

# Carbon Mineralizability Determines Interactive Effects on Mineralization of Pyrogenic Organic Matter and Soil Organic Carbon

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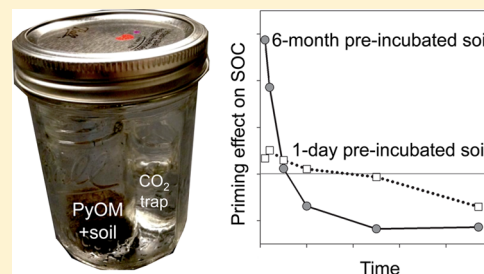
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## Supporting Information

**ABSTRACT:** Soil organic carbon (SOC) is a critical and active pool in the global C cycle, and the addition of pyrogenic organic matter (PyOM) has been shown to change SOC cycling, increasing or decreasing mineralization rates (often referred to as priming). We adjusted the amount of easily mineralizable C in the soil, through 1-day and 6-month preincubations, and in PyOM made from maple wood at 350 °C, through extraction. We investigated the impact of these adjustments on C mineralization interactions, excluding pH and nutrient effects and minimizing physical effects. We found short-term increases (+20–30%) in SOC mineralization with PyOM additions in the soil preincubated for 6 months. Over the longer term, both the 6-month and 1-day preincubated soils experienced net ~10% decreases in SOC mineralization with PyOM additions. Additionally, the duration of preincubation affected interactions, indicating that there may be no optimal preincubation time for SOC mineralization studies. We show conclusively that mineralizability of SOC in relation to PyOM-C is an important determinant of the effect of PyOM additions on SOC mineralization.



## INTRODUCTION

Soil organic carbon (SOC) stocks are a globally important pool of C, holding more than twice as much C as the atmosphere. The addition of exogenous organic inputs, such as fresh organic matter, plant root exudates, or pyrogenic organic matter (PyOM), are known to affect the cycling of existing soil organic C stocks, sometimes increasing and at times decreasing SOC mineralization rates.<sup>1,2</sup> These changes in SOC mineralization rates are often referred to as “priming”,<sup>3–5</sup> but we will use the specific terms “increased or decreased mineralization” here. Additionally, we use the term “mineralizability”, rather than “labile” or “recalcitrant” in order to be as neutral as possible about the causes of higher or lower mineralization, for example, physical protection vs chemical properties vs microbial community composition. “Mineralizability” is, thus, a relative term, and reflects the specific physical, chemical, and biological characteristics of a given soil.<sup>6</sup> Numerous mechanisms have been invoked to explain these observations, and these mechanisms have been revealed to be more complex than initially expected.<sup>7,8</sup> While the body of work that has focused on fresh organic matter or plant root inputs has certainly helped inform and form the basis for our understanding of PyOM–SOC interactions, PyOM as an input brings a suite of new complexities. These include high heterogeneity within and between PyOM materials, possible effects of PyOM on soil physical properties, particularly when applied at high rates, chemical effects of PyOM on the soil, particularly pH shifts and mineral nutrient additions in PyOM-associated ash, and the sorptive capacity of PyOM materials. Isolating and controlling for these diverse

factors in order to understand what drives PyOM–SOC interactions is necessarily complex, and is one reason much study to date has focused primarily on identifying the phenomena, rather than systematically testing for specific mechanisms that may inform model building.<sup>4</sup> Still, within the past few years (particularly, since work by Wardle et al.<sup>9</sup>), investigation into PyOM effects on SOC cycling has grown substantially, expanding our understanding of these dynamics. Reviewed recently by Maestrini et al.<sup>10</sup> and Whitman et al.,<sup>2</sup> diverse mechanisms can explain both increased and decreased SOC mineralization with the addition of PyOM materials. In this study, we focus on whether and how the mineralizability of the PyOM vs SOM can determine the impact of PyOM on SOC mineralization, and vice versa. We also consider the sorption of SOC on the surface of PyOM<sup>2,10</sup> as a proposed mechanism for observations of decreased SOC mineralization in the presence of PyOM.<sup>5,11–13</sup>

PyOM is produced naturally during fires,<sup>14</sup> as well as intentionally for C management.<sup>15</sup> Because of its heterogeneity, its interactions with SOC cycling are complex, and may depend on the properties of the specific combination of PyOM and soil. It has been postulated that the easily decomposable components of PyOM function similarly to fresh organic matter additions.<sup>2,10</sup> Thus, the amount of easily mineralizable C compounds

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in PyOM vs soil organic matter (SOM) could be an important predictor of these interactions. Specifically, if the addition of easily mineralizable PyOM alleviates an energy constraint for soil microbes, soils with less mineralizable SOM may be more prone to enhanced C mineralization.<sup>16</sup> Similarly, the mineralizability of the SOM and the associated microbial activity of a given soil could influence the mineralization of added PyOM-C. However, achieving a gradient of mineralizability of PyOM-C or SOC is not straightforward. For example, Keith et al.<sup>17</sup> used additions of fresh organic matter along with PyOM to investigate these effects. They found that increasing fresh organic matter additions to soils increased the decomposition of added PyOM, and that the soils with more fresh organic matter experienced decreased SOC mineralization rates with PyOM additions, whereas soils with no fresh organic matter additions experienced increased mineralization over a 3 month time period. However, fresh organic matter can be quite different from bulk SOM stocks, and this approach does not necessarily conclusively inform us how soils of different C composition would interact with PyOM additions. Another approach is to compare a diversity of soil types with PyOM produced from different feedstocks and temperatures. For example, Zimmerman et al.<sup>5</sup> found a larger increase in SOC mineralization when easily mineralizable PyOM materials were added to soils. The complicating factor with this approach is that using different soils and PyOM materials, by necessity, means other key properties, such as pH or ash mineral contents, will differ as well, necessitating a very large sample set in order to control for all potential confounding variables. Therefore, we attempted to manipulate the mineralizability of SOC and PyOM while keeping as many other properties constant as possible, by using preincubations of varying lengths for the soil and altering the amount of water-dissolvable compounds in the PyOM (DPyOM). It is important to note that in this approach, the changes in the SOC mineralizability will reflect both changes in the chemical and physical availability of the SOC, as well as changes in the soil microbial community.

Preincubations are often used to attempt to control for the disruptive effect of sampling, sieving, and/or drying and re-wetting soils when investigating SOC mineralization dynamics, but there is no commonly accepted protocol. For example, across 12 recent studies of the effects of PyOM additions to soil, seven studies did not report any preincubation, and those that did ranged from overnight to 23 days, with an average of 10 days.<sup>5,8,12,13,17–25</sup> If the amount of easily mineralizable SOC determines PyOM-induced changes to SOC mineralization, then the duration of preincubation may also affect the extent and direction of these effects.

We therefore studied the effects of PyOM additions on the mineralization of existing SOC as a function of the mineralizability of both PyOM and SOC. We hypothesized that (1) increased mineralizability of PyOM will result in greater increases in SOC mineralization, and soils with less mineralizable SOC will be more susceptible to increased mineralization with PyOM additions; (2) more PyOM-C will be mineralized in the soils with larger amounts of mineralizable SOC and greater microbial activity; and (3) preincubating soil for longer durations will change the mineralizability of SOC, thereby changing the impact of PyOM additions.

## MATERIALS AND METHODS

**Soil Type and PyOM Properties.** The soil (see<sup>25</sup>) was collected from a mixed deciduous forest in Dryden, NY, which

has not been burned within recorded history. 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 (<2 mm) (Supporting Information (SI) Table S1). The PyOM was produced from sugar maple (*Acer saccharum*) twigs grown under a regularly pulse-labeled <sup>13</sup>C atmosphere and mineral <sup>15</sup>N additions,<sup>26</sup> milled <2 mm and pyrolyzed at 325 °C in a modified muffle furnace under Ar gas (SI Table S2).

**Soil Preincubations.** To adjust the amount of easily mineralizable SOC without changing other soil properties such as pH, texture, or mineralogy, soils were preincubated for two different lengths of time—6 months, leaving SOC with lower mineralizability and 1 day, retaining the easily mineralizable SOC—before initiating the experiment. The same air-dried, <2 mm sieved soil was divided into two equal portions. Both were hand-mixed with 27% (w/w<sub>dry</sub>) water (57% WFPS), kept in a covered bucket that was opened regularly to ensure O<sub>2</sub> levels were not depleted while maintaining moisture levels (thereby mirroring conditions later induced during incubations in jars), and incubated at 30 °C in the dark for either 6 months or 1 day before beginning the experiment. This resulted in a 60% reduction in water-extractable C in the 6-month incubated soil (SI Table S1). Just before the initiation of the experiment, the water contents of both soils were determined and re-adjusted to be equal at 27% (w/w<sub>dry</sub>). Soils were sieved through a 4 mm sieve immediately before experimental initiation for optimal mixing.

Varying preincubation length to alter the characteristics of SOM has multiple implications. While preincubation effectively manipulates the SOM status, it would also be expected to affect the soil microbial community composition and activity. It is therefore difficult and even undesirable to separate or attribute the effects of one from those of the other, and should rather be seen as connected. A strong benefit of this approach is that it allows us to keep many variables as constant as possible, including pH, nutrients, management history, texture, and mineralogy. It would be challenging to find soils that contrast in organic matter status but not in one or many other ways.

**Water-Extractable PyOM and pH Adjustment.** To alter the amount of easily mineralizable PyOM,<sup>21</sup> water-extractable compounds were removed from PyOM through a series of three sequential DIW extractions. PyOM (60 g) was shaken with deionized water (DIW) (300 mL), after which it was syringe filtered through a <0.45 μm C-free glass filter. The resulting extracts were retained, and second and third DIW extractions were performed on the remaining PyOM, resulting in 2 mg dissolved C g<sup>-1</sup> PyOM. The remaining PyOM was divided into three equal masses. The extract was then returned to the PyOM at rates of 2x (“high DPyOM”), 1x (“medium DPyOM”) or 0x (“low DPyOM”), with the remaining liquid made up with nonextract DIW, so each received an equivalent volume of liquid. Thus, the three treatments received an addition of +1.3 mg DPyOM-C g<sup>-1</sup> PyOM, no change, or a decrease of -1.3 mg DPyOM-C g<sup>-1</sup> PyOM. Then, the pH of the resulting PyOM slurries was adjusted to match that of the soil (3.9 in 0.01 M CaCl<sub>2</sub>) using HCl additions, in order to control for pH effects and potential release of inorganic C from the PyOM (see SI for more detail). The resulting PyOM materials were dried at 70 °C.

**Soil and PyOM Incubations.** The incubations took place in 473 mL glass Mason jars. Each Mason jar received a 60 mL glass jar with the soil or soil–PyOM mixture and a 20 mL glass

vial with 15 mL 0.09 M KOH made with CO<sub>2</sub>-free DIW to trap CO<sub>2</sub> emissions, and 5 mL CO<sub>2</sub>-free DIW was added to the bottom of the jar to maintain a humid atmosphere. Each jar received 40 g moist (31.6 g dry) soil. The soils with PyOM additions received 200 mg PyOM (4.4 mg PyOM-C g<sup>-1</sup> soil). Jars were temporarily capped and rolled to mix soil and PyOM and then placed in the Mason jars. (Jars receiving no PyOM were also rolled.) KOH traps were added to the Mason jars immediately before sealing and incubating at 30 °C in the dark. Eight replicates were established for each treatment, and eight blanks with no soil additions—only DIW and a KOH trap—were also established. At the same time, a standard curve was created by sealing KOH traps in a series of jars with rubber septa in their lids and injecting a range of volumes of CO<sub>2</sub> gas. After at least 24 h of equilibration, the electrical conductivity (EC) of the traps was measured and linearly correlated with known CO<sub>2</sub> volumes to create a standard curve.<sup>27</sup>

A second incubation was run with PyOM alone in order to determine whether and how the δ<sup>13</sup>C signature of the mineralized PyOM-C changed over time, and a third incubation was run in order to answer two questions: (1) Are the effects of PyOM on SOC mineralization due to a nutrient subsidy? (2) Is there a short-term effect of soil preincubation on PyOM decomposition and its effect on SOC mineralization? (Details of both trials are in the SI.)

**Sampling Protocol for Determining C Mineralization and δ<sup>13</sup>C Values.** On days 1, 2, 5, 10, 25, and 47, jars were opened and the electrical conductivity (EC) of the KOH traps was measured at a constant temperature of 23.0 °C (EC is sensitive to temperature). The traps were then poured into 50 mL centrifuge tubes containing 5 mL 0.3 M BaCl<sub>2</sub>. Traps were replaced with fresh vials of KOH solution. DIW previously added to the bottom of the Mason jars was poured out and replaced with 5 mL fresh CO<sub>2</sub>-free DIW. Mason jars were resealed and returned to the incubation chamber. The BaCl<sub>2</sub> mixed with the KOH trap results in the precipitation of absorbed CO<sub>2</sub> as BaCO<sub>3</sub>. Precipitated solutions were centrifuged at 2500 rpm for 5 min. Supernatant solution was decanted, leaving the precipitate. The remaining precipitate was rinsed with 10 mL DIW, centrifuged again, and solution decanted, for a total of three rinses. The remaining precipitate was dried at 70 °C. BaCO<sub>3</sub> samples were acidified using H<sub>3</sub>PO<sub>4</sub> and the released CO<sub>2</sub> was analyzed for δ<sup>13</sup>C on a Thermo Scientific DELTA V isotope ratio mass spectrometer interfaced with a Gasbench II (Thermo Scientific, West Palm Beach, FL).

**Determining Total CO<sub>2</sub> Mineralization and δ<sup>13</sup>C Values.** To remove the effect of the small amount of CO<sub>2</sub> present in the air in the jar at the time of setup, measurements from “blank” jars with no soil additions were used. To determine total C mineralized by the sample, the average (*n* = 8) blank EC value for the corresponding sampling day was subtracted from each jar’s EC measurement. This delta EC value was then converted into total CO<sub>2</sub> released by the sample, using the standard curve (more details provided in the SI). To determine the δ<sup>13</sup>C signature (δ<sup>13</sup>C<sub>sample</sub>) of the C derived only from the sample (CO<sub>2-sample</sub>), we used the following equation:

$$\delta^{13}\text{C}_{\text{sample}} = \frac{(\delta^{13}\text{C}_{\text{total}} \cdot \text{CO}_{2\text{-total}} - \delta^{13}\text{C}_{\text{blank}} \cdot \text{CO}_{2\text{-blank}})}{\text{CO}_{2\text{-sample}}}$$

where

$$\text{CO}_{2\text{-total}} = \text{CO}_{2\text{-blank}} + \text{CO}_{2\text{-sample}}$$

To determine the amount of CO<sub>2</sub> sorbed by the blanks (CO<sub>2-blank</sub>), we subtracted the average EC value of the blank KOH traps after 24 h under lab conditions from the initial EC of a CO<sub>2</sub>-free KOH solution and converted it to a mass of CO<sub>2</sub> using standard curves. (This is equivalent to 548 ppm of CO<sub>2</sub> in the laboratory.) δ<sup>13</sup>C<sub>blank</sub> was calculated using the mean δ<sup>13</sup>C value of all blanks generated throughout the incubation experiment. The δ<sup>13</sup>C values of PyOM and SOC end-members changed over time, and were determined through incubations of each component on its own (see SI and Figure S1 for details).

**Nanoscale Secondary Ion Mass Spectrometry.** In order to investigate whether SOM is located on PyOM surfaces, we examined soil-incubated PyOM particles using nanoscale secondary ion mass spectrometry (nanoSIMS). The final incubated soil–PyOM mixtures were flash-frozen in sterile Whirl-Paks in liquid N<sub>2</sub> and stored at –80 °C. A subsample of one of the replicates from the 1 day preincubated soil with increased water-extractable PyOM treatment was air-dried, and a set of PyOM particles were removed with tweezers. They were laid on the surface of indium foil and then pressed into the soft metal under a glass slide using a PanaPress. The resulting sample was coated with ~10 nm Au to reduce any possible charging and imaged using a scanning electron microscope (Hitachi TM-1000, Krefeld, Germany) to find areas of interest. Then the sample was loaded into the Cameca NanoSIMS 50L spectrometer (AMETEK, Inc., CAMECA SAS, Paris, France). A ~2 pA Cs<sup>+</sup> beam (16 keV) was focused onto a ~150 nm sized spot and rastered over a 30 × 30 μm area. Secondary ions of <sup>12</sup>C<sup>-</sup>, <sup>13</sup>C<sup>-</sup>, <sup>16</sup>O<sup>-</sup>, <sup>12</sup>C<sup>14</sup>N<sup>-</sup>, <sup>12</sup>C<sup>15</sup>N<sup>-</sup> and <sup>28</sup>Si<sup>-</sup> were simultaneously detected at high mass resolution (*M*/Δ*M* > 7000 for <sup>12</sup>C<sup>14</sup>N<sup>-</sup>). 256 × 256 pixels were used for all images. Each measurement consists of 200 frames, and each frame was obtained over 131.072 s. Presputtering was required to remove surface contamination and Au-coating, as well as preparation of a mature crater with adequate Cs implantation. Presputtering was carried out on an area of ~35 × 35 μm to avoid crater effects in the analysis area.

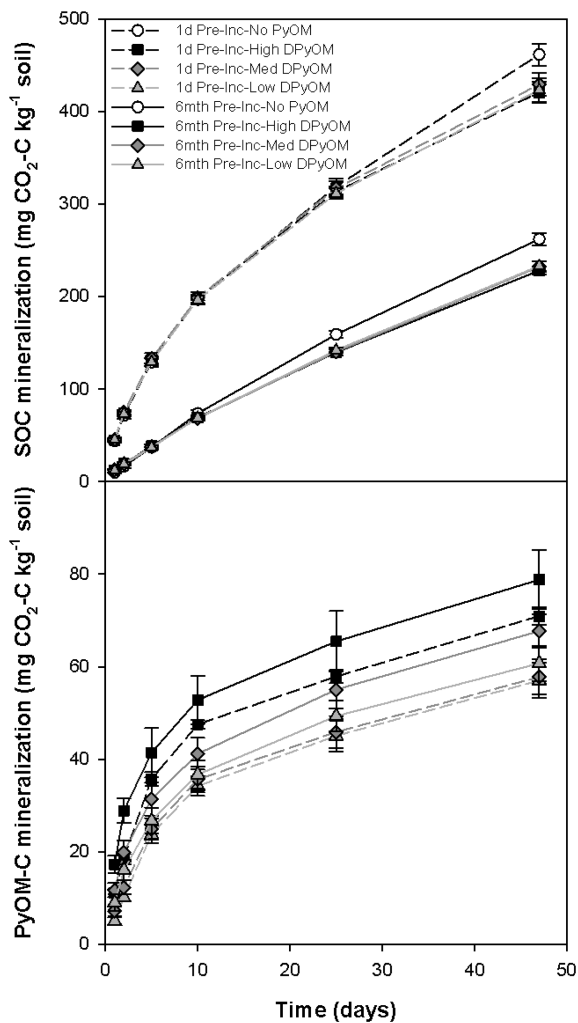
Image processing was performed using ImageJ software with MIMS plug-in (developed by Prof. Claude Lechene’s group at Harvard Medical School). For each secondary ion, frames were aligned (shift correction), then all 200 frames were added together as a stack. To map the relative <sup>15</sup>N enrichment of the samples, the stacked image for the <sup>12</sup>C<sup>15</sup>N<sup>-</sup> ion was divided by the stacked image for the <sup>12</sup>C<sup>14</sup>N<sup>-</sup> ion, returning a new calculated image of <sup>15</sup>N/<sup>14</sup>N ratios in the sample.

**Statistics.** If it was suspected that a jar leaked, because it was found to be a very strong outlier (11–21 standard deviations away from the mean of remaining samples), it was excluded from final analyses. This resulted in the exclusion of 8 samples out of 384, resulting in *n* values for each treatment at any given time point ranging from 6 to 8. All statistical analyses were performed in R.<sup>28</sup> We fit a linear mixed effects model to the cumulative SOC-derived CO<sub>2</sub> emissions and the PyOM-C-derived CO<sub>2</sub> emissions, with PyOM addition, soil, day, and jar ID as a random effect as factors, an interaction between PyOM and day (does the effect of PyOM change over time?), an interaction between PyOM and soil (do the PyOM additions affect the two soils differently?), and an interaction between soil and day (does the effect of soil preincubation change over time?) using the R package lme4.<sup>29</sup> To determine whether the interactions were significant, we performed a log-likelihood ratio comparison of a model with vs without the interaction term. To make posthoc comparisons within the models, we

performed pairwise comparisons between the different PyOM additions or soils for a given day with a Tukey adjustment of *p*-values, using the lsmeans R package.<sup>30</sup>

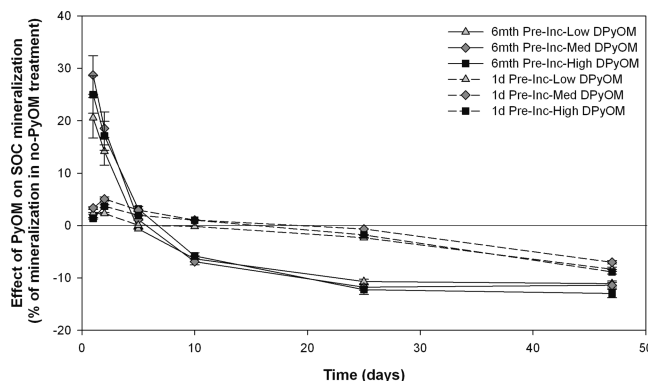
RESULTS

**SOC Mineralization.** SOC mineralization was significantly greater after the short-term than the long-term incubation (Figure 1, top; SI Table S5).



**Figure 1.** Cumulative mean C mineralization over time for SOC (top) and PyOM-C (bottom) for different amounts of water-dissolvable PyOM (DPyOM) and preincubation durations. Dashed lines indicate 1 day preincubated soils; solid lines indicate 6-month preincubated soils. Black squares, dark gray diamonds, light gray triangles, and white circles indicate high DPyOM, medium DPyOM, low DPyOM, and no PyOM, respectively. Note different scales on *y*-axes, to show detail. Error bars  $\pm 1SE$ , *n* = 6–8. Exact values and significant differences listed in SI Tables S5 and S6.

Cumulative SOC mineralization was initially (days 1 and 2) greater in the soils that received PyOM additions in comparison to those that did not receive PyOM additions (Figure 2; SI Table S5). Over time this trend reversed, with significantly less cumulative SOC mineralized in the soils that received PyOM compared to soils with no additions by day 25 of the experiment for the 6-month preincubated soil and by day 49 for the 1 day preincubated soil (Figures 1 and 2; SI Table S5). The day by PyOM addition interaction term in the mixed effects model



**Figure 2.** Mean cumulative relative effect of PyOM additions on SOC mineralization over time:  $(SOC_{PyOM} - SOC_{no\ PyOM}) / SOC_{no\ PyOM}$ . Dashed lines indicate 1-day preincubated soils; solid lines indicate 6-month preincubated soils. Black squares, dark gray diamonds, and light gray triangles indicate high DPyOM, medium DPyOM, and low DPyOM, respectively. Error bars  $\pm 1SE$ , *n* = (6–8).

was significant (*p* < 0.0001), indicating that this shift in the effect of PyOM on CO<sub>2</sub> emissions over time is significant. The soil by PyOM addition interaction had *p* = 0.052, indicating that the PyOM additions may have affected the total SOC-derived CO<sub>2</sub> emissions from the two soils differently, although it is not significant.

**PyOM Mineralization.** Cumulative PyOM-C mineralization was greatest with additions of more water-extractable PyOM and lowest with depleted water-extractable PyOM (Figure 1, bottom; SI Table S6). For a given level of water-extractable PyOM, cumulative PyOM-C mineralization was significantly greater in the 6-month preincubated soil mixtures, except for the PyOM with depleted water-extractable compounds, which were not significantly different between the two soils on days 1, 5, and 10 (SI Figure S2 and Table S6). This effect was initially greatest for the PyOM with depleted water-extractable C and lowest for the PyOM with increased water-extractable C (although the absolute effect is greatest in the PyOM with greater water-extractable C), and decreased and stabilized at a positive value over time.

**PyOM–SOC Mineralization Correlations.** Initial PyOM-C mineralization was positively correlated with SOC mineralization for the 6-month preincubated soil but not for the 1 day preincubated soil (Figure 3).

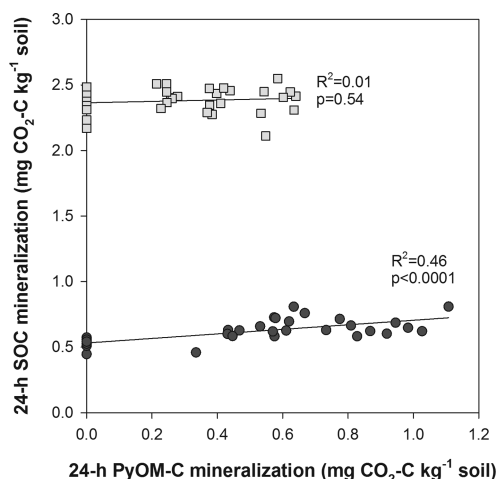
Cumulative PyOM-C mineralization over the first 2 days was negatively correlated with SOC mineralization across the range of studies and preincubation times (Figure 4).

The duration of preincubation affected the 48 h change in SOC mineralization with PyOM additions, with longer preincubation times resulting in greater increases in SOC mineralization, while short preincubation times yielded negative or no changes to SOC mineralization (Figure 5). There is not a clear “levelling-off” effect over the 6-month range investigated here.

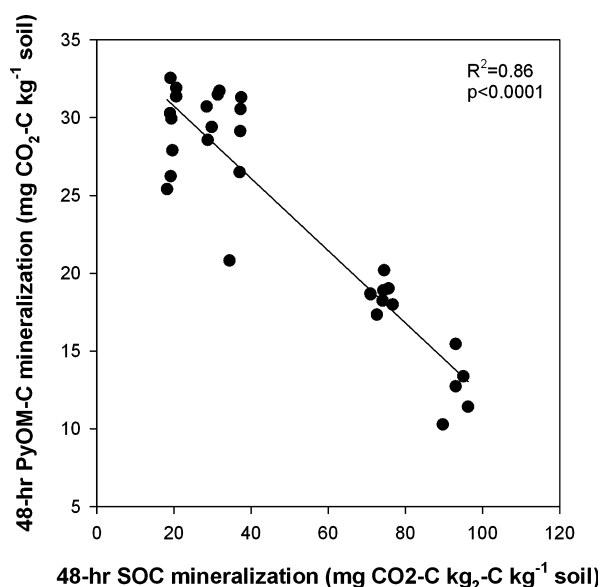
**NanoSIMS.** The SEM and nanoSIMS image map of the <sup>15</sup>N/<sup>14</sup>N ratios on the soil-incubated PyOM sample (SI Figure S3) indicates areas of relatively <sup>15</sup>N-depleted SOM regions on top of the relatively <sup>15</sup>N-enriched PyOM.

DISCUSSION

**Short-Term Increase in SOC Mineralization with PyOM Additions.** The observed short-term increases in SOC mineralization with PyOM additions after 6 months of preincubation are consistent with the growing array of PyOM–SOC

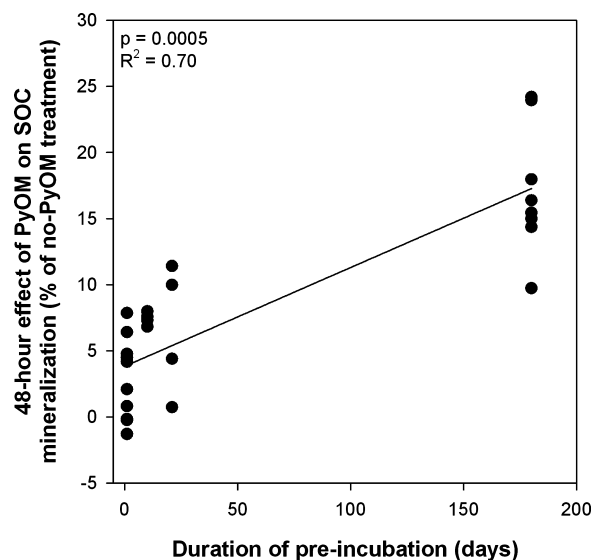


**Figure 3.** Relationships between SOC mineralized and PyOM-C mineralized over the first day of incubation within soils after 6 month (dark circles) and 1 day (light squares) preincubation. Lines indicate linear regressions: 1 day:  $y = 2.36 + 0.05x$ ,  $p = 0.54$ ,  $R^2 = 0.01$ ; 6 month:  $y = 0.53 + 0.173x$ ,  $p < 0.0001$ ,  $R^2 = 0.46$ .



**Figure 4.** Relationship between SOC mineralized and PyOM-C mineralized in the first 2 days across soil preincubations of 1, 10, 20, and 180 days and with high DPyOM additions. Line indicates linear regression,  $y = 35.38 - 0.23x$ ,  $p < 0.0001$ ,  $R^2 = 0.86$ .

mineralization studies that find that any increases in SOC mineralization with PyOM additions occur over relatively short time scales, with decreased SOC mineralization taking longer to emerge (e.g., refs 12, 17, 21, and 23). However, this study expands on previous studies in that it explicitly controlled for possible pH and nutrient addition effects of the PyOM additions on SOC, lending stronger support to the hypothesis that the short-term increased SOC mineralization is driven largely by the easily mineralizable fraction of PyOM. While this effect has largely been described in general terms by stimulation of the microbial community, resulting in increased enzyme production and metabolic activity, and shifts toward members of the microbial community that are best able to take advantage of the added substrate,<sup>1,5,7,17,21</sup> there are certainly deeper mechanistic layers to this explanation. Outstanding questions



**Figure 5.** Effect of preincubation duration on relative effect of PyOM (High DPyOM) on SOC mineralization. Line indicates linear regression,  $y = 3.82 + 0.0747x$ ,  $p = 0.0005$ ,  $R^2 = 0.70$ . Excluding the 180 day time point still yields a significant positive correlation  $y = 2.748 + 0.235x$ ,  $p = 0.031$ ,  $R^2 = 0.25$ .

include, among others: (1) What specific soil and PyOM properties lead to such a stimulation of the microbial community? (2) Which members of the soil microbial community are responsible for this response, and how does the community differ in the soils with lower mineralization? While this experiment does not address the second question, we are able to make some inferences with regard to the first.

The 6-month preincubated soil experienced greater increases in mineralization with PyOM additions. This could be explained in at least two ways: (1) If, in the 1-day preincubated soil, the microbial community is not C-limited. Thus, the addition of PyOM does not alleviate any constraint and does not result in increased SOC mineralization. (2) If, while SOC mineralization was actually inhibited by direct mineral N additions (SI Figure S4), the accumulated mineral N in the 6 month preincubated soil could have allowed for increased access to the added PyOM, which, in turn, stimulated the microbial community and increased SOC mineralization. These two explanations are not incompatible—increased mineral N in the 6 month preincubated soils could have alleviated the N constraint on PyOM-C mineralization (SI Figure S7), allowing access to the PyOM-C, which, in turn, alleviated the C limitation in the 6-month preincubated soils. The fact that short-term increased PyOM mineralization is positively correlated with increased SOC mineralization for the 6-month preincubated soil (Figure 3), while across soils, less PyOM was mineralized in the 1-day preincubated soil (Figure 4) is consistent with this interpretation. These observations may indicate that the mineralizability of the SOC determines whether PyOM-C is a more attractive substrate (with less easily mineralizable SOM being more susceptible to increased mineralization by PyOM additions), since increasing the amount of easily mineralizable PyOM increases SOC mineralization over the short-term. This interpretation is consistent with the work of Fontaine et al.,<sup>16</sup> who found that SOC decomposition in the subsoil was limited by available C. Thus, lower-temperature PyOM materials, which are more readily mineralized,<sup>31,32</sup> may be more likely to cause increased SOC mineralization. This is also consistent

with the work of Keith et al.,<sup>17</sup> who found that the addition of fresh organic matter along with PyOM resulted in a net reduction in the (fresh organic matter + SOM) decomposition. Our results therefore extend prior research to further support the hypothesis that the mineralizability of SOC affects the magnitude and direction of changes to SOC mineralization induced by PyOM additions.

**Longer-Term Decrease in SOC Mineralization with PyOM.** Over time, PyOM additions resulted in a net decrease in SOC mineralization for both soils. This decrease emerged later for the 1 day preincubated soil. There are at least five potential mechanisms<sup>2,10,33</sup> that we believe we can rule out. First, we suggest that in this case, the decrease in SOC mineralization was not caused by substrate-switching or dilution effects.<sup>25</sup> Both of these effects depend on a greater or equal “appeal” of the PyOM as compared to SOC as a substrate, and should be driven largely by the better available fractions of PyOM-C that are mineralized initially. Thus, under these mechanisms, the effects would likely be expected to occur in the first few days of the incubation, and would not be expected to emerge only after a week or more, as was seen here. Second, because we adjusted the pH of the PyOM to match that of the soil, we do not expect that these changes reflect the release of inorganic C, or are due to the shift of the soil’s pH to one less favorable for microbial activity. Third, because the PyOM was only added at a rate of 0.3% by mass, we would argue that changes to the physical properties of the soil are unlikely to be driving these effects, especially because the water contents were optimized. Fourth, while N inhibition is commonly observed in soils,<sup>34,35</sup> this is not likely the case in these soils; while SOC mineralization in the studied soils was also inhibited by N additions (SI Figure S4), the amount of mineral N added with the PyOM was only between 0.01 and 0.1% of existing soil mineral N. Finally, it may be worth considering whether other nutrient additions could explain this inhibition of SOC mineralization. We did see some inhibition of the cumulative SOC mineralization over the first 2 days with the addition of nutrients equivalent to those added in the increased water-extractable PyOM treatment (SI Figures S5 and S6). However, the Mehlich III-extractable nutrients varied almost 2-fold across the three PyOM treatments, and the effect of PyOM on the SOC mineralization did not change proportionally—indeed it was not significantly different across the three treatments. Thus, if we thought that nutrients were primarily responsible for this effect, we would likely also predict that the effect might be proportional to the level of nutrients in the PyOM and the soil (SI Figure S5). However, we see significant differences in changes to SOC mineralization between the soils, which had relatively similar levels of nutrients, but no significant differences between the types of PyOM additions, across which the levels of nutrients varied almost 2-fold. Thus, we would argue that this mechanism, while potentially active, is not primarily responsible for these effects.

We suggest that sorption of SOC by PyOM,<sup>36,37</sup> making it less available to microbes, may play an important role in decreasing SOC mineralization over time.<sup>7</sup> If we postulate that it is the bulk PyOM, and not the water-extractable PyOM that is largely responsible for any sorption of SOM, then we would not necessarily expect to see significant differences between the different PyOM additions, which is consistent with our findings. It might be more difficult to postulate how the SOC properties would be expected to affect PyOM sorption, as physical and chemical properties of the PyOM and the SOC both likely

change over the course of the incubation. We could predict that there is a limited quantity of sorption sites on the PyOM, and, once filled, stabilization would stop. The rate at which this would be expected to occur would depend on the kinetics of sorption—thus, we might predict that the SOC in the soil with a higher concentration of easily mineralizable SOC would be sorbed more quickly, which we cannot confirm from our data. However, it is also possible that this sorption is more limited by the rate of formation of sorption sites on the PyOM as it oxidizes in the soil, and thus we would not necessarily predict that we should see differences in the two soil types. Zimmerman et al.<sup>5</sup> consider sorption mechanisms in detail, citing sorption of SOC on PyOM surfaces and within pores as well as sorption and inactivation of enzymes on PyOM as mechanisms for reduced SOC mineralization with PyOM additions.

While we did not test explicitly to differentiate between sorption mechanisms, we did find some indication using nanoSIMS that SOM was located on PyOM surfaces (SI Figure S3). Still, it is important to interpret this image within the constraints of the technique: this is only a single  $30 \times 30 \mu\text{m}$  region of a single sample of soil-incubated PyOM. Thus, while fine-scale observation techniques are inherently required to directly observe phenomena that occur at very small scales, we should not extrapolate these findings to make a statement about their importance at larger scales. What it does indicate is that PyOM surface-SOM interactions may be occurring in the soil, and are worth further investigation. For future nanoSIMS investigations, we would recommend that researchers take care to design experiments using materials with high (i.e., >10 atom % range) enrichments, which would allow for higher throughput analyses.

**Soil C Status Effects on PyOM-C Mineralization.** Cumulative PyOM-C mineralization was greatest in the incubations that had increased DPyOM and lowest in the treatments that had depleted DPyOM (Figure 1, bottom; SI Table S6). The amount of cumulative mineralized PyOM-C was proportional to the amounts of DPyOM-C (SI Figure S7). This confirms that a detectable proportion of the easily mineralizable PyOM-C was in the form of water-extractable compounds, although a large portion of rapidly mineralizable PyOM-C certainly remained postextraction, as indicated by the PyOM mineralization in the DPyOM-depleted treatment. The finding that PyOM-C mineralization was higher in the 6 month preincubated soil is somewhat in opposition to the results of Keith et al.,<sup>16</sup> who found that increasing additions of fresh organic matter to soils along with PyOM resulted in increased mineralization of the PyOM. While we might have predicted that the more active soil would have generally higher microbial activity, and thus result in greater PyOM mineralization, this did not occur. Because the bulk of this effect occurred over the first couple of days, one possible explanation is that the effect was driven by the relative appeal of the C substrates in the two soils. The easily mineralizable PyOM was used preferentially in the 6 month preincubated soil, but was less appealing in the 1 day preincubated soil. This effect likely largely acted upon the DPyOM-C, and diminished after a few days as this source was depleted in both soils. Additionally, the 6-month incubated soil had higher mineral N, which could have further facilitated the mineralization of the added PyOM-C, the decomposition of which is likely N-limited (SI Figure S8).

**Effects and Implications of Preincubation Duration on SOC–PyOM Interactions.** Preincubations are common practice in studies of SOM cycling, employed in order to allow C mineralization in the soils to stabilize to some extent after the liberation of fresh SOM during experimental procedures that

may include drying, rewetting, sieving, and temperature changes. While the reasoning that the changes induced temporarily by these manipulations are artificial and not germane to the processes we most want to study is valid, the challenge is determining at what point of preincubation it is appropriate to initiate amendments or possibly any soil manipulation. The data in this study indicate that even if respiration rates seem to have leveled off, soils may still not have reached a meaningful “steady state”. The change in short-term SOC mineralization in this experiment was 9 times greater in the 6 month compared to the 1 day preincubation. Of course, it is impractical to recommend 6-month preincubations, and even then, no indication exists that a final point was reached, at which we could determine the “true” effect of PyOM on SOC mineralization. Perhaps it is best to (1) think of the state of the soil as a continuum, rather than imagining it possible to allow it to “equilibrate” in a meaningful way, (2) discount or only carefully evaluate the practical importance of any very short-term effects, (3) attempt to design studies where soils are maintained in as natural conditions as possible, such as intact soil cores (challenging for studies involving the introduction of amendments), or (4) tie the manipulations or time scale to analogous real-world processes (e.g., PyOM additions to agricultural systems while tilling or disking soils).

In summary, we found that the 6 month preincubated soil, with overall lower SOC mineralization and microbial activity, was more susceptible to short-term increases in SOC mineralization with PyOM additions, which were proportional to the mineralization in the added PyOM. Both soils experienced net long-term decreases in SOC mineralization with PyOM additions, possibly due to stabilization of SOC on PyOM surfaces. Additionally, we showed that the duration of preincubation of soils before PyOM additions between 1 day and 6 months resulted in a 9-fold increase in the changes to short-term SOC mineralization, indicating that there may be no optimal duration of preincubation for SOC mineralization studies.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Additional information as noted in the text. This material is available free of charge via the Internet at <http://pubs.acs.org/>

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### Notes

The authors declare no competing financial interest.

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