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Pyrogenic carbon additions to soil counteract positive priming of soil carbon mineralization by plants

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ABSTRACT

Important due to both its role in fire-affected ecosystems, and also its proposed intentional production and application for carbon (C) management, pyrogenic organic matter (PyOM) is thought to contain very stable forms of C. However, the mechanisms behind its interactions with non-PyOM soil organic C (SOC) remain speculative, with studies often showing short-term positive and then long-term negative "priming effects" on SOC decomposition after PyOM applications. Furthermore, studies of these interactions to date have been limited to systems that do not include plants. This study describes results from a 12-week greenhouse experiment where PyOM-SOC priming effects with and without plants were investigated using stable isotope partitioning. In addition, we investigated the optimal $\delta^{13}C$ proxies for sources of SOC, PyOM, and plant-derived CO_2 emissions. The two-factorial experiment included the presence or absence of corn plants and of ^{13}C-labelled PyOM. In order to control for pH and nutrient addition effects from PyOM, its pH was adjusted to that of the soil and optimal nutrient and water conditions were provided to the plants. The $\delta^{13}C$ of PyOM sub-components were significantly different. Significant losses of 0.4% of the applied PyOM-C occurred in the first week. We find evidence for a "negative priming" effect of PyOM on SOC in the system (SOC losses are 48% lower with PyOM present), which occurred primarily during the first week, indicating it may be due to transient effects driven by easily mineralizable PyOM. Additionally, while the presence of corn plants resulted in significantly increased SOC losses ("positive priming"), PyOM additions counteract this effect, almost completely eliminating net C losses either by decreasing SOC decomposition or increasing corn C additions to soil. This highlights the importance of including plants in studies of PyOM-SOC interactions.

1. Introduction

Pyrogenic organic matter (PyOM) plays a critical but poorly understood role in the global carbon (C) cycle. PyOM is the product of biomass heated to relatively high temperatures (<700 °C) under low or no oxygen, and includes a spectrum of materials from lightly charred biomass to soot (Masiello, 2004; Lehmann, 2007; Laird, 2008; Keiluweit et al., 2010; Bird and Ascough, 2012). On a global scale, 50–500 Tg of PyOM are produced through wildfires annually (Kuhlbusch and Crutzen, 1995; Forbes et al., 2006), and data are emerging that PyOM is a more important natural pool of C in soils than previously thought (Skjemstad et al., 2002; Krull et al., 2006; Lehmann et al., 2008; Mao et al., 2012). Because PyOM is a more persistent form of C in comparison to the original biomass from which it is produced (Schmidt and Noack, 2000; Masiello, 2004; DeLuca and Aplet, 2008; Keiluweit et al., 2010; Bird and Ascough, 2012), its production and management have been proposed as a strategy for reducing atmospheric CO_2 stocks, in which case it is often referred to as "biochar" (Kuhlbusch and Crutzen, 1995; Forbes et al., 2006; Lehmann, 2007; Laird, 2008). However, interactions of PyOM with soil organic carbon (SOC) are still poorly understood.

PyOM additions have been shown to cause SOC to mineralize at a different rate than it would without the PyOM application, with the magnitude and direction of these interactions changing over time (Cross and Sohi, 2011; Jones et al., 2011; Keith et al., 2011; Luo et al., 2011; Zimmerman et al., 2011). Similarly, increased SOC mineralization in the presence of plant roots has been observed in many systems (e.g., Cheng et al., 2003; Dijkstra and Cheng, 2007; Pausch et al., 2013). These interactions are often described as "priming", where "positive priming" means a C pool (such as SOC)

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mineralizes more quickly when in the presence of another substrate (such as PyOM), while "negative priming" indicates it mineralizes more slowly (Bingeman et al., 1953). Recent papers on PyOM-SOC priming, examining different combinations of PyOM types and soils over different timescales, have shown mixed effects. For example, Luo et al. (2011) observed predominantly positive priming of SOC for different PyOM types in both low and high pH soils over 180 days, Cross and Sohi (2011) saw insignificant or negative priming over a range of soils and PyOM types over two weeks, while Zimmerman et al. (2011) observed initial positive (for low-temperature and grass PyOM) or neutral priming effects becoming negative (for higher-temperature and hardwood PyOM) over one year in a range of soils. However, we are aware of no published studies that have explicitly considered these effects in systems where plants are present. Since plant roots have been found to dramatically affect SOC cycling, it is likely that they also affect PyOM-SOC interactions, and PyOM additions may, in turn, affect plant root-SOC interactions (Major et al., 2010; Slavich et al., 2013). For example, Slavich et al. (2013) found that PyOM additions to soils planted with ryegrass increased total SOC in the top 75 mm more than could be explained by the PyOM additions alone, after three years. However, they were not able to partition the soil C between the ryegrass, original SOC, and added PyOM, and so could not conclusively determine how much each component contributed to total SOC.

To explain positive priming of SOC by PyOM, at least three key mechanisms have been proposed (Blagodatskaya and Kuzyakov, 2008; Jones et al., 2011; Zimmerman et al., 2011): (1) Co-metabolism – microbial mineralization of the easily mineralizable fraction of PyOM allows for the direct simultaneous mineralization of SOC and increases active extracellular enzyme levels, resulting in additional SOC mineralization. This effect can also be understood in terms of classic Michaelis–Menten enzyme kinetics, where rate of reaction is not linearly proportional to substrate concentration. If the concentration of the substrate (here, C) is initially limiting, and is then increased, a non-linear increase in reaction rate could occur, resulting in positive priming; (2) N or other nutrient stimulation – the addition of N or other nutrients in PyOM alleviates some microbial constraint, resulting in generally increased activity; (3) General stimulation – PyOM additions result in a beneficial pH shift or alleviation of physical constraints, resulting in generally increased microbial activity. In addition, Blagodatskaya and Kuzyakov (2008) describe an "apparent [positive] priming effect", where changes to the system result in increased microbial biomass turnover (appearing as increased CO_2 emissions), but do not affect the SOC mineralization rate. It can be challenging to distinguish this mechanism from the others, particularly since this increase in microbial activity may subsequently result in SOC mineralization, or "real" priming.

At least four general mechanisms have been proposed to explain the negative priming of SOC by PyOM (Blagodatskaya and Kuzyakov, 2008; Jones et al., 2011; Zimmerman et al., 2011): (1) Substrate switching – although much of the PyOM is highly stable, there is an easily mineralizable portion of PyOM (Cross and Sohi, 2011; Whitman et al., 2013) which may be used preferentially by microbes as a C substrate, resulting in decreased SOC mineralization; (2) Stabilization – PyOM may adsorb or otherwise physically or chemically stabilise SOC in the soil, making it more difficult for microbes to decompose; (3) General inhibition – PyOM additions may have a general inhibitory effect on the microbial community, decreasing total mineralization rates. For example, this could occur if PyOM additions shift the soil pH out of the optimal range, added toxic chemicals, or if PyOM inactivates microbial enzymes necessary for mineralization; (4) N inhibition – the sometimes inhibitory effect of N on SOC mineralization has long been noted (Fog, 1988), and the reasons behind this phenomenon are still not settled (Ramirez et al., 2012). However, although "black N" may play an important role in SOM cycling (Knicker, 2007), PyOM tends to have low available N. In fact, a low C:N ratio of some PyOM could lead to the immobilization of N during any PyOM mineralization, resulting in the opposite effect. In addition, we would add another potential mechanism, which is a variation on substrate switching: (5) "Dilution" – microbes may not use labile PyOM preferentially, but if it is used as readily as SOC, over very short timescales (hours to days), microbial populations are faced with a larger pool of C substrate, but have not yet grown to take full advantage of it – hence, a similar amount of total C is respired, but because a fraction of it is supplied by PyOM, less total SOC is respired. This mechanism would only be expected to be important over short time scales.

It is reasonable to expect that any or all of these mechanisms could take place given the right set of conditions, and many of the above mechanisms have analogues in plant root-SOC priming interactions. Thus, it is likely that the effects of plant roots and PyOM additions on SOC cycling could enhance, offset, or interact with each other. To investigate this gap in our knowledge, we ask how PyOM effects on SOC mineralization change with and without plants, hypothesizing that (i) important interactions may occur between plants, PyOM, and SOC in these three-part systems; (ii) PyOM will initially exert positive priming of SOC, becoming negative over time; (iii) plants will cause positive priming of SOC; and (iv) PyOM will counteract positive priming of SOC mineralization by plants. In addition, we investigated the optimal $\delta^{13}C$ proxies for sources of SOC, PyOM, and plant-derived CO_2 emissions.

2. Materials and methods

2.1. Soil type and PyOM production

Soil was collected from a mixed deciduous forest in Dryden, NY, which has not been burned within recorded history. It is dominated by oaks (*Quercus sp.*), red maple (*Acer rubrum*), sugar maple (*Acer saccharum*), white ash (*Fraxinus americana*), beech (*Fagus sp.*), basswood (*Tilia americana*), and hickories (*Carya sp.*), while understory species include hop hornbeam (*Ostrya virginiana*), musclewood (*Carpinus caroliniana*), and witch hazel (*Hamamelis virginiana*). The soil is a Mardin channery silt loam – a coarse-loamy, mixed, active, mesic Typic Fragiudept. It was collected from the top 0.5 m and was air-dried and sieved (<10 mm). PyOM was produced from maple twigs grown under a labelled ^{13}C atmosphere (see Horowitz et al., 2009), milled <2 mm and pyrolyzed at 325 °C in a modified muffle furnace under Ar gas. Initial soil and PyOM properties are listed in Tables 1 and 2, and Mehlich III-extractable nutrients in the Supplementary Table S1.

2.2. Treatments and experimental design

We used a 2 by 2 factorial design with corn (*Zea mays* (L.)) plants and PyOM as the two factors and 6 replicates for each of the 4 treatments. Pots were designed (Supplementary Fig. S1) based on those used by Yang and Cai (2006). Pots were constructed from 7.5-L white plastic buckets with a PVC tube fixed in the centre, extending 50 mm into the soil, into which the corn seeds were planted. This central tube was surrounded by an ethylene propylene diene monomer (EPDM) rubber cover, which could be stretched over the rim, sealing the chamber, or pulled back, leaving the chamber open to the air. In addition, a chamber vent 29 mm long with an internal diameter of 1.8 mm was installed to prevent pressure changes upon capping the chamber that could affect CO_2 evolution (Hutchinson and Mosier, 1981). Each pot received 7 kg soil. After bringing soil to 60% water-filled pore space (WFPS) with

Table 1
Initial soil properties.

Property (units)	Value
Texture	(Channery) silt loam
Bulk density (packed at) (g cm^{-3})	1.28
60% WFPS (g water g^{-1} dry soil)	0.28
Soil microbial biomass C (g kg^{-1} dry soil)	0.01
Soil microbial biomass C (g kg^{-1} SOC)	0.51
pH (0.01 M $CaCl_2$)	3.9
Total C (%)	1.1
Total N (%)	0.12
C:N	9.2
Water-extractable C (g kg^{-1} total)	5.1
100% WFPS (g g^{-1} dry soil)	0.45
Extractable NO_3^- and NO_2^- (mg kg^{-1})	3.73
Extractable NH_4^+ (mg kg^{-1})	20.48
Available P (Mehlich III, mg kg^{-1})	1.0
Particle size (mm)	<10
% sand	28.1
% silt	54.7
% clay	17.2

reverse osmosis water, pots were incubated in a greenhouse for 2 weeks, allowing for the flush of C made available through soil processing and the resulting microbial death to be respired. PyOM and corn seeds were added after this pre-incubation (day 0). 22 g PyOM was added (equivalent to ~3 t ha^{-1}) and mixed by hand throughout the soil. Pots that did not receive PyOM additions were also mixed by hand. In planted pots, two corn seeds were sown per pot and thinned to one plant after 7 days. All pots received a modified Hoagland's nutrient solution (Supplementary Table S2) every other day for the first 38 days, after which only pots with plants were fertilized, to reduce salt build-up in the unplanted soils. Pots of soil were maintained at 60% WFPS with reverse osmosis water over the course of the experiment by watering to weight every other day initially, and every day once plants had grown substantially.

2.3. Gas sampling

Chambers were sealed for gas sampling by stretching the EPDM cover over the pot rim and sampling with a syringe through a rubber septum in the side. When sampling was not taking place, the cover was retracted so the soil was exposed to the air and greenhouse light. CO_2 emitted from the soil was measured for each pot between 10 AM and 2 PM over a period of three months. After PyOM application and seed planting, measurement of CO_2 emission rates took place on days 1, 5, 9, and then every 7 days thereafter for the first month, after which sampling took place every 2 weeks. For each pot, 10 mL samples were taken every 11–15 min after sealing the chamber, depending on the rate of respiration, using syringes, which were sealed with rubber stoppers, for a total of 4 or 5 samples per pot. All samples were measured for CO_2 concentrations within 6 h of collection in a LI-6200 portable photosynthesis system coupled to a LI-6200 infra-red CO_2 analyzer (LI-COR Biosciences, Lincoln, NE). Measurements for isotopic analysis were taken less frequently, on days 1, 7, and then every 2 weeks thereafter. All pots were capped at the same time, and then each pot within a given treatment was sampled once to represent a different time point for that treatment, giving 6 data points from which to construct the associated Keeling plot (Pataki, 2003). This approach was designed to reduce the impact of drawing CO_2 from soil pores by advection during sampling (Nick Nickerson, personal communication). 15 mL of sample were injected into 12.5 mL evacuated exetainers (Labco). Samples were analyzed for CO_2 concentrations and $\delta^{13}C$ using a Gasbench II unit coupled with a Thermo Delta V Advantage Isotope Ratio Mass Spectrometer and a Temperature Conversion Elemental Analyzer (Thermo Scientific, West Palm Beach, FL). The $\delta^{13}C$ signature of emitted CO_2 was calculated using a Keeling plot ($[CO_2]^{-1}$ vs. $\delta^{13}C$) and extrapolating to the intercept. Only plots with an $R^2 > 0.90$ were interpreted.

Table 2
Initial properties and production conditions of PyOM.

Property (units)	Value
pH (0.01 M $CaCl_2$)	Initial pH 8.95; Adjusted to soil pH (3.9)
Total $C_{organic}$ (%)	61
Total $C_{inorganic}$ (%)	none
Total N (%)	0.87
Total H (%)	3.55
Total O (%)	21.63
C:N (by mass)	69
H:$C_{organic}$ (molar)	0.69
O:C (molar)	0.26
Water-extractable C (g kg^{-1} total)	0.61
Extractable NO_3^- and NO_2^- (mg kg^{-1})	0.4
Extractable NH_4^+ (mg kg^{-1})	2.0
Available P (Mehlich III, mg kg^{-1})	360.1
Feedstock	Sugar maple twigs
Particle size (mm)	<2
Heating rate (°C min^{-1})	2
Final temp (°C)	350
Residence time (hours)	2
Surface area (m^2 g^{-1})	50.5
Ash (%)	4.26
Volatiles (%)	40.12
Fixed carbon (%)	55.62

2.4. Biomass and soil sampling

All pots were destructively sampled at the end of the experiment. Soil samples for microbial biomass measurements were taken by using a soil probe to collect 2 cores from the full depth of each pot, totaling about 100 g moist soil. These samples were sieved <2 mm and stored at 4 °C until analysis, which took place within 48 h. Initial and final microbial biomass was measured using chloroform fumigation extraction (Vance et al., 1987), with 0.5 M KCl used as the extractant. After extractions, samples were dried in a 60 °C oven until only crystals remained, which were then ground with a mortar and pestle and weighed into tin capsules and analyzed for $\delta^{13}C$. Soil sub-samples were sieved to <2 mm and then ground in a Retsch mixer mill to a fine powder. The corn plants were divided into shoots and roots, washing soil from roots with water. Plant matter and soils were dried at 60 °C and stored in the dark until analysis. Plant samples were progressively ground in a Viking hammer mill, a Wiley mill, and a Retsch mixer mill, until a fine and homogeneous sample remained. Root sugars were extracted, following Brugnoli et al. (1988), as modified by Richter et al. (2009).

2.5. DOC and volatile PyOM analysis

DOC was extracted from soils and PyOM by shaking 100 g air-dried soil with 200 mL DIW or 7 g PyOM with 45 mL DIW for 10 min at 120 rpm, centrifuging for 15 min at 3000 × g, filtering the supernatant using a 0.70-μm glass fiber syringe filter, and then freeze-drying the filtrate for solid $\delta^{13}C$ analysis (Zsolnay, 2003). Volatile PyOM (the collected condensate released as a gas during pyrolysis, henceforth referred to as "tar") was collected and freeze-dried to remove any water before analysis for $\delta^{13}C$.

2.6. Solid sample $\delta^{13}C$ analysis

All dried and ground solid samples were weighed into tin capsules and analyzed in a Thermo Delta V Advantage Isotope Ratio

Mass Spectrometer and a Temperature Conversion Elemental Analyzer (Thermo Scientific, West Palm Beach, FL) to determine $\delta^{13}C$ values. For the PyOM tar samples, elemental analysis tins designed for liquid samples were used.

2.7. $\delta^{13}C$ of root-derived CO_2 and PyOM-derived CO_2

In order to determine the $\delta^{13}C$ of root respiration alone, we grew corn plants in a "no-C soil". We created the soil by ashing the same soil used for the greenhouse trial at 550 °C for 2 h, with the goal that this setup would best mimic the physical conditions of the soil. This resulted in a soil containing <0.05% C, which was negligible in relation to the magnitude of root respiration, allowing us to determine the $\delta^{13}C$ of the root respiration using three pots and the same gas sampling approach as in the main experiment. However, a similar approach was not successful for PyOM additions, because the CO_2 emissions from the <0.05% C of the ashed soil were not negligibly small in comparison to PyOM-derived emissions. This was evident because the initial $\delta^{13}C$ of the CO_2 emissions from the pot with ashed soil and PyOM additions was calculated to be +16 ± 5‰. This value is substantially lower than we would expect it to be if the CO_2 emissions were only derived from PyOM (bulk PyOM $\delta^{13}C$ of +27.2 ± 0.2‰, Table 4), indicating that SOC, with a lower $\delta^{13}C$ (initial bulk $\delta^{13}C$ of −25.93 ± 0.1‰, Table 3), is clearly a contributor.

Consequently, in order to determine the $\delta^{13}C$ of microbially-respired PyOM, we designed a PyOM incubation in a sand matrix (PyOM-sand). Six identical mixtures of 19.20 g quartz sand (ashed at 550 °C for 2 h to remove any C) and 0.80 g PyOM were created in amber glass 50 mL vials, with Hoagland's nutrient solution (Supplementary Table S2), a microbial inoculum, and deionized water added to bring the mixture to 60% WFPS. Vials were sealed, and each was sampled through a polytetrafluoroethylene silicone septum for $^{13}CO_2$ analysis on the GC–MS at a different time point over the course of a day. Their $[CO_2]$ and $\delta^{13}C$ values were used to construct a Keeling plot in order to determine the $\delta^{13}C$ of respired PyOM. In order to ensure that any C added in the microbial inoculum did not affect the $\delta^{13}C$ values, the microbial inoculum was derived from a water extraction of a PyOM-sand incubation, which, in turn, had been inoculated with a water extract of another PyOM-sand incubation, which had originally been inoculated with a 1:50 (m:v) soil–water extraction from the pots with PyOM additions. Thus, we aimed to ensure that the $\delta^{13}C$ of the microbial addition would resemble the $\delta^{13}C$ of the PyOM.

2.8. Data analysis

Soil CO_2 emission rates were calculated by fitting a quadratic curve to a $[CO_2]$ vs. time plot, and using the slope of the first derivative (*i.e.*, slope at $t = 0$) to represent the emissions rate. Where possible, these emissions were partitioned between SOC-derived CO_2 and PyOM-derived CO_2 using stable isotopic partitioning (Werth and Kuzyakov, 2010):

$$f_{PyOM} = \left(\delta^{13}C_{Total} - \delta^{13}C_{Soil}\right) \Big/ \left(\delta^{13}C_{PyOM} - \delta^{13}C_{Soil}\right)$$

and

$$f_{Soil} = 1 - f_{PyOM},$$

where f_{PyOM} represents the fraction of total CO_2 emissions attributable to PyOM, $\delta^{13}C_{Total}$ is the measured $\delta^{13}C$ signature of the combined sources, $\delta^{13}C_{Soil}$ is the measured $\delta^{13}C$ signature of the soil, and $\delta^{13}C_{PyOM}$ is the $\delta^{13}C$ signature of the PyOM. The end-member $\delta^{13}C$ values chosen to represent SOC and PyOM were the $\delta^{13}C$ signature of the emissions from the pots without PyOM on the corresponding day and the $\delta^{13}C$ value measured during the PyOM-sand incubation. (Further details and background on stable isotope partitioning is provided in the Supplementary Information.)

Final total belowground C was partitioned between SOC and either corn- or PyOM-derived C for the two-component pots, using the $\delta^{13}C$ of the final soil-only pot, the corn roots, or the bulk PyOM, respectively, as the end-member $\delta^{13}C$ values. We cannot conclusively partition the final C stocks in the pots that contained both plants and PyOM into three pools using only two isotopes. However, we can partition the total belowground C from these pots between (PyOM) and [SOC + corn-derived C] by making reasonable assumptions about the range of $\delta^{13}C$ values expected for the [SOC + corn-derived C] pool. We might predict that it would lie between a lower value equivalent to that of the final soil-only pots and an upper value that represents some combination of the soil and the corn. We could use the final value from the soil + corn pots, but if the corn deposited more C in the soil in the three-component pots or those pots experienced greater SOC mineralization, corn-derived C would account for a larger fraction of the total belowground C and shift its $\delta^{13}C$ signature to a higher value. To account for this fact, we also considered an extreme scenario where corn-derived C makes up 10% of the total [SOC + corn-derived C] (as compared to <1% observed in the soil + corn only pots). We then partitioned the total C in the three-component pots using both the upper and lower possible $\delta^{13}C$ values to represent the $\delta^{13}C$ of the [SOC + corn-derived C] fraction.

Where applicable, ANOVAs were used to detect significant treatment effects, after which Tukey's HSD was used to compare treatments. For paired or single comparisons, *t*-tests were used. Significance levels are $p < 0.05$ unless otherwise stated. Statistical analyses were performed using JMP 9 software (SAS Institute Inc., Cary, NC).

Table 3
Mean $\delta^{13}C$ signature of SOC proxies. Letters indicate significant differences (Tukey's HSD, $p < 0.05$).

Component	$\delta^{13}C$ (SE) (‰)	*n*
Initial bulk SOC	−25.93 (0.10) c	4
Final bulk SOC	−25.98 (0.04) c	6
Initial DOC	−24.05 (0.04) a	6
Final DOC	−24.31 (0.04) b	6
Microbial biomass	Not significantly different from bulk SOC	3
CO_2 evolved from soil-only pots (Day 1; Day 8)	−26.73 (0.30) ($R^2 = 0.99$); −24.80 (0.34) ($R^2 = 0.99$)	6

Table 4
$\delta^{13}C$ signature of PyOM components (*A. saccharum* pyrolyzed at 350 °C). Different letters indicate significant differences (ANOVA, Tukey's HSD, $p < 0.05$).

Component	$\delta^{13}C$ (SE) (‰)	*n*
DOC	+67.4 (1.7) a	5
Bulk PyOM	+27.2 (0.2) b	5
CO_2 evolved during PyOM-sand incubation ($R^2 = 0.99$)	+27.0 (0.6) b	6
Original material	+26.5 (1.0) b	3
Tars evolved and condensed during PyOM production	+22.4 (0.1) c	6

3. Results

3.1. CO_2 emission rates and plant growth

Total CO_2 emission rates were significantly higher with PyOM additions at days 1 and 4 for all pots (Fig. 1). CO_2 emission rates

decreased over the first three weeks in all pots. Corn plants emerged on day 3, and after two weeks, the CO_2 emissions from pots with plants were significantly higher than without plants ($p < 0.001$) as the corn plants grew larger, indicating a significant contribution from root respiration.

Although there seems to be a slight trend toward increased plant growth in soils that received PyOM, PyOM additions did not significantly increase above-ground ($p = 0.25$), below-ground ($p = 0.15$), or total ($p = 0.19$) plant biomass (Supplementary Fig. S2).

3.2. $\delta^{13}C$ signature proxies for soil, PyOM, and corn respiration

Soil DOC was significantly enriched in ^{13}C relative to the bulk SOC (Table 3). The final $\delta^{13}C$ of the DOC in soil without plants or PyOM was significantly depleted in ^{13}C compared to its initial value. The CO_2 evolved from soil without plants or PyOM was depleted in ^{13}C relative to the bulk SOC on day 1, but was enriched in ^{13}C by day 8 (Table 3).

The $\delta^{13}C$ of the CO_2 evolved from the incubation of PyOM alone was not significantly different from the $\delta^{13}C$ value of a bulk sample of PyOM (Table 4). However, the DOC from PyOM was significantly more enriched in ^{13}C, while the tars evolved and captured during PyOM production were significantly depleted in ^{13}C.

The $\delta^{13}C$ values of bulk corn shoots or roots did not differ at the end of the trial within or between pots with or without PyOM. Therefore, the combined mean values for pots with and without PyOM are reported (Table 5). Root sugars were significantly enriched in ^{13}C compared to bulk plant tissue. The sugars extracted from plant roots with PyOM additions were slightly enriched in ^{13}C compared to the sugars from those without PyOM additions (by 0.42‰). However, because replicate root sugar samples had to be combined to achieve sufficient mass for analysis, it is not possible to determine whether these two values are significantly different. Because root respiration signatures are only reported from sampling dates where the R^2 value of the Keeling plot was greater than 0.90, they are only available for later sampling dates, when the plants had reached relative maturity and substantially overwhelmed any trace emissions from remaining soil C in the ashed soil pots (initially 0.05% C). The $\delta^{13}C$ of the CO_2 evolved from the roots in the ashed soil was enriched in ^{13}C relative to final bulk plant tissue on day 21 and depleted on day 36. However, because the plant tissue was not sampled until the end of the trial, it is not necessarily appropriate to compare these values directly.

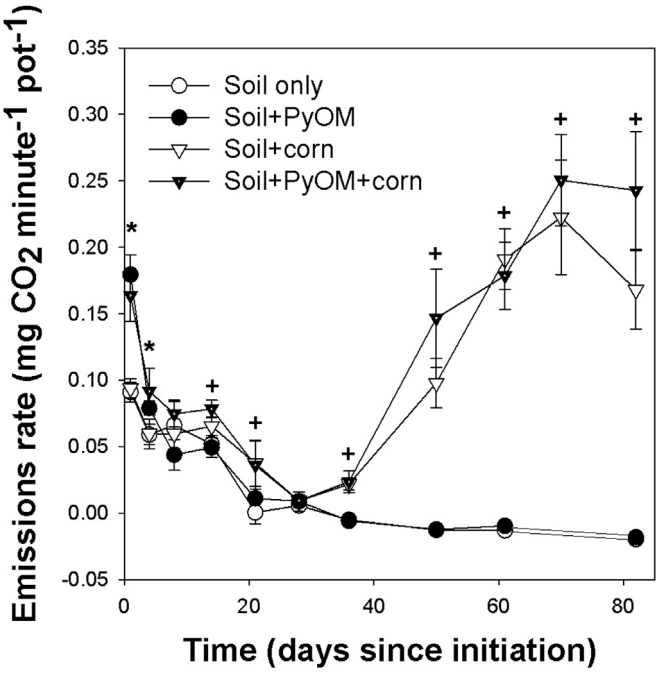

Fig. 1. Emissions rate over time. Error bars are ±1SE. * indicates significant differences between the +PyOM and -PyOM pots (t-test, $p < 0.05$, $n = 12$), and + indicates significant differences between the +corn and −corn pots (t-test, $p < 0.05$, $n = 6$).

Table 5
Mean $\delta^{13}C$ signature of *Zea mays* L. components, combined for plants grown with or without PyOM.

Component	$\delta^{13}C$ (SE) (‰)	n
Bulk shoots	−13.92 (0.05)	11
Bulk roots	−13.90 (0.05)	11
Root sugars	−12.81 (0.12)	1 (6 pooled samples)
CO_2 evolved from plant in ashed soil (Day 21)	−13.50 (0.25) ($R^2 = 0.99$)	3
CO_2 evolved from plant in ashed soil (Day 36)	−14.68 (0.37) ($R^2 = 0.99$)	3

3.3. ^{13}C partitioning of CO_2 emissions

SOC-derived CO_2 emissions were lower with than without PyOM additions on day 1 (t-test, $p < 0.05$) (Fig. 2). However, SOC-derived CO_2 emissions were not affected by PyOM additions by day 8. This indicates that short-term negative priming of SOC by PyOM occurred. Because emissions were partitioned between PyOM and SOC only on dates where the R^2 values for the calculated Keeling plot were greater than 0.90, partitioning data are only presented for days 1 and 8. These were also the only two sampling days where PyOM additions significantly increased respiration rates, suggesting that results from these dates might capture the bulk of any short-term PyOM priming effects due to additions of easily mineralizable C.

3.4. ^{13}C Partitioning of bulk soil C

Only PyOM additions significantly shifted the final $\delta^{13}C$ values of bulk SOC ($p < 0.0001$), while for DOC, both PyOM additions ($p = 0.014$) and corn plants ($p < 0.0001$) significantly changed the final $\delta^{13}C$ values (Fig. 3).

Total C significantly decreased from initial values in pots without PyOM additions (paired t-test, $p < 0.0001$), but did not decrease in pots with PyOM additions (paired t-test, $p = 0.21$). Partitioning the final total C between SOC and corn-derived C or PyOM revealed significant SOC losses in the pots with no additions (t-test, $p = 0.006$) and with corn plants (t-test, $p = 0.0005$), while pots with PyOM additions experienced no significant SOC losses over the course of the experiment (t-test, $p = 0.57$). Significantly less SOC remained in the pots with only corn (t-test, $p = 0.04$) (Fig. 4).

Partitioning the final soil C in the three-component pot yields a range of 84.1 ± 1.2 to 84.3 ± 1.3 g of [SOC + corn-derived C]. Both of these values are significantly higher ($p = 0.007$ and $p = 0.005$, respectively) than the final total C measured in the pots with corn plants (79.3 ± 0.8 g) (Fig. 4).

4. Discussion

4.1. CO_2 emission rates

Our finding that a non-negligible fraction of PyOM is very labile and easily metabolized by microorganisms during the first week is consistent with other studies (Nguyen and Lehmann, 2009;

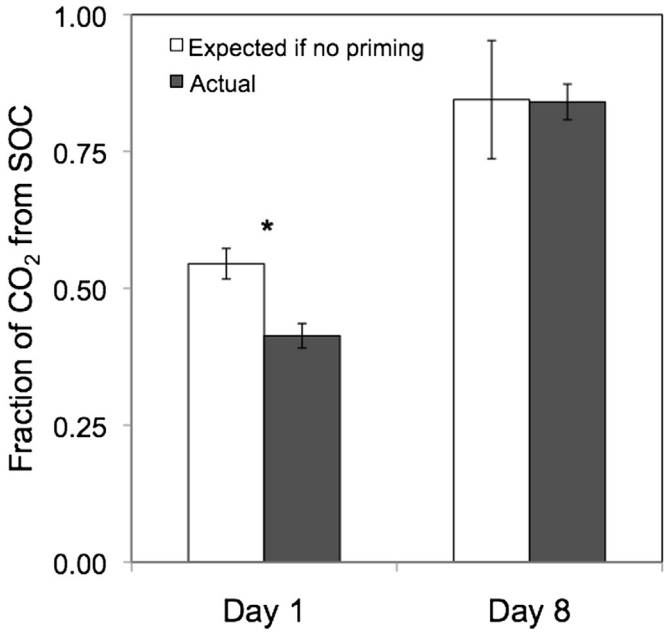

Fig. 2. Fraction of CO_2 emissions from SOC in soils with PyOM predicted under a no-priming scenario (white bars) and actual fraction, calculated using $\delta^{13}C$ partitioning (grey bars), on days 1 ($n = 12$) and 8 ($n = 6$: emission rates from day 8 only include pots without corn plants). Error bars represent ±1SE. * indicates a significant difference between the two treatments (t-test, $p < 0.05$).

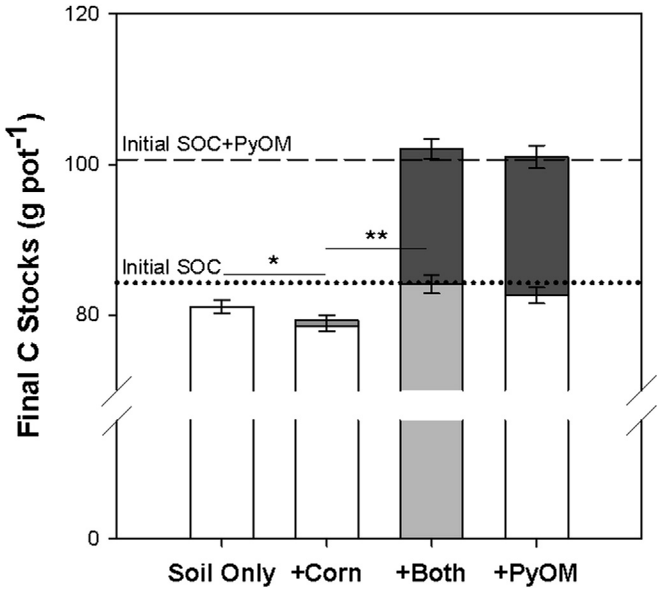

Fig. 4. Total final C stocks after ^{13}C partitioning. SOC is represented by the white bars, corn and PyOM-derived C are represented by dark grey bars, and the unpartitioned [SOC + corn-derived C] in the +Both treatment is represented by the light grey bar. Dashed line represents initial SOC + PyOM-C stocks, while dotted line represents initial SOC stocks. Horizontal lines and stars indicate significant differences in SOC (*) or [SOC + corn-derived C] stocks (**). Error bars represent ±SE ($n = 6$, except for the +PyOM + corn, for which $n = 5$). Note y-axis is broken to show detail.

Nocentini et al., 2010; Jones et al., 2011). This fraction was likely quickly depleted, mirroring the decrease in net respiration rate over time. This is a common pattern in incubated soils, where emissions decrease over time as easily mineralizable C — such as that released from protective aggregates during sieving and the drying-rewetting process — is depleted.

There was no detectable effect of PyOM on net emission rates by the time the corn was sufficiently large to contribute substantially to the soil CO_2 emissions, which supports the biomass data in showing that the PyOM did not have a significant effect on corn growth. However, this lack of an apparent PyOM effect on net soil CO_2 emissions in the pots with corn plants could also be the result of processes with opposite effects. *E.g.*, if PyOM additions increased plant growth, and, thus, root respiration, but at the same time exerted negative priming pressure on either SOC or root exudate-C, the net impact on CO_2 emissions could be unchanged. This highlights the need for three-part partitioning or other approaches (*e.g.*, Kuzyakov and Bol, 2004; Albanito et al., 2012) to detect such complex effects in future studies.

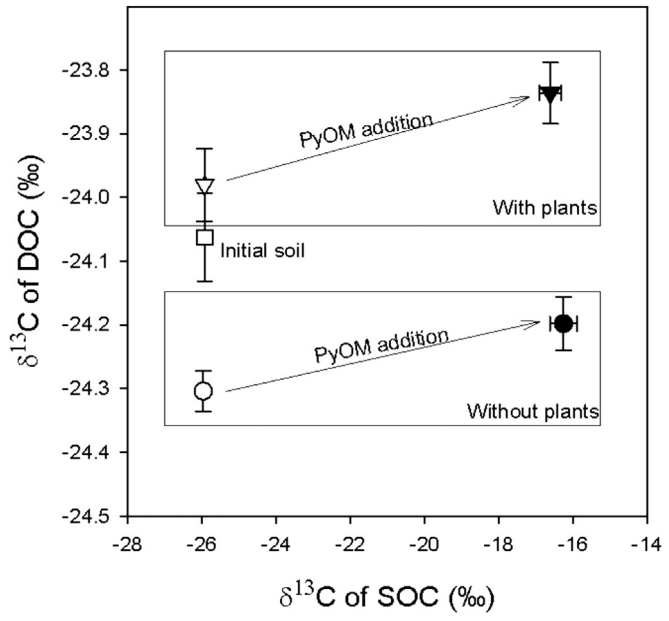

Fig. 3. $\delta^{13}C$ values of DOC and SOC at the end of the experiment (black symbols = with PyOM; white symbols = without PyOM; circles = no corn; triangles = with corn; white square = initial soil). Error bars represent ±1SE ($n =$ in Table 3).

The shift to negative net CO_2 emission rates in the pots without plants after 30 days could be explained by at least two factors. Initially, we speculated that this may have been a result of microbial nitrification — an autotrophic, C-fixing process where microbes use NH_4^+ as an electron donor for respiration, producing NO_3^-. The high nutrient levels in the pots from repeated fertilizer applications, without plants present to take up the nutrients, would have created favourable conditions for nitrification. Assuming a ratio of between 0.04 and 0.07 mol C fixed per mol NO_2 or NO_3 produced (Glover, 1985), this would predict nitrification rates of between 8 and 17 mg N consumed kg dry soil^{-1} day^{-1}. While these values are 1.5–3× higher than measured rates in some natural systems (*e.g.*, Cheng et al., 2011; Zhang et al., 2011), they are of the same order of magnitude, and would be expected to be high in such a low-C high-N environment, which would strongly favour nitrification. The second explanatory hypothesis is that some degree of photosynthesis was taking place. Upon disassembly of the pots, small green patches of algae or cyanobacteria were discovered growing on the insides of the pots, which must have allowed some fraction of photosynthetically active radiation to reach the soils through their white plastic walls. If these photosynthesized at the same rate as the maximum observed by Su et al. (2012) for algal-cyanobaterical crusts, then only 2% of the pot's sides would need to be covered to account for the observed CO_2 depletion. Even though the rates

shown by Su et al. (2012) are much higher than what would be expected on pot walls in this trial, it demonstrates that even a small amount of photosynthesis could also account for the observed net negative CO_2 flux from the soil. Thus, either explanation – nitrification or photosynthesis – may be plausible. However, while this is an interesting finding, it does not impact our conclusions regarding short-term negative priming, as the net negative fluxes only occurred during the latter two months, and the fluxes were not significantly different between the pots with and without PyOM.

4.2. $\delta^{13}C$ signature proxies for soil, PyOM, and corn respiration

During this study we also determined which isotopic proxies were optimal to represent soil, PyOM, and plant root respiration. While DOC was significantly enriched in ^{13}C relative to bulk SOC, the $\delta^{13}C$ of the respired CO_2 from the soil-only pots was initially depleted relative to both of these values (day 1), but was relatively enriched in ^{13}C compared to bulk SOC by day 8. These findings are consistent with Werth and Kuzyakov's review (Werth and Kuzyakov, 2010), where they found that DOC tends to be marginally enriched in ^{13}C relative to bulk SOC, but that CO_2 emitted from soils ranges between being depleted and enriched. Thus, while for day 1, the bulk SOC would have been a more appropriate proxy, for day 8, DOC would have been a better proxy. However, no matter which proxy is used in the isotopic partitioning calculations, we still detect negative priming on day 1. Thus, while our data support the importance of directly measuring the $\delta^{13}C$ values of soil respiration, they also indicate that if that is not possible, using either SOC or DOC as a proxy may not be a problem, depending on the system.

Similarly to soil respiration, we found that the $\delta^{13}C$ of root-respired C changed significantly over the course of the study, becoming more depleted in ^{13}C between days 21 and 36. This could represent shifting metabolic sources for the corn roots as the plants matured. The $\delta^{13}C$ values of respired CO_2 at these times were enriched and depleted in ^{13}C, respectively, in relation to the final $\delta^{13}C$ values of the corn shoots and roots (which were not significantly different from each other). This is consistent with previous studies, which have found a range of effects, with a number reporting both ^{13}C depletion and enrichment in root-respired CO_2 compared to root biomass (Werth and Kuzyakov, 2010). Contrary to our expectations, root sugars were not a good proxy for the respired CO_2, because they were significantly enriched in ^{13}C. This agrees with the observation of previous researchers that sugars tend to be enriched in ^{13}C compared to other plant tissues (Badeck et al., 2005; Bowling et al., 2008). Still, because the bulk roots and sugars were measured destructively only at the end of the experiment, some caution should be taken when comparing them to root respiration measurements taken during the experiment.

For PyOM proxies, we found that different sub-components had significantly different $\delta^{13}C$ signatures, the most striking being strong ^{13}C enrichment in DOC from PyOM and a depletion in the condensed PyOM tars. Materials such as cellulose, starches, and sucrose tend to be enriched in ^{13}C (Ehleringer et al., 2000), some of which would be expected to be extracted in the water-soluble PyOM fraction. Because lipids and lignins tend to be depleted in ^{13}C (Bowling et al., 2008), this might indicate that the tars were derived more from one or both of these compound types. Additionally, fractionation could have occurred while the tars were being volatilized: during both chemical and physical fractionation, the heavier isotope is usually discriminated against (Hobbie and Werner, 2004), resulting in a product (here, tars) that is depleted in that isotope. These results are somewhat compatible with those of Czimczik et al. (2002), who found that volatiles were depleted in ^{13}C relative to PyOM for lower-temperature PyOM materials, although their data show the opposite trend for PyOM created at temperatures within the range of those used in this experiment. What was surprising to us, given these differences in DOC from PyOM and tars, was that the $\delta^{13}C$ of what was actually respired in the PyOM-sand incubation was not significantly different from that of the bulk PyOM. We expect that this does not indicate that all portions of the bulk PyOM were respired proportionally to their abundance: Zimmerman et al. (2011) found that the $\delta^{13}C$ signature of respired lower-temperature PyOMs after 15–21 days tended to be depleted in comparison to the original bulk materials, but not by a consistent amount. In our case, we suggest that the combination of sub-components that were respired just happened to match the bulk signature of PyOM in this case (and would expect it to change over time, as was seen by Zimmerman et al. (2011)).

We considered the possibility that these differences in $\delta^{13}C$ values of PyOM materials were an artifact of the isotopic labelling process. For example, if the ^{13}C label was incorporated disproportionately into sugars and other water-soluble components, we might see these results. However, shifts of a proportional magnitude observed in PyOM produced from unlabelled *Acer saccharum* wood under the same production conditions indicate this is not the case (Supplementary Fig. S3 and Table S3). Thus, this effect of isotopic fractionation between PyOM sub-components does not seem to be simply an artifact of biomass labelling, and should be accounted for in all PyOM studies.

4.3. Priming of SOC by PyOM without plant influence

The observed negative priming appears to have taken place primarily during the first few days after the experiment's initiation, since SOC-derived CO_2 emissions were not affected by PyOM additions on day 8, and total emission rates declined in pots without plants over time. Indeed, by the end of the experiment, there was not a significant difference in total SOC remaining in pots with or without PyOM. The short-term negative priming in this system would be consistent with the model proposed by Blagodatskaya and Kuzyakov (2008), who find that when the easily available organic C added is in excess of 50% of the microbial biomass C, negative priming effects may be observed. The DOC from PyOM additions was equal to 1.3 times the extracted initial microbial biomass C (Tables 1 and 2). Because this effect was seen so rapidly and transiently, it may give clues as to the mechanism responsible for this priming. Jones et al. (2011) and Zimmerman et al. (2011) suggest that PyOM sorption of SOM or enzymes may be the most likely explanation for their observations of negative priming, which occurred later in their incubation experiments. Depending on the mechanism through which direct PyOM stabilization of SOC takes place, we might not predict that this was the dominant mechanism observed in this experiment: if direct stabilization was the mechanism driving the negative priming effect seen here, it would indicate that the stabilization occurred within the first 24 h (since negative priming was seen on day 1), but by day 8, this postulated sorption effect was no longer detectable in soil emissions. If this were the case, it would seem to indicate that the sorption capacity of the PyOM was rapidly filled, which is conceivable, although not consistent with the prolonged and later-appearing negative priming effects observed in previous studies. We suggest that a more likely candidate mechanism in this study would be a combination of substrate switching and the "dilution" effect. Because the soils were pre-incubated for two weeks before the corn seeds and PyOM were applied, in order to bring them to a relatively stable state, we might predict that the fraction of the PyOM additions that was readily accessible by microbes was either equivalent to (dilution effect) or "more appealing" than (substrate switching) the SOC. Because total emissions increased with the addition of PyOM, this indicates that the dilution effect could not be entirely responsible,

and that some degree of true substrate switching would have occurred, should these mechanisms be the correct ones. Because these soils developed under forested conditions, we may expect that the microbial community would have been regularly exposed to relatively complex C substrates, such as tree litter-derived lignin. This could lend support to both the substrate switching hypothesis – existing SOC may be less appealing than added PyOM – and the dilution effect hypothesis – dilution might occur even if the available PyOM was somewhat "unappealing". We suggest that N inhibition of SOC mineralization is not a likely reason for the negative priming because (1) substantial levels of N were added to all pots as fertilizer, and (2) PyOM additions would have had a negligible effect on available N levels (Tables 1 and 2).

4.4. PyOM effects on priming with plants

The positive priming of SOC by corn is consistent with previous studies (Dijkstra and Cheng, 2007; Bird et al., 2011), where active plant roots increased SOC losses. However, when PyOM was present along with plants, there was no significant loss in total C, suggesting that the increased SOC losses with plants present were counteracted by the presence of PyOM. This is consistent with the findings of Slavich et al. (2013), who noted increases in total soil C with PyOM additions in a ryegrass system beyond the increase expected by the addition of the PyOM. However, because of their experimental design, they were not able to partition their total soil C between SOC, PyOM, and plant sources. Our findings further expand our understanding of these interactions, by conclusively revealing no significant losses of PyOM while confirming SOC losses under corn plants without PyOM additions. Partitioning the total C between PyOM and [SOC + corn-derived C] revealed that significantly more [SOC + corn-derived C] remained in the pots with PyOM than those without, indicating that PyOM additions counteracted the positive priming of SOC caused by corn. However, it is not possible to say conclusively whether (1) the addition of PyOM to the planted soil changed the effects of corn on SOC, (2) the positive priming of SOC by corn remained the same but was offset by negative priming of SOC by PyOM, (3) the PyOM increased the contribution of corn C to total C (this is somewhat unlikely, as PyOM additions did not significantly increase plant root respiration or total biomass), or (4) some combination of the above occurred. We suggest that the presence of active plants in the system should be expected to alter priming dynamics between PyOM and SOC, but that more complex methodological approaches are needed to isolate the three different components.

4.5. Conclusions

PyOM additions to soil caused short-term negative priming of SOC, primarily occurring during the first week after PyOM additions. This negative priming of SOC by PyOM may be due to transient effects of labile PyOM additions to the system, through a combination of substrate switching and dilution. Additionally, PyOM additions counteracted the SOC losses incurred in the presence of corn plants, by decreasing SOC mineralization, increasing corn-derived C additions to the soil or a combination of the two. These findings have important implications for future studies, since no PyOM-SOC priming studies of which we are aware to date that allowed partitioning between PyOM and SOC have included plants.

The $\delta^{13}C$ values of different sub-components of PyOM are significantly different. While in this study, the best proxy for PyOM-derived CO_2 emissions was simply the bulk PyOM $\delta^{13}C$ signature, these findings highlight the need for further research into what fractions of PyOM are respired by microbes. An interesting direction of future study would be to further investigate this phenomenon, measuring the $\delta^{13}C$ values of different sub-components of PyOM, developing a fractionation process that is functionally meaningful, and to identify which sub-components of PyOM contribute the most to respiration, over varying timescales. Systematically exchanging extractable fractions between PyOM samples that are identical except for their $\delta^{13}C$ signatures, similar to a "reciprocal transplant" approach, and subsequently measuring the $\delta^{13}C$ of respired CO_2 would be an ideal approach.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2014.02.009.

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