



Microbial mineralization of pyrogenic organic matter in different mineral systems



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ABSTRACT

The objectives of the study were to (i) investigate whether short term mineralization varies to a greater extent by charring organic matter (OM) or by exposure to different mineral surfaces, (ii) examine the effect of clay mineralogy on mineralization of either charred or uncharred OM and (iii) quantify these differences in mineralization in the context of C evolution during charring. Mineralization of different combinations of OM types (corn stover, cellulose, glucose, lignin and arginine) or their corresponding pyrogenic organic matter (PyOM) with minerals (quartz, corundum, goethite, kaolinite and kaolinite mixed with goethite) were assessed in incubation experiments at 30 °C for 196 days. All PyOM materials showed lower mineralization extent (3.8–7.5% of initial C) than uncharred OM (7.6–89% of initial C). Charring decreased mineralization to a greater extent (by 4.7–95%) than differences in the minerals (by 3.7–70%). The relative standard deviation of C mineralization of individual charred OM materials caused by differences in mineralogy was smaller (8.7–16%) than that of uncharred OM (8.7–65%). For the ratio of C remaining after both mineralization and charring to mineralization of uncharred biomass, the relative standard deviation was higher for different OM types (51–84%) than mineral types (1.1–47%), suggesting that mineralogy was less important than OM properties. Therefore, the importance of mineralogy was lower for charred than uncharred OM and the type of PyOM more strongly controlled mineralization than the type of mineral during the initial decomposition process.

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1. Introduction

Soil organic carbon (SOC) is important in the global C cycle because soils contain about three times more C than the atmosphere (Lal, 2004). It has been recognized that small changes in the SOC stock can have a significant impact on atmospheric CO₂ concentration (Eglin et al., 2010). Properties of plant residues determine short term mineralization and decomposition (Cotrufo et al., 2013), but do not account for long term C persistence in soil (Schmidt et al., 2011). Rather, organic matter (OM) is stabilized in soil mainly through interaction with minerals (Jones and Edwards, 1998; Baldock and Skjemstad, 2000; Lalonde et al., 2012; Barré et al., 2014; Kleber et al., 2015a).

Pyrogenic OM (PyOM), the residue of incomplete combustion of biomass or fossil fuel, has recently attracted growing attention as an important component of slow cycling SOC (Preston and

Schmidt, 2006; Knicker, 2011; Woolf and Lehmann, 2012; Wang et al., 2015; Weng et al., 2015). Charring biomass has been proposed as a way of diverting C from a rapid biological C cycle into a slow geological C cycle (Kuhlbusch and Crutzen, 1995) and thereby to sequester atmospheric CO₂ into soil (Lehmann, 2007; Woolf et al., 2010). It is unclear by how much charring reduces OM mineralization compared with interaction with soil minerals, which is known to be key for the protection of uncharred OM.

Even though PyOM was found to have long mean residence time compared with uncharred OM, initial mineralization of PyOM after addition to soil can be significant (Lehmann et al., 2015a; Wang et al., 2015). In contrast to litter decomposition (Miltner and Zech, 1998), there have been few studies of short term decomposition of PyOM as affected by soil mineral properties, despite findings that PyOM may be bound to mineral particles (Brodowski et al., 2005). Indeed, soil PyC content has been shown to correlate with (Cusack et al., 2012), and be mineralized more slowly (Santos et al., 2012) in the presence of, short range order minerals. In addition, mineralization of PyOM was significantly reduced with increasing clay content from 11 to 23% in three

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Danish soils (Bruun et al., 2014). However, Kuzyakov et al. (2009) observed similar mineralization of PyOM in an incubation experiment with loess or with soil, and Liang et al. (2008) did not find any difference in mineralization of PyOM-rich terra preta soils as a function of soil texture. Thus, the importance of interaction of PyOM with minerals for its short term decomposition remains poorly understood.

OM in general is known to be protected in soil through physical occlusion within soil aggregates and/or sorption of OM onto mineral surfaces (Wershaw, 1993; Sollins et al., 1996; Baldock and Skjemstad, 2000; Kleber et al., 2007; Barré et al., 2014). SOM stabilization does not depend only on the type of mineral but also on the interaction between the form of OM and mineral due to their different functional groups. Polymethylene structures were preferentially sorbed to kaolinite and montmorillonite (Ghosh et al., 2009), while lignin had a low affinity for them (Asselman and Garnier, 2000). Carboxyl groups preferentially interact with goethite, while amide groups of proteins interact with montmorillonite (Ghosh et al., 2009). Ferrihydrite and aluminium hydroxide reduced the mineralization of bulk OM by 12% and 65%, respectively, probably also by adsorption to the oxide or hydroxide groups (Miltner and Zech, 1998). The addition of goethite to kaolinite particles reduced cumulative C mineralization by 11% for a 144 day incubation (Saidy et al., 2012). The extent to which short term decomposition of PyOM also depends on both the type of PyOM and mineral is not known. In addition, C loss during charring is influenced by OM properties (Knicker, 2011; Enders et al., 2012). Whether the combined C loss from charring and mineralization is more dependent on OM type or mineral type, has not been studied.

The aim of this study was to understand the combined effects of charring, type of OM and mineralogy on short term decomposition. The objectives were to: (i) investigate whether short term mineralization varies to a greater extent by charring OM or by exposure to mineral surfaces, (ii) examine the effect of clay mineralogy on C mineralization of charred and uncharred OM with different properties and (iii) evaluate the differences in mineralization in relation to C evolution during charring.

2. Material and methods

2.1. Materials

The following were obtained from chemical companies: glucose (Sigma G5767), cellulose (Aldrich 435236), lignin (Aldrich 370959), arginine (Aldrich A5006), quartz (Sigma 274738), corundum (Sigma 234745), goethite (Sigma 71063) and kaolinite (Fluka 03584). Corn stover was collected in Wisconsin from a local supplier and crushed to fine powder prior to the experiments. In order to supply nutrients to microorganisms during incubation, a mixed nutrient powder (Hoagland's modified basal salt medium) was obtained from MPBio (Solon, OH, USA).

2.2. PyOM production

PyOM was produced from the five OM feedstocks using a lab-scale pyrolysis reactor. An aliquot of ca. 500 g feedstock was manually placed into a main chamber, which was thoroughly purged with N₂ while the mixer was running. The material was pyrolyzed in a uniformly heated chamber for 250–260 min. The period included both raising the temperature from ambient to target (600 °C) at 2.5 °C/min, and holding at the target temperature for 30 min. After completion of pyrolysis, the furnace was turned off and the main chamber was allowed to cool before the PyOM was unloaded under N₂ purge to reduce rapid oxidation and auto-ignition. The PyOM was preserved in closed plastic bottles after

weighing. Both PyOM and uncharred OM were ground with mortar and pestle and sieved with mesh #400 (37 µm) prior to analysis and use for the incubation experiments.

2.3. Physical and chemical analysis

Proximate analysis of PyOM was conducted using ASTM D1762-84 Chemical Analysis of Wood Charcoal after modification (Joseph et al., 2009) to quantify ash, volatile matter and fixed carbon (carbon is written out to indicate that this is an operational definition and does not refer to the element C). For elemental analysis, total C and N were determined by way of Dumas combustion using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). Hydrogen was determined by combustion using a Hekatech HT oxygen analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Total O was derived by subtraction according to the ASTM method.

The pH was measured at a 1:20 w/v ratio using deionized water under continuous stirring after agitation on an orbital shaker table for 1.5 h. Water extractable OM was quantified gravimetrically by mixing 0.2 g OM or PyOM with 10 ml deionized water and shaking at 150 rpm for 24 h. The solution was moved onto Al trays after filtering and the weight difference was measured before and after drying at 105 °C for 24 h.

Water holding capacity of minerals was measured from the weight difference before and after drying the saturated samples with deionized water at 105 °C for 24 h. All analyses described above were performed in duplicate.

Particle size distribution of the mineral and PyOM powder was determined using a Cilas 1064 laser particle size analyzer with a measurement range from 0.04 to 500 µm in wet dispersion mode. BET specific surface area and pore volume of minerals and PyOM were determined on the basis of N₂ adsorption at 77.3 K by using a surface area analyzer (ASAP 2010, Micromeritics, USA). The specific surface area was calculated according to the Brunauer–Emmett–Teller (BET) method (Supplementary Online Table S1).

2.4. Measurement of CO₂ evolution

The CO₂ evolved during incubation was measured by absorbing it in a KOH solution and measuring the conductivity with a conductivity meter (Orion E011010 and Orion EC 115A+, Thermo Scientific, USA). To establish a standard curve, a known volume of 99.8% pure CO₂ was injected into gas-tight vessels containing 15 ml of 0.5 M KOH and the conductivity was measured after equilibration. The equilibration condition was examined under three conditions, using (i) a 60 ml Teflon-lined screw cap borosilicate vial shaken for 5 h, (ii) a 235 ml Mason jar mixed with stirring bar for 5 h and (iii) the same Mason jar but mixing for 30 h. For all cases the conductivity data fell into a single standard curve (r^2 0.998) for a range of 0.164–2.63 g CO₂-C/l KOH was selected due to its higher sensitivity than NaOH. The conductivity values were similar to literature values (Strotmann et al., 2004).

2.5. Incubation experiments

A 235 ml Mason jar was filled with 10 g mineral and 0.3 g OM, which were mixed homogeneously. Five uncharred OM samples (corn stover, cellulose, glucose, lignin and arginine) and the corresponding PyOM samples were used with each of 5 different minerals (quartz, corundum, goethite, kaolinite and the mixture of kaolinite and goethite at 10% w/w goethite). In total, 50 different combinations were prepared in four replicates for each combination. Each jar was filled with 2.5 ml of tenfold concentrated Hoagland's solution that had been mixed with 20% v/v inoculum as

described below. In order to adjust the water holding capacity to 35% and to achieve good mixing properties, 0.3 ml for quartz and 0.8 ml for kaolinite and kaolinite + goethite were added. The pH was not adjusted to maintain the values associated with the different combinations of OM and minerals. Each jar was incubated in the dark at 30 °C for 196 days.

As an inoculum, soil was sampled at a historic charcoal storage site in Alabama (Cheng et al., 2008) to provide an inoculum with a microbial population adapted to a PyOM-rich soil environment. It was added to Hoagland's solution at 10 g/100 ml and cultivated in a rotary shaker at 30 °C for 2 weeks. An aliquot (20 ml) of the solution was transferred into 100 ml of fresh Hoagland's solution with and without 0.3 g corn stover PyOM and incubated again for one month. The two cultures were mixed (1:1, v/v) and filtered with Whatman #2v (8 µm pore size) to remove soil and PyOM particles, and used for the incubation experiments as an inoculum.

The CO₂ absorbed by the KOH was measured with the conductivity meter at appropriate intervals during incubation. Since the conductivity of 0.5 M KOH decreases by 1.4 mS/cm with a 1 °C decrease, the jars were moved to a measuring room for 3 h to allow them to reach temperature equilibrium before measurement. When a significant amount of CO₂ was absorbed, the vial was replaced; otherwise, it was placed back into the jar. The volume of KOH solution lost due to attachment to the probe surface during each measurement (0.0705 ml) was corrected for during the calculation of actual CO₂ evolution.

2.6. Calculation of a partial C balance

Since C in biomass is volatilized during pyrolysis, a partial C balance for PyOMC remaining after pyrolysis and mineralization can be calculated from the following equations, respectively:

$$C_{S,PyOM} = C_{PyOM}(1 - f_{C,M}) \quad (1)$$

$$C_{PyOM} = \frac{Y_{C/PyOM} \cdot f_{C,PyOM}}{f_{C,BM}} \quad (2)$$

where $C_{S,PyOM}$ is the fraction of C sequestered after pyrolysis and mineralization, C_{PyOM} the fraction of C remaining in PyOM after pyrolysis, $f_{C,M}$ the fraction of C lost during mineralization, $Y_{C/PyOM}$ the yield of PyOM generated from biomass, and $f_{C,PyOM}$ and $f_{C,BM}$ the C fractions of PyOM and biomass, respectively.

Reduction of mineralization by charring/mineralogy (%)

$$= \frac{(f_{C,M,j,base} - f_{C,M,j})}{f_{C,M,j,base}} \times 100 \quad (3)$$

where $f_{C,M,j}$ is the fraction of C lost for OM type j , $f_{C,M,j,base}$ the fraction of C lost for the base condition. The j denotes the charred OM j and the base condition is for the uncharred OM j when the reduction of mineralization by charring is calculated, while the base condition is that for the mineral with the highest mineralization extent when the reduction by exposure to different minerals is calculated.

2.7. C debt or credit ratio

The ratio of the net C remaining ('C debt or credit ratio') was calculated by comparing the C remaining in the incubated PyOM with the C remaining in the incubated original feedstock used to generate the PyOM (Whitman et al., 2013).

$$\text{C debt or credit ratio} = \frac{\text{C remaining of PyOM after charring and mineralization}}{\text{C remaining of original feedstock after mineralization}} \quad (4)$$

A value > 1 indicates that more C remained after mineralization of the PyOM, including C loss by pyrolysis than after mineralization

of the unpyrolyzed feedstock (i.e. C credit), while a value < 1 indicates that less C remained (i.e. a C debt). The ratio was used here to evaluate the combined effects of charring and mineralization of both the PyOM and the OM it was produced from. The ratio changes as a function of the duration of observation (Whitman et al., 2013), which was not investigated.

2.8. Statistical analysis

Analyses of variance were carried out using IBM SPSS Statistics 20 to compare the effect of OM or PyOM and mineralogy on C mineralization. To separate the mean of C mineralization, post hoc least significant difference was used with Tukey's HSD test at $P < 0.05$. The relative variation was evaluated using the proportion of standard deviation (%RSD) as follows:

$$\%RSD = \frac{\text{standard deviation}}{\text{average}} \times 100 \quad (5)$$

Average %RSD values of either uncharred and charred OM types ($n = 5$) or the different mineral types ($n = 5$) were used to evaluate which factor had a greater effect on mineralization as well as the C debt or credit ratio and compared using t -tests for independent samples.

3. Results

3.1. Properties and yield of PyOM

The PyOM yield from pyrolysis at 600 °C depended on the type of feedstock. The monosaccharide glucose showed a much lower PyOM yield (11.9%) than that of its polymeric form cellulose (21.5%), corn stover (24.9%), amino acid arginine (20.7%), or lignin (53.9%). Among the charred materials (Table 1), arginine (20.5%) and lignin (18.7%) possessed a larger proportion of volatile matter than corn stover (13.2%), cellulose (9.6%) and glucose (9.4%). The largest proportion of ash was found after charring of lignin (20.1%), while others had ash content < 6.7%. The pH values of the uncharred OM were slightly acidic, ranging from 5.3 for cellulose to 6.6 for corn stover and lignin, with the exception of arginine (11.2) that was basic. After pyrolysis, the pH values of most materials increased from 6.5 for cellulose to 10.8 for lignin, with the exception of arginine, whose pH decreased from 11.2 to 6.3.

Total C content was similar for the different uncharred materials (40.0–44.4%), with the exception of lignin that had a higher content (58.9%). Arginine had a large N content (32.2%) due to its amine moiety, while others had a low N content of < 1.3%. Total C content of charred corn stover, cellulose and glucose was similar (80.1–88.8%), whereas lignin (70.8%) and arginine (62.7%) had lower C content. The molar O/C ratio showed that oxygen in lignin was preserved during pyrolysis in higher proportion than oxygen in other organic materials, which coincided with a greater ash content (Table 1).

3.2. Cumulative C mineralization

Cumulative C mineralization was significantly ($P < 0.001$; $n = 20$) affected by the type of OM regardless of mineralogy (Fig. 1a). Uncharred corn stover (52–59% of initial C) and glucose (80–89% of initial C) were mineralized more rapidly than cellulose, lignin and arginine (7.5–25% of initial C; full data in Supplementary material Fig. S1). Mineralization of PyOM was also significantly ($P < 0.001$; $n = 20$) affected by the type of OM used to make the PyOM (Fig. 1b), which was mostly related to its volatile matter content (r^2 0.332–0.938) and less to H/C or O/C ratios (r^2 0.000–0.649; Supplementary Fig. S2).

Table 1
Characteristics of uncharred and charred OM ($n = 2$).

		pH	WE ^b (% w/w)	Volatiles (% w/w)	Fixed carbon (% w/w)	Ash (% w/w)	C (%)	N (%)	O (%)	H (%)	O/C ^c	H/C ^c
Uncharred material	Corn stover	6.6	3.5	86.7	12.2	1.2	42.1	1.28	52.1	7.14	0.93	2.03
	Cellulose ^a	5.3	0.33	na	na	na	44.4	0.00	49.4	6.17	0.83	1.67
	Glucose ^a	5.7	100	na	na	na	40.0	0.00	53.3	6.67	1.00	2.00
	Lignin	6.6	14	60.0	36.3	3.8	58.9	0.51	33.1	7.15	0.42	1.46
	Arginine ^a	11.2	100	na	na	na	41.4	32.2	18.4	8.05	0.33	2.33
Charred material	Corn stover	9.0	0.79	13.2	80.2	6.7	80.1	0.48	11.4	2.77	0.11	0.42
	Cellulose	6.5	0.04	9.6	90.4	0.0	88.8	0.06	5.00	2.73	0.042	0.37
	Glucose	8.2	0.16	9.4	85.3	5.3	86.1	0.00	5.93	2.92	0.052	0.41
	Lignin	10.8	23.1	18.7	61.2	20.1	70.8	0.00	15.9	1.74	0.17	0.30
	Arginine	6.3	0.04	20.5	79.6	0.0	62.7	18.0	4.37	2.58	0.052	0.49

^a Elemental content calculated from chemical formula.

^b Water extractable.

^c Molar ratio; na, not available.

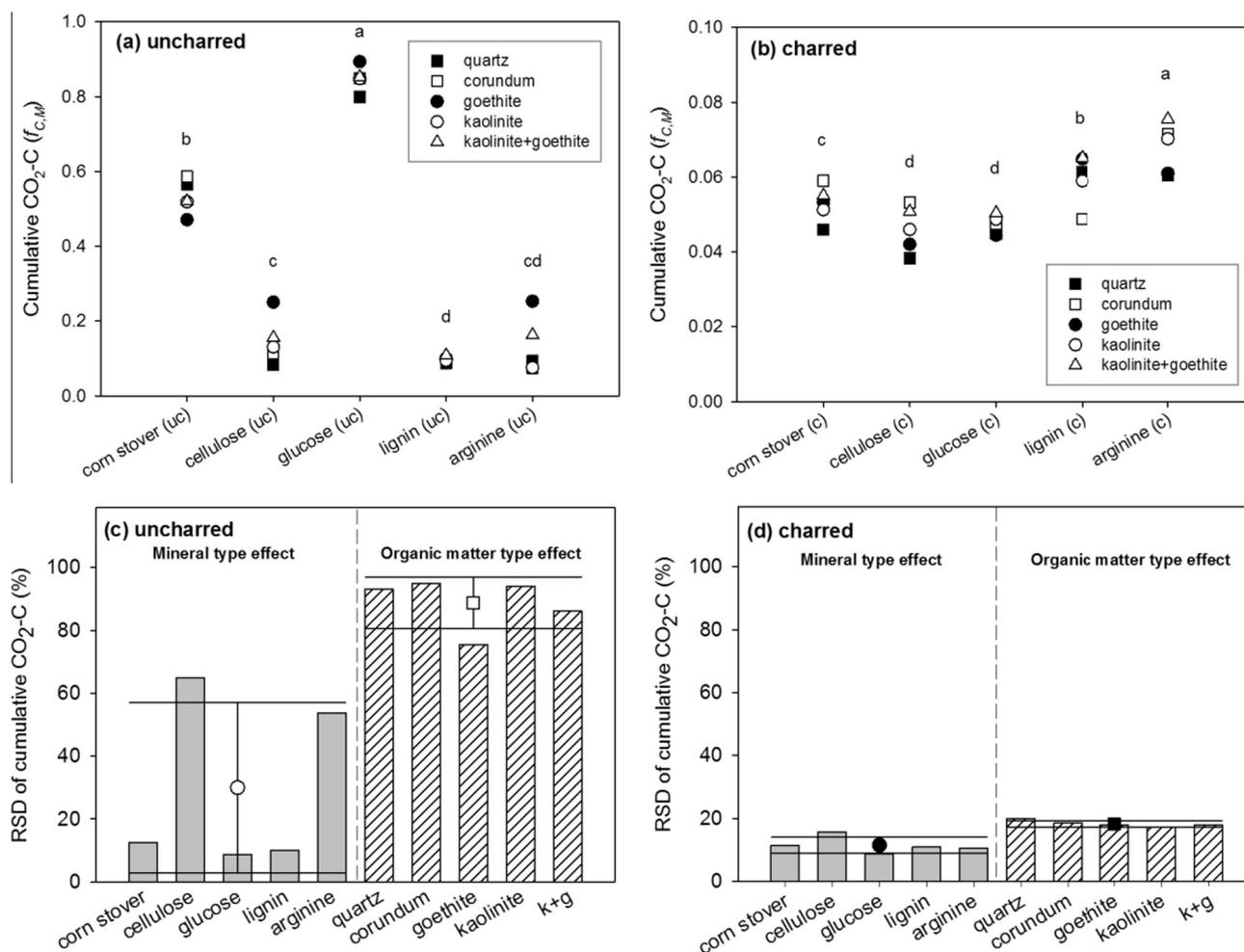


Fig. 1. Cumulative CO₂ evolution ($f_{C,M}$, see Eq. 3) after 196 days of incubation with various combinations of minerals and uncharred (a) or charred (b) organic matter ($n = 4$; standard deviation values are presented in Supplementary Fig. S1; identical letters above symbols indicate no statistical difference between the treatments based on the Tukey's HSD test at $P < 0.05$), and the effect of mineral type or OM type on relative standard deviation (RSD) of cumulative mineralization of uncharred (c) and charred (d) OM (symbols are means and standard deviations of $n = 5$; bars means of $n = 20$ (detailed data in Supplementary Table S2)).

All PyOM samples showed slow mineralization, ranging from 3.8 to 7.6% of initial C (Fig. 1b). The reduction of mineralization by charring ranged from 4.7 to 95.0% over the 196 days of incubation, which increased asymptotically with greater mineralization of uncharred OM (Fig. 2a). For easily mineralizable feedstock, 94% of mineralization of glucose and 90% of that of corn stover could be avoided by charring (see also Supplementary Fig. S3). For the OM that was mineralized slowly when uncharred, the reduction

was proportionally lower. For example, the reduction in lignin mineralization by charring only ranged from 30 to 48%. The reduction in mineralization by different minerals ranged from 3.7 to 70.4% (compared with the mixture that caused the greatest mineralization), smaller than that by charring (Supplementary Fig. S3). In contrast to the charring effect, a lower reduction in mineralization caused by different mineralogy was found with easily mineralizable feedstock, such as glucose and corn stover.

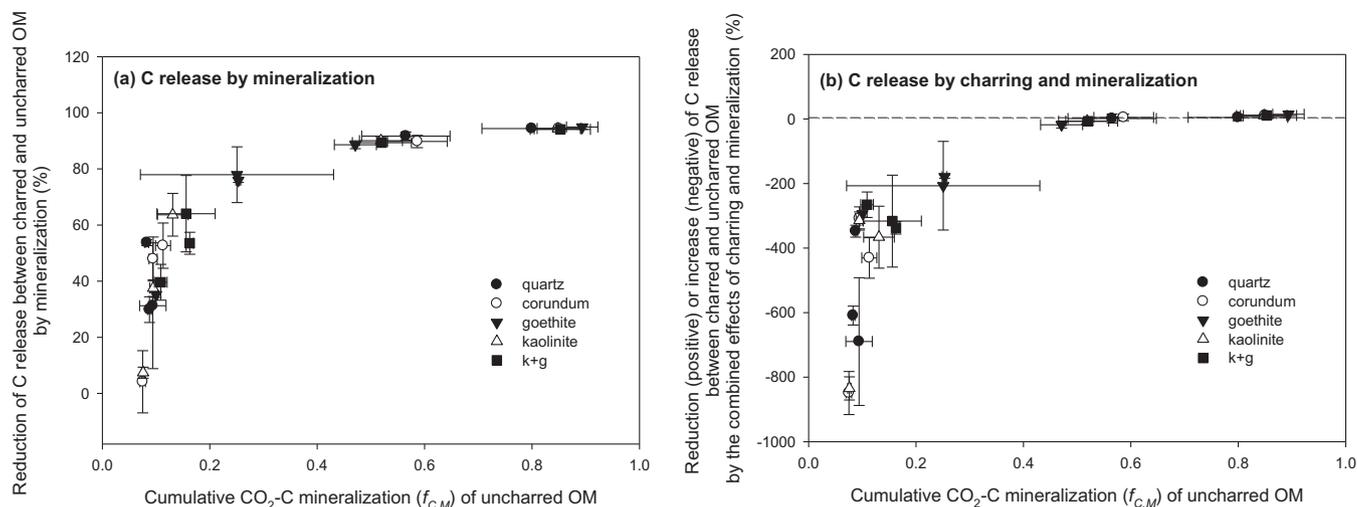


Fig. 2. Reduction or increase of C release by either comparing (a) the difference in mineralization between charred and uncharred OM, or (b) the difference in both C losses during the charring process as well as the difference in mineralization between charred and uncharred OM ($n = 4$).

The effect of clay mineralogy on mineralization of uncharred materials was on average not significant ($n = 20$; $P 0.980$) but differed depending on OM type ($n = 4$; $P 0.059, 0.603, 0.456, 0.052$ and < 0.001 for corn stover, cellulose, glucose, lignin and arginine, respectively; [Supplementary Table S2](#)). Mineralization of corn stover was slower in the presence of goethite than other minerals: 9.2% lower than kaolinite and 19.7% lower than corundum. In the case of kaolinite mixed with 10% goethite, the mineralization ratio was lower than for kaolinite alone but higher than for goethite alone. The goethite effect depended on the type of OM and was not found for lignin, cellulose, glucose or arginine.

The effect of mineralogy on the charred OM was quite different from that on uncharred OM, with higher significance ($n = 4$; $P 0.010, 0.007, 0.363, < 0.001$ and 0.004 for PyOM from corn stover, cellulose, glucose, lignin and arginine, respectively). Goethite or the mixture of kaolinite and goethite did not change C mineralization of any PyOM. For the entire PyOM set ($n = 20$, $P 0.061$; [Supplementary Table S2](#)), lower mineralization was found with quartz (5.0%) than other minerals (5.3–5.9%).

Since the total amount of mineralizable C of uncharred OM was much higher than that of PyOM, we used the relative standard deviation (%RSD) to assess the effect of mineralogy. Both uncharred ([Fig. 1c](#); $n = 5$; $P 0.006$) and charred ([Fig. 1d](#); $n = 5$; $P 0.001$) OM showed larger %RSD ($88.7 \pm 8.2\%$ for uncharred; $18.3 \pm 1.0\%$ for charred) for the type of OM than the %RSD ($30.0 \pm 27.1\%$ for uncharred; $11.5 \pm 2.6\%$ for charred) for the type of mineral. The

overall effect of OM type on C mineralization was greater ($n = 5$; $P < 0.001$) for uncharred OM with greater variation (%RSD 75.3–95.0%; [Fig. 1c](#)) than for PyOM (%RSD 17.2–19.9%; [Fig. 1d](#)). The %RSD for C mineralization of a specific uncharred OM caused by different mineralogy ranged from 8.7 to 64.8% ([Fig. 1c](#)), which was only marginally ($n = 5$; $P 0.202$) greater than that of PyOM, ranging from 8.7% for glucose PyOM to 15.7% for cellulose PyOM ([Fig. 1d](#)). For cellulose and arginine, %RSD values of uncharred material were significantly higher than those of the respective PyOM material, while corn stover, glucose and lignin showed smaller differences in %RSD between uncharred and charred OM.

3.3. Net effect of charring and mineralization

Even though the charred material was mineralized much more slowly than feedstock, the loss of C during pyrolysis reduces the net C remaining ([Whitman et al., 2013](#)). Therefore, the overall C remaining was evaluated under two scenarios: (i) after mineralization of uncharred OM (Scenario 1) and (ii) after pyrolysis and subsequent mineralization of the charred material (Scenario 2 in [Table 2](#)). Calculated C remaining after mineralization of PyOM including the C loss during pyrolysis (Scenario 2) was not always greater than after mineralization of uncharred OM (Scenario 1). This overall C remaining after pyrolysis and mineralization of PyOM was less variable (24–62% remaining) than the C remaining after mineralization of the uncharred OM (11–93%).

Table 2
Overall carbon remaining (fraction of initial carbon, dimensionless) after either mineralization of uncharred OM (Scenario 1) or both pyrolysis and mineralization of charred OM (Scenario 2; Q, quartz; C, corundum; G, goethite; K, kaolinite).

	Carbon remaining															
	Scenario 1 (mineralization of uncharred OM ^a)					Scenario 2 (charring and mineralization of charred OM)										
	Q	C	G	K	K + G	Charring ^b	Mineralization ^a					Charring and mineralization ^c				
							Q	C	G	K	K + G	Q	C	G	K	K + G
Corn stover	0.435	0.413	0.529	0.481	0.480	0.473	0.954	0.941	0.947	0.949	0.945	0.451	0.445	0.448	0.449	0.447
Cellulose	0.917	0.887	0.749	0.869	0.844	0.430	0.962	0.947	0.958	0.954	0.949	0.413	0.407	0.412	0.410	0.408
Glucose	0.201	0.150	0.107	0.152	0.147	0.256	0.955	0.953	0.956	0.951	0.950	0.245	0.244	0.245	0.244	0.243
Lignin	0.912	0.906	0.900	0.905	0.891	0.648	0.939	0.951	0.935	0.941	0.935	0.608	0.616	0.606	0.609	0.605
Arginine	0.906	0.925	0.747	0.924	0.837	0.313	0.939	0.929	0.939	0.930	0.925	0.294	0.291	0.294	0.291	0.290

^a Experimental data at 196 days ($1 - f_{C,M}$).

^b C_{PyOM} .

^c Calculated by multiplying the fraction remaining after charring and mineralization ($C_{S,PyOM}$).

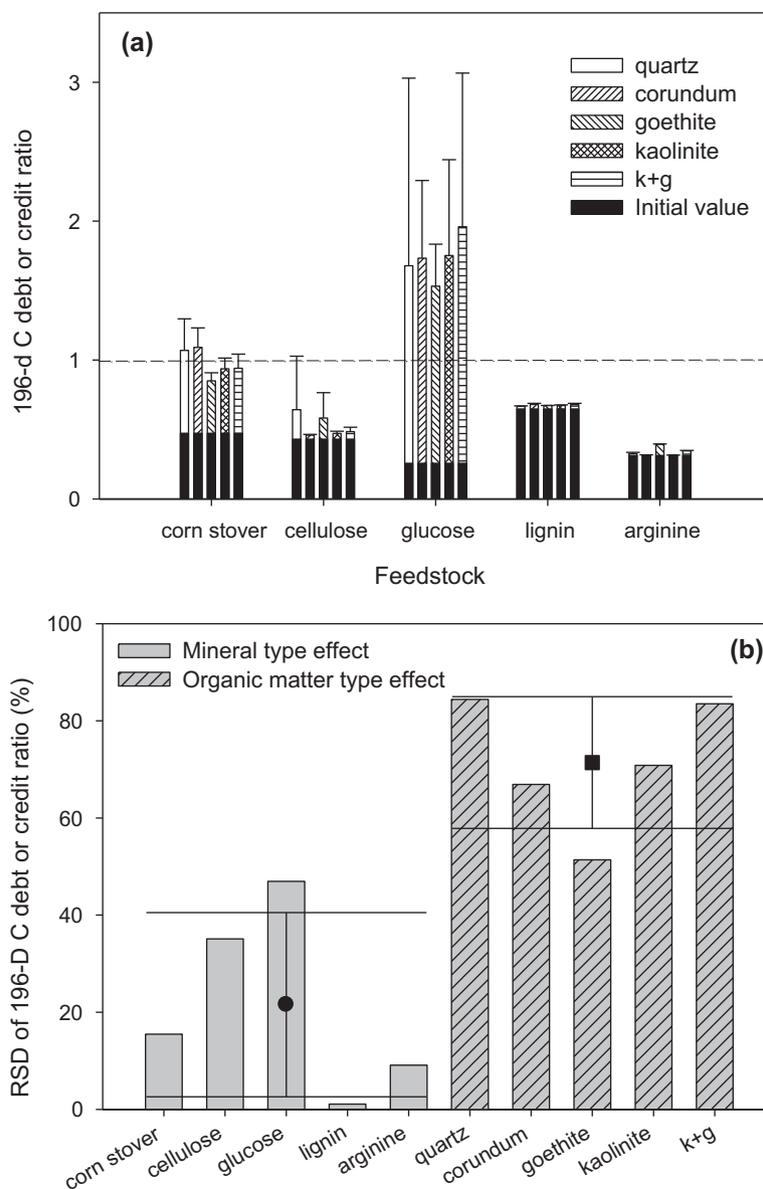


Fig. 3. Ratio of C remaining after charring and mineralization to mineralization of uncharred biomass ('C debt or credit ratio') after 196 days incubation (a) (means and standard deviation; $n = 4$) and its RSD (b) ($n = 20$ for bars; means and standard deviation for symbols, $n = 5$). Black portion of the stacked bars indicates initial values directly after PyOM production and before mineralization. Horizontal line indicates the "break-even" point above which more C remains after mineralization of PyOM, including C loss by charring than after mineralization of uncharred biomass, where more C remains after 196 days if the OM is charred.

From the results of the scenarios, ratio values of net carbon remaining were compared for each combination of mineral and OM (Fig. 3), often called 'C debt or credit ratios'. Glucose, the most easily biodegradable OM when not charred, showed a ratio > 1 (1.5–2.0) after charring and mineralization irrespective of mineral properties (Fig. 3a), since a significant amount of C remained in the PyOM after pyrolysis, which would otherwise be rapidly mineralized if uncharred. In the case of corn stover, the C ratio depended on the type of mineral; only incubation with quartz and corundum resulted in values > 1 , while all others showed values < 1 . Less degradable OM such as cellulose, lignin and arginine had low values (0.32–0.68; Table 2) and the reduction in C release increased with greater mineralization of the uncharred OM (Fig. 2b). RSD values were compared to evaluate whether the type of mineral or type of OM had a greater effect on the C ratio values (Fig. 3b; $n = 20$; $P < 0.001$). RSD values were higher (51–84%; $71.4 \pm 13.6\%$) when OM was varied than when mineral type was varied

(1.1–47%; $21.5 \pm 19.0\%$), suggesting that mineralogy had a lower influence on C remaining after pyrolysis and mineralization than the type of OM.

The contribution of C remaining from either PyOM or original feedstock to the C debt or credit ratio was evaluated by calculating the ratio of RSD with the data sets for the same feedstock but different mineral type (Supplementary Table S3). The RSD of C remaining from the original feedstock with variation in mineral composition ranged from 1.1% for lignin to 34.1% for glucose, whereas the RSD of PyOM was much less (0.43–0.76%). The contribution of variation in mineralogy to the C ratio compared with variation in original feedstock type, represented by the ratio of the RSD, was very small (0.01–0.09) with the exception of lignin (0.65). It should be noted that the ratio for easily mineralizable OM such as glucose was in this experiment more strongly affected by mineralogy than OM, that was mineralized more slowly, but this observation disappeared after charring.

4. Discussion

4.1. OM and charring effect on C mineralization

Varying the type of OM affected the mineralization of charred OM less than that of uncharred OM. The total C mineralization of PyOM fell into a much narrower range (3.8–7.5%) than that of the different original feedstock (7.6–89%). The C mineralization of PyOM was similar to that found in other incubation studies, with similar or different feedstock (Zimmerman, 2010; Whitman et al., 2013; Bruun et al., 2014). Compared with other properties of PyOM, ASTM volatile matter content was strongly related to short term mineralization, which reflects results from Zimmerman (2010). The unexpected lack of a strong correlation with the H/Corg ratio found in a review of published data by Lehmann et al. (2015a) may be explained by the sample set used in this experiment as well as the duration of the experiment: (i) pyrolysis temperature which alters the H/Corg ratio the most was identical, but feedstock varied dramatically, which has the largest effect on ASTM volatile content (Enders et al., 2012); (ii) the relatively short incubation time of 196 days here compared with > 1 year in the data reviewed by Lehmann et al. (2015a) means that the C forms mineralized in the present study are not in fused aromatic ring structures, and the H/Corg ratio may be less able to capture differences in these C forms than the ASTM method.

4.2. Effect of different mineralogy on PyOM mineralization

Mineralogy significantly changed the mineralization of PyOM, which varied depending on the combination of the type of PyOM and mineral. The mineralization of lignin PyOM showed the opposite behavior as a result of mineralogy than the other PyOM samples, i.e. greater mineralization in the presence of quartz but lower mineralization in the presence of corundum. The reason may lie in the high oxygenation of the studied lignin PyOM and different surface charges due to different pH values associated with different PyOM samples. The pH for the minerals ranged from 5.7 for quartz to 8.6 for corundum in comparison with 6.3 in the original solution (Supplementary Table S1). The surface bond of quartz can be broken to interact with water molecules to form silanol groups that gain or lose H⁺ depending on the solution pH (Rosenbrand et al., 2012). Therefore, the surface charge is negative when the solution pH is higher than the point of zero charge of quartz (< 3; Kosmulski, 2014). Adding lignin PyOM with a pH of 10.8 (the highest pH value among the PyOM samples) would increase negative surface charge of most mineral surfaces, which might lower their affinity to electron-rich oxygen in lignin PyOM, leading to lower interaction of OM to minerals such as quartz. On the other hand, corundum, with a high point of zero charge above pH 9 (Beak et al., 2006), would still have positive surface charge and may interact with negatively charged lignin PyOM, thereby leading to lower mineralization extent. Unfortunately, the original or bulk pH does not fully capture which pH and therefore which charge occurs at the interface (Lehmann et al., 2015b), which would be desirable, albeit experimentally challenging, information to obtain. Additionally, the much smaller size of corundum might enhance the ability of PyOM to interact with the mineral surface (Supplementary Table S1).

In contrast to uncharred corn stover, goethite did not decrease the mineralization of charred corn stover. Rather than goethite, the presence of quartz reduced mineralization of PyOM most over the first few months, with the exception of lignin PyOM mentioned above. The reasons are unclear but may be a result of the only weakly charged surfaces of both fresh PyOM (Cheng et al., 2006, 2008) and quartz. An abundance of quartz in natural soil systems

may therefore reduce initial mineralization of fresh PyOM to a greater extent than that of uncharred OM.

4.3. Effect of variation in mineralogy and feedstock

The lower variability in mineralization after charring is a result of both (i) the lower effect of different OM types, as well as (ii) a lower overall effect of mineralogy on mineralization of charred than of uncharred OM. The reason for the lower importance of PyOM type for mineralization is that PyOM properties have a greater similarity to each other than those between the OM that they are produced from (Table 1), which is observed for several PyOM properties (Enders et al., 2012) and has been reported for mineralization (Zimmerman, 2010; Whitman et al., 2013). For charred OM (Fig. 1d), only a slightly larger %RSD for varying the type of PyOM than for varying the type of mineral was obtained, while it was much larger with uncharred OM (Fig. 1c). This suggests that variation in PyOM properties were still more important for inducing different mineralization by minerals than the type of mineral, but the variation is not as important for PyOM as it is for uncharred OM.

The importance of different mineralogy for mineralization was less pronounced among PyOM samples than among uncharred OM samples (Table 2 and Fig. 1). This comparison for the mineralization of both PyOM and the OM they were produced from in the presence of different mineralogy has not been reported before. Fang et al. (2014) observed, under similar incubation conditions, that the influence of soil properties of four contrasting soils on PyOM mineralization was less when the PyOM was charred at 550 °C than 450 °C. This aligns with our observations assuming that lower pyrolysis temperature produces PyOM that has properties with greater resemblance to the original OM. Santos et al. (2012) tested two soils with different mineralogy and found that PyOM mineralization did not differ between an andesitic and a granitic soil. Cusack et al. (2012) quantified relationships between PyC, SOC and the amount of short range order (SRO) minerals in 44 soil samples collected from different grasslands. They observed that PyC concentration correlated significantly with amount of SRO (r^2 0.46), but less strongly than SOC concentration (r^2 0.67), with a small but significant decrease in the PyC/SOC ratio with increasing SRO concentration. This may indicate that the amount of SRO minerals may reduce C mineralization of PyOM to a lesser extent than that of SOC, corroborating our results.

Some characteristics of PyOM that are different from uncharred OM may reduce the effect of different mineralogy on PyOM mineralization during the initial period studied here. Functional group composition among PyOM materials must have resembled each other to a greater extent than among uncharred OM (Sharma et al., 2004; Knicker, 2011). The structures in feedstock OM are decomposed during thermal treatment and many functional groups disappear, resulting in mainly aromatic networks (Kleber et al., 2015b). In addition, protection of uncharred OM will likely depend to a greater extent on the interaction of its metabolites (Schmidt et al., 2011), whereas the PyOM particle surfaces may to a greater extent interact directly with the minerals in the initial phases of decomposition studied here. For uncharred OM, many different types and sizes of intermediate metabolites may be rapidly produced by extracellular enzymes, which may increase the variability of the different organic compounds interacting with mineral surfaces (Sánchez, 2009). Furthermore, greater water solubility of uncharred OM and its biological metabolites compared with fresh PyOM (Graber et al., 2014) can make the interaction with minerals easier and more variable. This may be different for highly oxidized PyOM; however, significant oxidation may take longer than the period investigated in our study (Cheng et al., 2008), and was therefore not fully captured by our short term

study. The limited accessibility of minerals to functional groups in PyOM due to the presence in internal pores or filling of the pore entrance with mineral particles (Fang et al., 2014) may also reduce variability in mineralogy effects on PyOM mineralization.

4.4. Mineralogy and feedstock effect on C loss by both charring and mineralization

The ratio of C remaining after both mineralization and charring to mineralization of the original biomass was used as a tool to evaluate the C impact of both PyOM production and mineralization of charred and uncharred OM (also called “C debt or credit ratio”; Whitman et al., 2013; Herath et al., 2015). The strong dependence of this C ratio on the type of feedstock coincides with previous findings (Whitman et al., 2013), where feedstock type was a more important determinant than the temperature of PyOM production. The strong dependence of the C ratio on the type of OM is related to the fact that the degree of mineralization of uncharred OM affects the ratio more strongly than does that of PyOM due to the much slower mineralization of PyOM. The small variation in mineralization of PyOM contributes less to the calculation of the C ratio than the difference in mineralization between uncharred OM samples.

Similar to C mineralization alone, the effect of mineral type on the ratio was less important than that of OM type. In addition, the ratio for easily mineralizable OM was more affected by mineralogy than that for non-easily mineralizable OM such as lignin. This suggests that charring, as well as different mineralogy, reduces C release from fast-decaying OM after both charring and mineralization to a greater extent than that of slowly decaying OM.

5. Conclusions

Soil minerals had a more varied effect on the mineralization of uncharred OM than on that of PyOM, presumably because of a greater variety of mechanisms operating on the interaction with uncharred OM and its metabolites than with the PyOM particles that likely remained largely unaltered during the initial short term decomposition process. Rather than goethite, the presence of quartz reduced mineralization of the fresh PyOM the most over the first few months, possibly due to different surface charge characteristics between charred and uncharred OM. The greater sensitivity of PyOM mineralization to OM type used to produce the PyOM than to soil mineralogy indicates a greater control of PyOM production conditions and specifically feedstock properties than soil conditions on the short term. Since about half of the C is volatilized by thermal degradation during pyrolysis, the question whether pyrolysis or direct application will generate more C remaining in soil also depends on both the feedstock mineralization and the time horizon. Charring of more easily mineralizable OM reduces total C emission to a greater extent but is more dependent on mineralogy of the soil system than slow-decaying feedstock. Future research may need to test whether the observed short term results are also found over longer periods of time and include aged and therefore oxidized PyOM. These effects of different mineralogy on PyOM mineralization provide motivation for investigating PyOM–mineral interactions on a molecular scale, including the interaction between different OM forms.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.orggeochem.2016.05.006>.

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