight SRC willow plantations in England, with ages of 3–22 years, soil amended 18–22 months previously with PyC was compared with unamended control soil. Cumulative CO_2 flux was measured weekly from incubated soil at 0–5 cm depth, and soil-surface CO_2 flux was also measured in the field. For the incubated soil, cumulative CO_2 flux was significantly higher from soil containing weathered PyC than the control soil for seven of the eight sites. Across all sites, the mean cumulative CO_2 flux was 21% higher from soil incubated with weathered PyC than the control soil. These results indicate the potential for positive priming in the surface 5 cm of soil independent of changes in soil properties following LUC to SRC willow production. However, no net effect on CO_2 flux was observed in the field, suggesting this increase in CO_2 is offset by a contrasting PyC-induced effect at a different soil depth or that different effects were observed under laboratory and field conditions. Although the mechanisms for these contrasting effects remain unclear, results presented here suggest that PyC does not reduce LUC-induced SOC losses through negative priming, at least for this PyC type and application rate.

Keywords: biomass crops, carbon dioxide, land-use change, priming, pyrogenic carbon, short rotation coppice willow, soil organic carbon

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Introduction

Land-use change (LUC) from conventional agriculture to lignocellulosic biomass crop production has received considerable attention as a prospective carbon (C) abatement strategy (Smith *et al.*, 2000; Don *et al.*, 2012). Life cycle assessment studies indicate that substitution of fossil fuels for land-based renewables has significant greenhouse gas mitigation potential (Smith *et al.*, 2000; Hillier *et al.*, 2009). However, the effects of LUC to biomass crops on soil organic carbon (SOC) stocks remain uncertain (Elsayed *et al.*, 2003). Results from paired-plot studies are highly variable and the trajectory of SOC has been related to many factors such as biomass crop type, previous land use, climate and soil texture (Keoleian & Volk, 2005). Any alteration in SOC stocks will have a subsequent impact on the overall C abatement potential of biomass crops.

It has been suggested that the long-term C abatement potential of biomass crops could be enhanced if combined with pyrogenic C (PyC) production and use as a soil amendment (Case *et al.*, 2014). This PyC, also frequently termed 'biochar', has been proposed mainly as a strategy for long-term C sequestration (Pessenda *et al.*, 2001; Masiello, 2004; Krull *et al.*, 2006; Preston & Schmidt, 2006) that is simultaneously capable of improving soil quality (Joseph *et al.*, 2010; Woolf *et al.*, 2010; Montanarella & Lugato, 2013). However, some aspects of PyC function in soil remain poorly understood. For example, concerns persist over the impact of PyC on native SOC mineralization (Wardle *et al.*, 2008;

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Kuzyakov *et al.*, 2009; Keith *et al.*, 2011; Luo *et al.*, 2011). Alteration of the turnover rates of native SOC after the addition of any substrate is often referred to as 'priming', with increased and decreased rates referred to as positive and negative priming respectively. Both positive and negative priming following PyC application have been observed (Kuzyakov *et al.*, 2009; Spokas & Reicosky, 2009; Liang *et al.*, 2010; Jones *et al.*, 2011; Keith *et al.*, 2011; Zimmerman *et al.*, 2011). Effects are therefore likely to vary according to the nature and composition of the PyC used and the receiving soil type (Shneour, 1966; Spokas & Reicosky, 2009; Atkinson *et al.*, 2010; Lehmann *et al.*, 2011).

Although priming effects vary between studies, most evidence indicates that any PyC-induced increase in CO_2 production is likely to be short lived, with a negligible impact on SOC stocks in the longer term (Woolf & Lehmann, 2012). Due to the different timescales of the mechanisms responsible for positive and negative priming, incubation studies often report an initial positive priming effect followed by reduced or negative priming (Keith *et al.*, 2011; Zimmerman *et al.*, 2011; Singh & Cowie, 2014). These observations have been used to further substantiate the environmental benefits of PyC production and soil incorporation strategies (Singh & Cowie, 2014). PyC application to recently established biomass crops could not only offset any LUC-induced SOC losses with C sequestered in the stable aromatic portion of PyC, but may possibly further reduce such losses through negative priming. A few studies have assessed priming effects of PyC amendment to soil in the context of recently established biomass crops but none have directly investigated this potential.

In one study, PyC application to a 5-year-old *Miscanthus* × *giganteus* plantation was reported to decrease CO_2 flux in the field by 33% over 2 years and by 53% in a 120-day incubation experiment (Case *et al.*, 2014). Net CO_2 flux was reduced by up to 20% in a 90-day incubation experiment using soil from a 14-year-old short rotation coppice (SRC) willow plantation mixed with PyC (Prayogo *et al.*, 2013). While these results demonstrate considerable potential for PyC to decrease net soil CO_2 flux from biomass crops, both report only single-site observations. Due to remaining uncertainty over both the mechanisms involved and the conditions associated with positive and negative priming, observed priming effects are likely to vary for different PyC–soil combinations. The long-term direction of any priming effects is also unclear, since few studies have investigated the impact of environmental weathering of PyC on interactions with native SOC (Spokas, 2013). Furthermore, the establishment of biomass crops on former agricultural land can be expected to alter soil biological and physicochemical properties over time (McCormack *et al.*, 2013), which could affect the response of soil to PyC independent of changes in PyC itself. The aim of this study was therefore to assess the impact of environmentally weathered PyC on native SOC mineralization at different time points in LUC.

The focus of this study is on LUC from arable crops to SRC willow production since other studies indicate that this transition has considerable SOC accumulation potential, but with short-term losses owing to initial soil disturbance (Jug *et al.*, 1999; Lemus & Lal, 2005; Amichev *et al.*, 2012). One previous incubation study reported a reduction in CO_2 emissions when soil from a 14-year-old SRC willow plantation was mixed with fresh PyC (Prayogo *et al.*, 2013). In the present study, the effects of environmentally weathered PyC at various stages of LUC are considered. Using laboratory incubations and field flux measurements from SRC willow plantations of different ages, this study aimed to: (i) test the hypothesis that environmentally weathered PyC reduces native SOC mineralization through negative priming and; (ii) assess the sensitivity of priming effects to changes in soil properties following LUC and; elucidate any potential consequences for the timing of PyC application.

Materials and methods

Study sites and pyrogenic carbon characterization

This study focused on commercial SRC willow plantations established on former arable land in England. Eight field sites were selected across England to provide a range of stand ages and SOC status (Table 1) from those investigated as part of a wider study (McClean, 2015). Site climate was characterized using mean annual precipitation (MAP) and mean annual temperature (MAT), for 1981–2010 observations at the station of the UK Meteorological Office nearest to each study site (distance 7.9–57 km). The PyC used in this study was produced by slow pyrolysis of *Miscanthus* straw (Pyreg GmbH, Dörth, Germany) with a final production temperature of 800 °C. Particle size distribution of the PyC was measured using progressive dry sieving and was as follows: 18% was <0.5 mm, 20% was 0.5–1.0 mm, 35.8% was 1–2 mm and 26.2% was 2–5.6 mm. PyC was characterized by elemental and proximate analysis and the University of Edinburgh stable C (Cross & Sohi, 2013) and labile C (Cross & Sohi, 2011) and nutrient release toolkit assays (Angst & Sohi, 2013). PyC pH was measured using a ratio of 1.0 g of PyC in 20 ml of deionized water and shaking for 1.5 h before measuring pH to ensure sufficient equilibration between solution and PyC surfaces (Rajkovich *et al.*, 2011; Table 2).

Pyrogenic carbon field application and soil sampling

PyC amendment was carried out between July and November 2011. A grid of 100 intersections was overlain on each study site using a scale appropriate to the field size and then further

Table 1 Soil and climate characteristics for each study site

	Location	Age (years)	Field area (ha^{-1})	% Soil organic carbon ($n = 3$)	% Total nitrogen ($n = 3$)	Bulk density ($g\ cm^{-3}$) ($n = 3$)	Soil pH ($n = 3$)	% Clay ($n = 1$)	% Silt ($n = 1$)	% Sand ($n = 1$)	Mean annual precipitation (mm)	Mean annual temperature (°C)	Met Office weather station
1	Oxfordshire	22	0.37	7.46 ± 0.48	0.79 ± 0.04	0.78 ± 0.09	6.58 ± 0.22	36.3	55.6	8.1	659.7	10.8	Oxford
2	Oxfordshire	9	3.72	6.90 ± 0.71	0.72 ± 0.06	0.81 ± 0.03	7.25 ± 0.15	27.3	55.2	17.4	659.7	10.8	Oxford
3	Oxfordshire	9	0.76	9.02 ± 0.83	0.66 ± 0.05	1.12 ± 0.07	7.06 ± 0.08	16.4	56.1	27.5	659.7	10.8	Oxford
4	Berkshire	3	10	3.03 ± 0.18	0.26 ± 0.03	1.38 ± 0.20	5.94 ± 0.80	15.2	53.5	31.3	659.7	10.8	Wisley
5	Berkshire	4	10	2.45 ± 0.36	0.22 ± 0.03	1.49 ± 0.14	5.82 ± 0.35	12.2	47.6	40.2	659.7	10.8	Wisley
6	Norfolk	11	11.07	2.39 ± 0.26	0.16 ± 0.03	1.60 ± 0.05	5.98 ± 0.47	18.2	55.5	26.3	613.7	9.9	Wattisham
7	North Yorkshire	5	2.64	2.69 ± 0.33	0.19 ± 0.04	1.24 ± 0.25	5.08 ± 0.16	14.9	59.1	26.0	651.1	9.2	Durham
8	County Durham	4	7.05	4.57 ± 0.53	0.27 ± 0.02	1.10 ± 0.09	5.4 ± 0.16	18.6	60.5	20.9	651.1	9.2	Durham

divided into three areas of approximately equal size. Within each of the three areas, a pair of 2 × 2 m plots was established at a randomly selected intersection. For each pair, PyC was applied manually to the surface of one plot at an application rate of 16 t ha^{-1} and incorporated to 15 cm depth using a spading fork. The forking treatment was also applied to the corresponding control plot, located at a 5 m distance from the PyC amended plot. At site 8 only, three additional pairs of plots were established 2 weeks before sampling to compare the effects of weathered and fresh PyC.

In May 2013, 18–22 months after PyC amendment, soil cores (Ø 30 mm) were taken using an absorbing hammer and bipartite gouge auger (Van Walt, Haslemere, UK). Sampling was to 5 cm depth from the central 1 m^2 of each plot in a 'W' formation. Ten soil cores were collected from each plot to obtain sufficient material for the laboratory incubations and soil analysis. At site 3, only two pairs of plots could be sampled due to partial flooding of the field. Samples were combined by plot and stored in the dark at 4 °C for <30 days prior to the incubation experiment. An additional core (Ø 50 mm) was taken to 5 cm depth from each plot using a specialized ring corer kit to measure soil bulk density (BD) (Van Walt).

Soil laboratory incubations and carbon dioxide flux measurements

Prior to incubation, soil samples were sieved (<4 mm), with care taken to remove fine roots and stones, and adjusted with deionized water to 60% water holding capacity (WHC), which is considered optimal for soil microbial respiration (Howard & Howard, 1993). To determine the maximum WHC (WHC_{max}), a method adapted from Ohlinger (1995) was used. For each sample, triplicates of 20 g of field moist soil were weighed into cellulose filters (No.1, Whatman; Maidstone, Kent, UK; 11 μm retention), which were placed inside plastic funnels with the bottoms sealed with a stopper. The soil samples were kept in saturated conditions with deionized water for 1 h with the funnel covered with plastic film (Parafilm; Bemis, Oshkosh, WI, USA) and placed in a closed plastic box to limit evaporation. After 1 h the stoppers were removed from the funnel bottoms and samples were left to drain for 3 h. Samples were then weighed into foil cups, dried at 105 °C for 24 h, and then cooled in a desiccator and reweighed to determine gravimetric moisture content (GMC). WHC_{max} under laboratory conditions was assessed for each sample (Eqn 1). The moisture addition/reduction required to adjust 10 g (dry weight equivalent) of field moist soil to 60% WHC was determined. WHC_{max} was also calculated for the samples from the amended plots to determine the effects of PyC on WHC. Prior to incubation, samples from amended plots were adjusted to the GMC equivalent to 60% WHC of the control soil. The purpose of using equalized GMC was to remove indirect WHC-related effects of PyC amendment and instead focus on direct priming effects. Since PyC may alter both the distribution of a fixed amount of water within different soil pores as well as the bulk soil water-filled pore space (WFPS), using equalized GMC may assist in ascertaining the importance of these effects with respect to priming.

Table 2 Pyrogenic carbon (PyC) characteristics: % stable and labile C (n = 4) quantified using the Edinburgh stable C (Cross & Sohi, 2013) and labile C (Cross & Sohi, 2011) toolkit assays, total elemental C, hydrogen (H), nitrogen (N), and oxygen (O), molar oxygen-to-carbon (O/C), hydrogen-to-carbon (H/C) and carbon-to-nitrogen (C/N) ratios (n = 1), free, locked and total volatile matter (FVM, LVM, and TVM), ash content, black carbon (BC_{hypy}) (n = 1), and pH (n = 6). Percentages are expressed on a dry weight basis

(wt %)													BC_{hypy}	
Stable C	Labile C	C	H	N	O	O/C	H/C	C/N	FVM	LVM	TVM	Ash	(BC/SOC %)	pH
95.3 ± 0.06	0.11 ± 0.01	77.7	0.97	0.36	4.52	0.04	0.15	253	4.01	2.46	6.47	16.4	99.1	9.97 ± 0.10

$$\mathrm{WHC_{max}}(\%) = \frac{(\text{drained soil (g)} - \text{oven dried soil (g)})}{\text{oven dried soil (g)}} \times 100 \quad (1)$$

Incubations were carried out in triplicates of 10 g (dry weight equivalent) of each sample, weighed into 250-ml conical flasks, and maintained at 30 °C for 10 weeks in a temperature-controlled room. Conical flasks were sealed with a rubber stopper to minimize moisture loss. Cumulative CO_2 flux was assessed gravimetrically using the soda lime adsorption method. In each flask, 1.0–1.5 g of self-indicating, nonhygroscopic soda lime granules (1.0–2.5 mm size; Fisher Scientific, Loughborough, UK) was weighed into a 1.7-ml glass vial, dried at 105 °C for 24 h, and cooled in a desiccator before reweighing and incubation. The vial was suspended from the rubber stopper used to seal each flask. A blank flask containing a soda lime vial but no soil was used for every five flasks, to correct for CO_2 gained during preparation of the vials, from the flask headspace at closure and on redrying of the soda lime prior to reweighing. Each vial was weighed and replaced weekly to prevent saturation of the soda lime. The first week was considered as a pre-incubation period during which respiration rate stabilized following sieving and moisture adjustment (Fierer & Schimel, 2003). Mineralized C was determined gravimetrically as the quantity of CO_2 is proportional to the increase in soda lime mass as the CO_2 reacts with sodium and calcium hydroxides to form carbonates (Eqn 2) (Edwards, 1982; Grogan, 1998).

$$\begin{aligned}&\text{Mineralized C (mg } CO_2 - C)\\ &= \left(1.69 \times (\text{mass gain of soda lime}\right.\\ &\qquad \left. - \text{mass gain of blank flask}) \times \frac{12}{44}\right) \times 1000,\end{aligned} \quad (2)$$

where 1.69 is a conversion factor used to correct for water formed during chemical adsorption (Grogan, 1998) and 12/44 is the ratio of the molar mass of C to CO_2.

Carbon dioxide flux measurements in the field

Soil-surface CO_2 flux was measured in the field immediately before soil sampling in May 2013, using a dynamic closed chamber infra-red gas analyser (EGM-4 PP Systems, Amesbury, MA, USA). At each plot, the respiration chamber was gently rotated into the soil surface and five measurements were taken in a 'W' formation from the central 1 m^2. Using probes inserted to 5 cm depth, ambient soil temperature (HI993310 Hanna Instruments, Leighton Buzzard, UK) and soil moisture (Moisture Meter HH2; Delta-T Devices, Cambridge, UK) were also measured at five locations in each plot, again using a 'W' formation from the central 1 m^2. Soil moisture and temperature conditions at the time of gas sampling from each plot are provided in Table S1.

Soil chemical and physical analysis

After sieving (<4 mm) the composite sample from each plot, a subsample for C and N analysis was air-dried at room temperature for 7 days, before being crushed with a pestle and mortar, sieved (<2 mm) and milled to a fine powder using a MM200 ball mill (Retsch, Castleford, UK). 15–20 mg of the control plot samples and 5–10 mg of sample from PyC amended plots were analysed for total C and N by dry combustion using a NA 2500 Elemental Analyser (Carlo Erba, Milan, Italy). Inorganic C content was measured using an automated acidification module and coulometry (CM 5012 and CM 5130; UIC, Joliet, IL, USA). 50–100 mg of each sample was acidified using 8 ml of 2 M perchloric acid ($HClO_4$) and, as carbonates were released as CO_2, the acid-evolved gas was measured by coulometric titration. For each sample, SOC content was determined by subtracting the inorganic C from the total C content.

To determine soil pH, 10 g of sample was added to a beaker with 25 ml of deionized water, stirred rigorously and then left for 30 min, stirred again and pH was measured after suspending the electrode for 30 s before each measurement was taken. Cores collected for BD measurements were returned to the laboratory, oven dried at 105 °C for 48 h and sieved (<2 mm) to separate coarse fragments from fine earth. Collected samples were weighed to calculate BD of the fine earth (BD_{fe}) (Eqn 3), correcting for the volume of coarse fragments with an assumed density of 2.65 g cm^{-3} (Eqn 4). Soil WFPS was then calculated using GMC and BD (Eqn 5):

$$\mathrm{BD_{fe}}(\mathrm{g\,cm^{-3}}) = \frac{(\text{soil (g)} - \text{coarse fragments (g)})}{\text{volume of corer (cm}^{-3}) - \left(\frac{\text{coarse fragments(g)}}{2.65}\right)} \quad (3)$$

$$\mathrm{BD_{corrected}}(\mathrm{g\,cm^{-3}}) = \mathrm{BD_{fe}} \times \text{diameter of corer (cm)} \times 10 \times \left(1 - \left(\frac{\text{coarse fragments (g)}}{(2.65 \times \text{volume of corer (cm}^{-3}))}\right)\right) \quad (4)$$

$$\text{WFPS}\ (\%) = \left(\frac{(\text{GMC}(\%) \times \text{BD}(\text{g cm}^{-3}))}{\left(1 - \left(\frac{\text{BD (g cm}^{-3})}{2.65}\right)\right) \times 100} \times 100 \right). \quad (5)$$

Black carbon quantification

Primed CO_2 flux from incubated PyC-amended and control soil samples was expressed in relation to their non-black C (nBC) concentration. Hydrogen pyrolysis (hypy) was used to isolate and quantify black C (BC), with nBC calculated as the difference between BC and SOC (Ascough *et al*., 2009; Meredith *et al*., 2012). By expressing the CO_2 flux in terms of the nBC content, both the background BC at each site and the PyC in the amended plots were excluded from the calculations.

The BC content of the samples collected from each plot was isolated using hypy and quantified by dry combustion elemental analysis. The fresh PyC was also tested to assess thermochemical stability. The milled samples prepared for C and N analysis were used for hypy. Samples containing inorganic C > 0.01% by weight were pretreated to remove carbonates by acid digestion with 1 M hydrochloric acid (HCl) and heating at 80 °C for 24 h. For each sample, 500 mg was loaded with a Mo catalyst 5% by weight using an aqueous/methanol solution of ammonium dioxydithiomolybdate $[(NH_4)_2MoO_2S_2]$ and placed inside borosilicate glass reactor inserts, which were sealed at each end using quartz wool. Inserts were weighed both before and after hypy to measure the loss in sample weight. The samples were pyrolysed with resistive heating from 50 to 250 °C at 300 °C min^{-1}, then from 250 to 550 °C at 8 °C min^{-1}, and finally held at 550 °C for 2 min under hydrogen pressure of 150 bar. A hydrogen sweep gas flow of 5 l min^{-1}, measured at ambient temperature and pressure, ensured the nBC products were quickly removed from the reactor and trapped on cooled silica (Meredith *et al*., 2004).

The hypy residue for each sample was analysed for total C using a NA 2500 Elemental Analyser (Carlo Erba). BC_{hypy} content was quantified by comparing the initial and residual SOC contents (Eqn 6):

$$\text{BC}_{\text{hypy}}(\text{BC/SOC}\%) = \frac{\text{Residual SOC (mg in hypy residue incl. spent catalyst)}}{\text{Initial SOC (mg in catalyst loaded sample)}} \times 100 \quad (6)$$

Statistical analysis

Analyses were carried out using SPSS 19 software (IBM, Armonk, NY, USA). For the incubation experiment, linear mixed-effect models for weekly CO_2 flux were created using cumulative CO_2 flux data (mg CO_2-C g^{-1} nBC) and the restricted maximum likelihood procedure. The significance of amending soil with PyC and time in incubation were assessed. Models were first created for soils of each site and then for all soils from all sites. Flux measurements used in the models were the arithmetic treatment means obtained for triplicate incubation flasks. For soils from individual sites, PyC amendment and time in incubation were fixed effects and plot pair a random effect. Site was introduced into the model for all paired plots as another random effect. Since soils from site 8 were from plots with fresh as well as weathered PyC, individual models were created for each and another model was created for all site 8 data to assess the significance of PyC age as an additional variable.

A linear mixed-effect model was also created to assess the significance of variables affecting soil-surface CO_2 flux in the field (mg CO_2-C m^{-2} h^{-1}) expressed for each plot as the arithmetic mean of the five measurements taken. This model included PyC amendment as a fixed effect, together with measured soil temperature (°C) and WFPS (%). Random effects were for site and plot pair. Due to the limited number of observations per parameter, models were not created for individual sites. However, it was possible to create a model for site 8 plots with weathered and fresh PyC, testing the significance of PyC age.

Paired *t*-tests were used to assess the effects of PyC amendment after weathering on soil physicochemical properties across sites. A general linear model (GLM) was used to assess the difference in specific mineralization rate between soils with and without PyC added. These measures of additional C were used for intersite comparison of priming effects. For the purpose of assessing the influence of site properties on PyC–SOC interactions and possible priming effects, only the incubation flux data was used. This was because field fluxes include root as well as soil respiration. For all models, residuals were checked for normality using the Shapiro–Wilk test.

To further explore the relationships and unexplained variance from the GLM, correlations were carried out on soil and site variables with: (i) specific nBC mineralization rates (mg CO_2-C g^{-1} nBC) and; (ii) ratios of C mineralized in amended and control soil. Pearson correlation coefficients (r) were reported for normally distributed data and Spearman rank coefficients (r_s) for non-normally distributed data.

Results

Cumulative carbon dioxide flux under controlled conditions

The mean cumulative flux across sites was 86.9 ± 4.3 mg CO_2-C g^{-1} nBC from the soil with weathered PyC compared to 71.7 ± 3.5 mg CO_2-C g^{-1} nBC from the control, a difference of 21 ± 11%. Over the 10-week incubation period, PyC amendment had a significant impact on soil CO_2 flux. For seven of the eight sites, cumulative CO_2 flux (mg CO_2-C g^{-1} nBC) was significantly higher for soil containing weathered PyC (Table 3, Figs 1 and 2). There was also a significant effect across sites ($P < 0.001$, Table 3, Fig. 2). There was no significant difference in CO_2 flux for site 8 between soil with fresh and weathered PyC over the 10-week period ($P = 0.111$, Fig. 2). Weekly CO_2 flux significantly decreased over time for amended and control soils from all sites ($P < 0.001$, Table 3, Figs 1 and 2).

Table 3 Variables affecting weekly cumulative CO_2 flux [mg CO_2-C g^{-1} non-black C (nBC)] from soil with weathered and fresh pyrogenic carbon (PyC) incubated under controlled conditions for 10 weeks. Results are from linear mixed-effect models with fixed effects for treatment (PyC amended v control) and time (week of incubation) and random effects for site and plot pair (n = 27 for each site except site 3 where n = 18 and for all sites n = 207)

	Independent variable			
	Treatment		Time	
Dependent variable: CO_2 flux	*F*-statistic	*P* value	*F*-statistic	*P* value
Site 1	14.799	<0.001	41.094	<0.001
Site 2	13.193	0.001	11.529	<0.001
Site 3	7.861	0.008	18.246	<0.001
Site 4	12.114	0.001	18.438	<0.001
Site 5	26.661	<0.001	12.347	<0.001
Site 6	11.774	0.001	8.431	<0.001
Site 7	14.210	0.001	17.405	<0.001
Site 8: weathered PyC	0.394	0.533	9.620	<0.001
Site 8: fresh PyC	1.654	0.205	13.894	<0.001
All sites: weathered PyC only	47.130	<0.001	88.46	<0.001

Carbon dioxide flux measured in the field

The mean CO_2 flux from the soil with weathered PyC was 109 ± 6.5 mg CO_2-C m^{-2} h^{-1}, which was not significantly different to the control soil mean of 108 ± 6.1 mg CO_2-C m^{-2} h^{-1} (Fig. 3). The mean CO_2 flux from all PyC amended soil (both before and after weathering) was 109 ± 6.1 mg CO_2-C m^{-2} h^{-1}, which was also not significantly different to the control soil mean of 112 ± 6 mg CO_2-C m^{-2} h^{-1} (Fig. 4). No significant differences were observed in the field between soil-surface CO_2 flux (mg CO_2-C m^{-2} h^{-1}) from the plots with weathered PyC and the control plots (P = 0.191, Fig. 3). There was also no significant difference in CO_2 flux at site 8 between plots with fresh and weathered PyC (P = 0.583). Soil temperature and WFPS both had a significant impact on CO_2 flux (P = 0.023 and 0.025 respectively).

Changes in soil physicochemical properties

PyC amendment significantly altered various soil physicochemical properties (Table 4). Soil carbon was affected, with significantly higher BC and C/N (both P < 0.001, Table 4) in the PyC amended soil relative to the control, but with significantly lower nBC (P = 0.001, Table 4). PyC amendment also significantly increased soil pH, GMC, WHC and WFPS (P = 0.031, P < 0.001, P < 0.001, and P = 0.024 respectively, Table 4) relative to the control and significantly reduced soil BD (P < 0.001, Table 4). There was no significant difference in total nitrogen (TN) (P > 0.05, Table 4) between the amended and control soil.

None of the site properties had a significant effect on the additional C mineralization rate during incubation (mg CO_2-C g^{-1} nBC) (P > 0.05, Table 5). There were no statistically significant correlations between site properties and additional C, expressed in absolute or proportional terms (P > 0.05, Table 6). None of the observed correlation coefficients were indicative of a strong relationship (in all cases <0.4, Table 6).

Discussion

Effects of pyrogenic carbon on cumulative carbon dioxide flux under controlled conditions

In the incubation study reported here, field plots were sampled to a 5 cm depth and incubated under constant temperature and moisture conditions. This was designed to isolate the effect of PyC amendment on C cycling processes from that of prevailing environmental factors. The surface soil was expected to contain a high concentration of PyC, and thus, any priming effects that might be occurring in the soil should be evident here. At the end of the 10-week incubation period, cumulative CO_2 flux was significantly higher from soils with weathered PyC than control soils. These results indicate the potential for a sustained positive priming effect in the surface 5 cm of soil. Spokas (2013) also reported an increase in CO_2 production from soil incubated with weathered PyC relative to the control soil. This increase was attributed to microbial mineralization of either the weathered PyC or of labile C compounds sorbed to the surface of the PyC. Without direct source-partitioning, PyC mineralization could not be confirmed, but no alteration was observed in bulk O/C ratio or change in the physical appearance of PyC as a result of weathering (Spokas, 2013). Without source-partitioning, it was also not possible to preclude PyC mineralization in the present study. However, characterization of the PyC indicates a high stability, most likely due to the high production temperature (Bruun *et al*., 2011; Cross & Sohi, 2011).

The results of hypy indicate that 99.1% of the original PyC comprised a highly recalcitrant fraction of BC that is resistant to degradation in the environment over millennia (Ascough *et al*., 2010). Previous studies have shown that hypy reliably isolates a consistent part of the BC continuum, namely poly-aromatic structures with >7 rings and an atomic H/C ratio <0.5 (Ascough *et al*., 2010; Meredith *et al*., 2012). Testing after accelerated ageing designed to simulate oxidative degradation

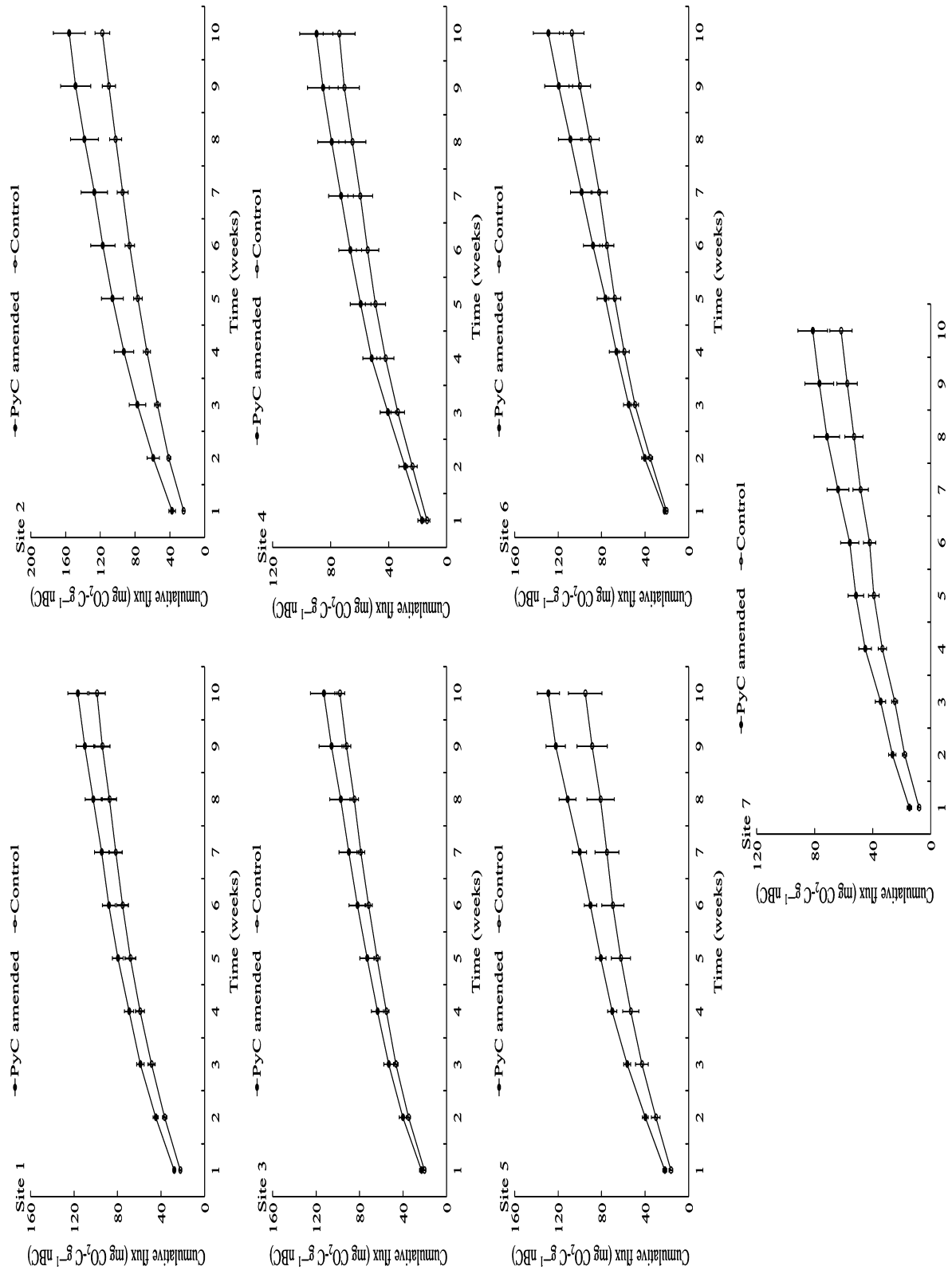

Fig. 1 Weekly cumulative flux [mg CO_2-C g^{-1} non-black C (nBC)] from incubated soil from sites with weathered pyrogenic carbon (PyC) and controls. Data points represent the mean ± standard error ($n = 9$ except for site 3 where $n = 6$).

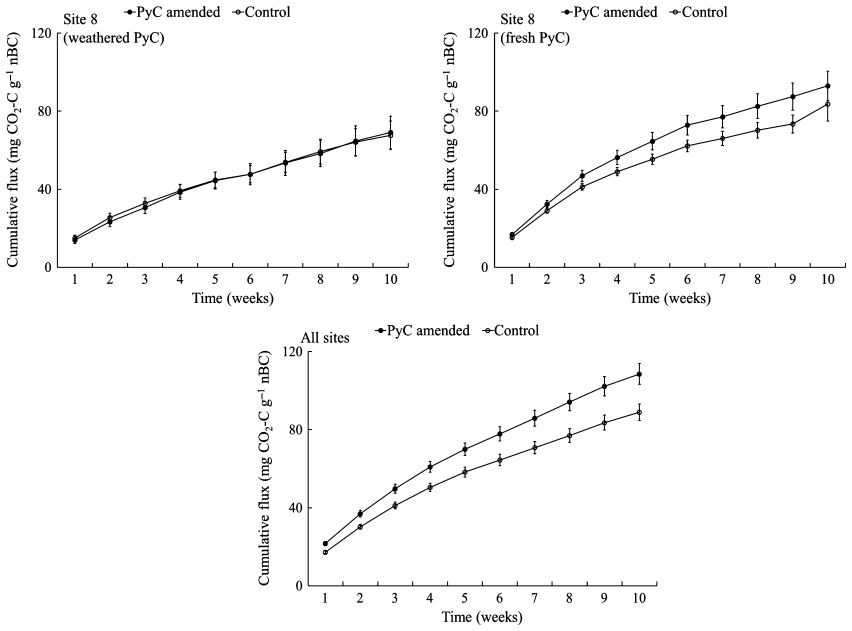

Fig. 2 Weekly cumulative flux [mg CO_2-C g^{-1} non-black C (nBC)] from incubated soil from site 8 and from all paired plots with weathered pyrogenic carbon (PyC) and respective controls across all sites. Data points represent the mean ± standard error (n = 9 for site 8 and for all sites combined n = 78).

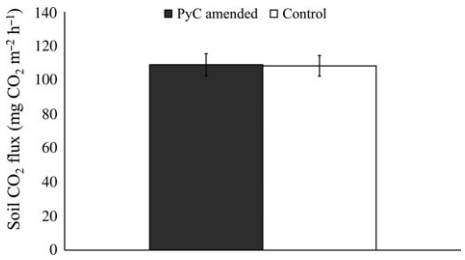

Fig. 3 Soil-surface CO_2 flux (mg CO_2-C m^{-2} h^{-1}) measured in the field from soil with weathered pyrogenic carbon (PyC) and control soil. Bars represent the mean across all sites ± standard error (n = 115).

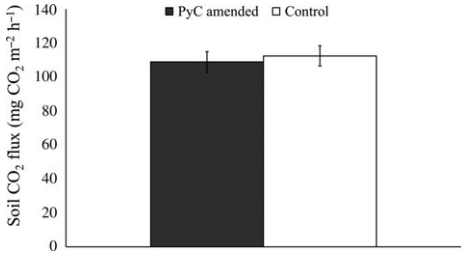

Fig. 4 Soil-surface CO_2 flux (mg CO_2-C m^{-2} h^{-1}) measured in the field from all pyrogenic carbon (PyC) amended soil and control soil. Bars represent the mean across all sites ± standard error (n = 130).

in soil indicated that 95.3% of the C would resist degradation for at least 100 years under temperate conditions (Cross & Sohi, 2013). Based on these results, the PyC is unlikely to have measurably degraded during the time frame of this incubation. In addition to being stable over the long term, this particular PyC does not display a fraction of C susceptible to short-term loss either. Fresh PyC amendment at site 8 had no effect on CO_2 flux in either the laboratory or the field. This most likely relates to the high production temperature (800 °C) of the PyC, which results in a very low labile C content of 0.11 ± 0.01%.

As Spokas (2013) suggested, it is also possible that the higher CO_2 flux measured here derives from the mineralization of labile C adsorbed to PyC surfaces in

Table 4 The effects of pyrogenic carbon (PyC) amendment after weathering on soil physicochemical properties. Results are from paired t-tests (n = 23). Data indicate mean ± standard error

Dependent variable	PyC amended	Control	t value	P value
% SOC	7.16 ± 0.78	4.68 ± 0.53	6.03	<0.001
% BC_{hypy}	3.80 ± 0.51	0.69 ± 0.18	6.29	<0.001
% nBC	3.36 ± 0.32	3.99 ± 0.43	−3.79	0.001
% TN	0.41 ± 0.05	0.39 ± 0.05	1.58	0.129
C/N	18.7 ± 0.8	13.3 ± 0.5	6.68	<0.001
pH	6.28 ± 0.15	6.09 ± 0.16	2.31	0.031
BD (g cm^{-3})	1.15 ± 0.06	1.21 ± 0.06	−18.1	<0.001
% GMC	36.9 ± 3.3	32.6 ± 3.2	5.51	<0.001
% WHC	116 ± 9.3	104 ± 8.1	4.12	<0.001
% WFPS	70.8 ± 3.8	66.7 ± 3.6	2.42	0.024

Table 5 The effects of various site properties on additional C [mg CO_2-C g^{-1} non-black C (nBC)]. Results are from a general linear model (n = 23)

	Dependent variable	
	Additional C (mg CO_2-C g^{-1} nBC)	
Independent variable	*F*-statistic	*P* value
Stand age	0.005	0.953
Initial SOC	1.789	0.208
% TN	0.943	0.353
Initial pH	1.855	0.201
Δ pH	0.555	0.475
Initial BD (g cm^{-3})	0.756	0.411
Δ BD (g cm^{-3})	0.849	0.379
Δ WFPS (%)	0.091	0.769
% clay	0.107	0.761
MAP (mm)	0.027	0.881
MAT (°C)	0.015	0.911

Table 6 Results of correlations between additional C (both absolute and relative amounts) mineralized from incubated soil with weathered pyrogenic carbon (PyC) and various site properties. Pearson's correlation coefficients (r) are displayed for normally distributed data and Spearman's rank coefficients (r_s) for non-normal data (n = 26)

	Additional C (mg CO_2-C g^{-1} nBC)	Additional C (%)
Age of stand	r_s = 0.151, P = 0.492	r_s = 0.128, P = 0.561
SOC conc.	r_s = −0.050, P = 0.819	r_s = −0.030, P = 0.893
TN conc.	r_s = −0.009, P = 0.968	r_s = −0.017, P = 0.939
pH	r = 0.259, P = 0.234	r = 0.183, P = 0.403
Δ pH (absolute)	r_s = 0.148, P = 0.501	r_s = 0.168, P = 0.445
Δ pH (relative)	r_s = 0.146, P = 0.506	r_s = 0.172, P = 0.433
Initial BD (g cm^{-3})	r = −0.033, P = 0.881	r = −0.048, P = 0.828
Δ BD (absolute)	r_s = 0.004, P = 0.985	r_s = 0.030, P = 0.891
Δ BD (relative)	r = 0.375, P = 0.078	r = 0.340, P = 0.112
Δ (WFPS (absolute)	r = 0.326, P = 0.128	r = 0.288, P = 0.183
Δ WFPS (relative)	r_s = 0.189, P = 0.388	r_s = 0.190, P = 0.386
Clay content	r_s = −0.070, P = 0.752	r_s = −0.092, P = 0.676
Silt content	r_s = −0.147, P = 0.502	r_s = −0.098, P = 0.656
Sand content	r = 0.047, P = 0.833	r = 0.053, P = 0.809
MAP	r_s = −0.016, P = 0.942	r_s = −0.013, P = 0.954
MAT	r_s = 0.026, P = 0.908	r_s = 0.001, P = 0.998
PyC weathering	r_s = 0.078, P = 0.724	r_s = 0.053, P = 0.809

the field. The large surface area and high porosity of PyC may provide a favourable habitat for micro-organisms, with access to labile substrates and refuge from predators (Neher *et al.*, 1999; Bardgett, 2005). However, it has also been argued that adsorption of labile C could also inhibit SOC mineralization if soluble constituents diffuse and adsorb in pores that are too small for micro-organisms to access (Hamer *et al.*, 2004; Hilscher *et al.*, 2009; Cross & Sohi, 2011; Zimmerman *et al.*, 2011). It is possible that the bioavailability of sorbed compounds could therefore vary with the physical properties of PyC. Although surface area and pore size were not measured, this high temperature PyC may have a fine pore size and a high sorption affinity for SOC as both are reported to increase with production temperature (Warnock *et al.*, 2007; Kasozi *et al.*, 2010). Since no reduction in CO_2 flux was observed in this study, it is possible that prior adsorption resulted in the mineralization of labile C compounds during incubation.

Few studies have investigated priming effects from PyC in soils of perennial biomass crops and only one was identified that used soil from a SRC willow plantation (Prayogo *et al.*, 2013). This incubation study using fresh PyC reported no net effect on CO_2 production for a low PyC application rate (0.5% w/w) and negative priming for a high application rate (2% w/w) to soil sampled from 0 to 30 cm depth (Prayogo *et al.*, 2013). A negative priming effect has also been observed following PyC amendment to a *Miscanthus* × *giganteus* plantation (Case *et al.*, 2014). In this study, following application of PyC at a rate of 49 t ha^{-1}, CO_2 flux was reduced by 53% in a 120-day incubation using soil collected from the field 10 months after PyC amendment (Case *et al.*, 2014). Since a low application was used in the present study and both of these studies reported negative priming at higher application rates, it is possible that this may indicate a threshold effect for priming mechanisms. However, the effects of increasing application rate are inconsistent with other studies reporting no effect for other land uses (Zhang *et al.*, 2012a). Further research is required to assess the effects of different application rates with environmental weathering of PyC for perennial biomass crops.

In the present study, an increase in soil pH was observed following PyC amendment and a liming effect has previously been identified as a potential cause for positive priming from PyC (Farrell *et al.*, 2013). However, both Case *et al.* (2014) and Prayogo *et al.* (2013) also reported an increase in pH following PyC amendment, neither of which was accompanied by positive priming. Since the mean soil pH of the sites in the present study (6.01) was lower than both of these studies (pH > 7), an alleviation of an existing pH constraint on C utilization is more likely to have occurred here, which may at least partially explain the higher CO_2 flux observed for PyC amended soil in the top 5 cm. However, this is unlikely to be the driving mechanism for

positive priming for all sites since soils with a range of pH were used in this study and positive priming was not significantly related to pH.

PyC amendment significantly altered other soil physicochemical properties in the present study. These effects may partially explain the increase in CO_2 flux observed in the top 5 cm through the alleviation of constraints on C utilization. PyC amendment reduced soil BD; hence, an increase in porosity and oxygen diffusion may have stimulated microbial activity (Torbert & Wood, 1992; Beylich *et al.*, 2010). Since the amended and control soils were adjusted to equalized GMC, the PyC may also have reduced water availability which could further have enhanced aerobic respiration. Case *et al.* (2014) also adjusted soils to equalized GMC prior to incubation and observed a reduction in both BD and WFPS following PyC amendment. However, in that study, these physical effects did not appear to stimulate microbial activity. In the present study, collected soil samples were disturbed by sieving prior to incubation, while Case *et al.* (2014) used intact cores. Although WFPS was reported to increase with PyC amendment in the present study, it is possible therefore that positive priming may have been caused by the removal of these controls on soil respiration rather than reflecting their *in situ* effect.

Effects of pyrogenic carbon on soil-surface carbon dioxide flux in the field

Soil-surface CO_2 flux measurements were made to confirm whether the effects observed in the laboratory are demonstrable under field conditions. Despite the increase in CO_2 flux from PyC amended soils incubated under controlled conditions, no significant differences in soil-surface CO_2 flux were observed between amended and control plots in the field. These contrasting results indicate that at least two mechanisms are occurring under different conditions and/or at different soil depths.

Similar WHC conditions were present in the incubations, where the control and amended soils received equalized GMC (equivalent to 60% WHC) and in the field (all sites were within 50–70% WHC), suggesting similarly optimal conditions for microbial activity in both the laboratory and the field. It was expected that PyC would increase aeration and oxygen diffusion (Torbert & Wood, 1992; Beylich *et al.*, 2010) in the field and the laboratory. However, soil cores sampled from the amended plots show an increase in WFPS ($P < 0.024$). It is possible that a reduction in soil aeration has occurred *in situ*, with the opposite occurring in the laboratory. Other studies have reported an increase in methanogenesis following PyC amendment (Knoblauch *et al.*, 2011; Zhang *et al.*, 2012b) but, although this was not measured in the present study, the rapid field flux rates suggest predominantly aerobic respiration.

Since soil-surface CO_2 flux also includes root respiration, it is possible that a reduction in root respiration could explain the differences observed between the laboratory and field flux measurements. PyC may impact plant productivity and possibly reduce root growth or even cause root mortality, thus indirectly affecting root respiration. PyC-induced changes to physicochemical soil properties and possible interference with plant chemical signalling have the potential to influence plant interspecific competition and root growth, particularly in biomass cropping systems with diverse understorey vegetation (McCormack *et al.*, 2013). It has been suggested that PyC absorption of secondary metabolites may lessen the plant's ability to establish mycorrhizal symbioses, which may reduce plant nutrient uptake (Bais *et al.*, 2006). Interference with plant defence chemicals may also increase plant susceptibility to disease, which would reduce primary productivity and subsequently root respiration (Bais *et al.*, 2006).

Priming effects observed during an incubation experiment carried out without leaf litter might have been expected to differ from those observed under field conditions. Studies have previously observed decreased positive priming and/or increased negative priming over time in the presence of labile C, indicating greater SOC stabilization by PyC with higher inputs of labile C (Keith *et al.*, 2011; Prayogo *et al.*, 2013; Singh & Cowie, 2014). Although negative priming was not observed in the field in the present study, it is still possible that contrasting effects observed in the laboratory and the field may relate to plant inputs since the nature of PyC–SOC interactions will vary both directly with substrate and indirectly through PyC-induced changes to soil physicochemical properties.

The effects of PyC may also vary with soil depth which may help to explain the contrasting effects observed in the laboratory and the field. Changes in the distribution of SOC may occur, either directly through PyC–SOC interactions such as adsorption or increased aggregation, or indirectly by altering the physicochemical properties of the soil such as BD and thermal conductivity. For example, it has previously been reported that a reduction in the supply of fresh SOC could prevent the decomposition of SOC in deeper soil layers (Fontaine *et al.*, 2007). Therefore, increased stabilization of labile C in the surface layer may reduce the delivery of labile C to the subsoil which would otherwise activate the mineralization of slower-cycling C in the deeper soil layers (Fontaine *et al.*, 2007). Further research is

required to determine how PyC may impact the distribution of labile C and SOC mineralization throughout the soil profile.

Sensitivity of priming effects to changes in soil properties following land-use change

Study sites were selected with different stand ages to assess the sensitivity of priming effects to changes in soil properties following LUC. It was expected that certain LUC-induced changes may have an impact on PyC–SOC interactions, however, stand age did not have a significant effect on additional C ($P > 0.05$). For example, soils in minimum till systems such as SRC willow can become compacted over time which affects soil invertebrates by reducing habitable pore space, fungal hyphae and water content (Whalley *et al*., 1995). Since PyC reduced BD, which may subsequently alleviate compaction, greater effects on microbial activity may have been expected for older sites. It has also been suggested that these biomass crops can increase soil acidity over time (Makeschin, 1994; Jug *et al*., 1999) due to reduced alkaline inputs and nitrification-induced loss of base cations (Vanmiegroet & Cole, 1985), which also impacts on soil organisms and plant productivity (Bardgett, 2005). Previous studies have observed differential effects of PyC for soils of different pH (Blagodatskaya & Kuzyakov, 2008; Luo *et al*., 2011), however, neither the initial pH of the receiving soil nor observed changes in pH (ΔpH) had an effect on additional C in the present study. Since fresh PyC was only applied to one site, it is also possible that LUC-induced changes in soil properties have a transient effect that has not been observed in the present study.

These results indicate that changes in soil properties during LUC from arable to SRC willow may not affect longer term PyC–SOC interactions. A relationship has previously been observed between the SOC status of a receiving soil and priming effects (Cross & Sohi, 2011) with indications that PyC may stabilize labile C in soils of higher SOC status. It may have been expected that increased C inputs and accumulation of leaf litter with stand age would affect PyC–SOC interactions and possibly even exhibit negative priming. Although the range of SOC between sites is similar to that of Cross & Sohi (2011), SOC content had no effect on additional C, indicating that changes in C quantity and quality had no demonstrable influence on priming effects. Soil texture might also have been expected to influence priming effects since PyC may provide a favourable habitat for micro-organisms, which may be important for soils with low clay content. However, for the range of soils used in this study, clay did not significantly affect additional C.

Results from the incubation presented here indicate the potential for a sustained positive priming effect for the surface 5 cm of soil that was detectable in soil collected 18–22 months after amendment with PyC. Across all sites, the mean cumulative CO_2 flux was 21% higher from soil incubated with weathered PyC than the control soil. This increase in C mineralization may relate to adsorption and subsequent mineralization of labile C compounds and/or PyC-induced changes in soil physicochemical properties such as increased soil pH or reduced water availability. However, no net effect on CO_2 flux was observed in the field suggesting that: (i) this increase is offset by a contrasting PyC-induced effect such as a reduction in either root respiration or SOC mineralization in the deeper soil layers or; (ii) different effects have been measured under different conditions in the laboratory and the field with a possible reduction in soil aeration in the field and the opposite occurring in the laboratory. For the PyC and application rate used in this study, results suggest that PyC does not reduce LUC-induced SOC losses through negative priming. Furthermore, positive priming observed in the laboratory incubation was not sensitive to changes in soil properties that follow LUC from arable crops to SRC willow.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Soil temperature and moisture conditions at the time of gas sampling from each plot. Data indicate mean ± standard error, $n = 5$.