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

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A B S T R A C T

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.

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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)

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