Supporting Information for

Carbon mineralizability determines interactive effects on mineralization of pyrogenic organic matter and soil organic carbon

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Soil collection site vegetation history

The site of soil collection 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*).

PyOM pH adjustment

Concentrated HCl was added dropwise while stirring the PyOM-DIW slurries. Once the pH was close to the target level, the jars were left to stir overnight, in order to allow the pH to re-equilibrate. This was repeated daily, and with lower concentrations of HCl, until the pH no longer shifted over time and the pH was at the target level.

Second incubation (PyOM)

200 mg of PyOM was mixed with 15.0 g ashed (550°C for 2 h) sand, with 8 replicates for each treatment. Samples were inoculated with 3.3 mL inoculum specific to the PyOM treatment. In order to ensure that the addition of the microbial inoculum did not affect the $\delta^{13}C$ signature of the respired CO_2, sequential pre-incubations and extractions were performed, where an initial soil extract was added to each type of PyOM-sand mixture and incubated for 48 hours. Then, these mixtures were extracted and used to inoculate subsequent PyOM-sand mixtures, etc., for a total of four times, before the final PyOM-sand mixtures were extracted and used as the microbial inocula for each type of PyOM.

Third incubation (effect of pre-incubation duration and nutrients)

This trial replicated the first, with the following exceptions. It was designed to elucidate the very short-term effects of experimental initiation, and so was only run for 48 hours. Soils were pre-incubated for 1, 10, or 20 days, and received one of three additions: PyOM, nutrients, or no additions. The added PyOM was the PyOM with increased water-extractable compounds described above for the main experiment. The nutrient treatment was added with a volume of 1 mL, and was designed to deliver a dose of nutrients equivalent to the plant-available (Mehlich III-extractable) nutrients quantified in the PyOM (Supporting Table S3). Any nutrient for which PyOM additions would have resulted in a > 3% increase from initial soil levels was included in the nutrient solution (Supporting Table S3 and Supporting Figure S3). Treatments that did not receive the nutrient solution received an equivalent amount of deionized water.

Notes on standard curve application

While the standard curves produced for each sampling episode were consistently linear, we noticed that equilibration of the traps with the jar atmosphere over time led to small increases in the slope of the standard curve with increasing incubation times. Thus, we incubated traps for a standard curve over increasing lengths of time, up to 25 days, to

create a linear regression for the slope of the standard curve. Over incubation lengths of 1-25 days, the slope (change in CO_2 (mL added) / change in EC ($\mu S\ cm^{-1}$)) ranged from 1.6 to 1.8. We used this adjusted slope equation to translate the measured EC values into CO_2 values. This does not significantly affect our general conclusions, but should increase the accuracy of the CO_2 measurements.

Nutrient addition experiment

In order to determine whether an alleviation of nutrient constraints could be responsible for the short-term increase in SOC mineralization with PyOM additions, we conducted a trial where nutrients were added in an amount equal to that added with the PyOM with increased water-extractable compounds. We included any nutrient for which PyOM additions increased total concentrations in the soil by more than 5% (see Supporting Figure S3). Supporting Figure S2 shows the results from this short-term incubation.

Determining $\delta^{13}C$ values of PyOM and SOC end-members

The procedure to subtract the effect of the blank jar on the $\delta^{13}C$ values of the PyOM-only incubation was the same as that used for the soil+PyOM incubation. This generated a set of $\delta^{13}C$ values for each PyOM treatment over time, corresponding to the mass of PyOM-C that had been mineralized over that interval. The $\delta^{13}C$ values of the mineralized PyOM were initially relatively enriched in ^{13}C, after which they all stabilized around +48‰. To account for this changing $\delta^{13}C$ signature over time, we assumed that the $\delta^{13}C$ signature changes consistently over the first period of the incubation and then stabilizes at +48‰. The challenge is that, in the PyOM-only incubation, larger masses of PyOM were respired per sampling interval than in the combined incubation. *I.e.*, while the average $\delta^{13}C$ value of PyOM with added extractable PyOM respired over the first timepoint in the PyOM-only trial was +60.28‰, this included 1.70 mL of CO_2. We know that the amount of respired PyOM in the combined incubation was less than this, and so, if we expect that the $\delta^{13}C$ of the PyOM decreased over time until it stabilized at +48‰, then we should predict that the average $\delta^{13}C$ signature for this smaller initial mass of mineralized PyOM would be greater than +60.28‰. However, if we take a higher value, then this changes the mass of PyOM we calculate as respired in the incubation experiment. To calculate the correct average $\delta^{13}C$ for the PyOM in each treatment at each sampling time, we assumed a linear decrease of $\delta^{13}C$ of mineralized PyOM-C over the course of the PyOM incubation until it reaches 48‰, after which it stabilizes. To calculate the correct $\delta^{13}C$ to use for each timepoint, we then iteratively adjusted the $\delta^{13}C$ value to represent the mean amount of PyOM respired (for each treatment at each timepoint) until it stabilized at a value that yields a mass of PyOM respired that would generate this same mean $\delta^{13}C$ value (Supporting Figure S1). This process was used to generate the appropriate $\delta^{13}C$ values for PyOM-C for each soil and PyOM combination at each timepoint (Supporting Table S4). Soil $\delta^{13}C$ values in the soils without PyOM additions did not show major shifts over time, but for each timepoint the corresponding mean $\delta^{13}C$ value from the 24-hour or 6-month pre-incubated soils without PyOM additions were used as the end-members for SOC mineralization.

Table S1. Initial soil properties

Property (units)	**Value**	
	6 months	**24 hours**
Texture	(Channery) silt loam	
100% WFPS (g water g^{-1} dry soil)	47	
pH (0.01M $CaCl_2$)	3.9	
Particle size (mm)	< 2	
Sand (%)	28.1	
Silt (%)	54.7	
Clay (%)	17.2	
Total C (%)	1.07	1.16
Total N (%)	0.11	0.11
C:N (mass)	9.76	10.41
2M KCl extractable NO_3^- and NO_2^- (mg kg^{-1} dry soil)	19.02	3.73
2M KCl extractable NH_4^+ (mg kg^{-1} dry soil)	35.37	20.48
Available P (Mehlich III, mg kg^{-1} dry soil)	1.0	1.0
Microbial biomass C ± SE (mg kg^{-1} dry soil)	18.7±10.4	17.6±14.8
Water-extractable C <2.5µm ± SE (mg kg^{-1} dry soil)	68.7±9.3	171.3±7.7
Water-extractable N <2.5µm ± SE (mg kg^{-1} dry soil)	60.4±4.8	37.6±1.1

Table S2. Production conditions and initial properties for PyOM and after removal and additions of water-extractable compounds

Property (units)	**PyOM treatment**		
	High DPyOM	**Medium DPyOM**	**Low DPyOM**
Total $C_{organic}$ (%)	71	70	71
Total $C_{inorganic}$ (%)	0	0	0
Bulk $\delta^{13}C$ (‰)	+77.8	+77.0	+78.5
Total N (%)	0.9	0.9	0.9
Bulk $\delta^{15}N$ (‰)	+1038.4	+1027.1	+1050.6
Total H (%)	4.3	4.3	4.4
Total O (%)	20	21	21
C:N (by mass)	79	77	79
$H:C_{organic}$ (molar)	1.4	1.4	1.4
$O:C_{organic}$ (molar)	0.21	0.23	0.22
2M KCl extractable NO_3^- and NO_2^- (mg kg^{-1})	3.37	0.41	0.32
2M KCl extractable NH_4^+ (mg kg^{-1})	0.96	1.46	0.70
Available P (Mehlich III, mg kg^{-1})	49.35	34.22	28.51
pH	Initial pH 8.9; Adjusted to soil pH (3.9)		
Feedstock	Sugar maple twigs		
Particle size (mm)	< 2		
Heating rate (°C min^{-1})	2		
Final pyrolysis temperature (°C)	325		
Residence time (hours)	2		
Surface area (m^2 g^{-1})	115	117	116
Ash (%)	4	4	3
Volatiles (%)	46	45	45
Fixed C (%)	50	51	52

Table S3. Nutrient solution for nutrient effect experiment

Nutrient	Form added	Amount added per jar (μmol)
Ca	$CaCl_2$	18.7
K	KCl	17.2
P	H_2KPO_4	0.3
Mg	$MgCl_2$	3.2
Sr	$SrCl_2$	2.9

Table S4. Isotopic $\delta^{13}C$ signatures (‰) for PyOM-C for each timepoint and soil-PyOM combination

Pre-incubation	Water-extractable PyOM adjustment	Day 1	Day 2	Day 5	Days 11+
6-month	Increased	65.36	54.91	48.95	48.95
	No change	61.47	54.81	48.95	48.95
	Decreased	60.47	53.80	48.95	48.95
1-day	Increased	67.68	61.07	52.33	48.95
	No change	63.05	58.85	52.76	48.95
	Decreased	62.20	57.88	48.95	48.95

Table S5. Cumulative CO_2 fluxes from soils (mg CO_2-C kg^{-1} soil). Different letters indicate significant differences between addition types for a given day (mixed effects model, $p<0.05$, Tukey correction).

		Day					
Soil	**Addition**	**1**	**2**	**5**	**10**	**25**	**47**
6-month pre-incubated soil	High DPyOM	12.05 b	19.04 b	38.05 b	69.03 b	139.38 d	227.80 e
	Med DPyOM	12.41 b	19.27 b	37.31 b	68.23 b	140.13 d	231.92 e
	Low DPyOM	11.62 b	18.56 b	36.66 b	68.64 b	141.83 d	232.73 e
	No PyOM	9.64 b	16.26 b	36.88 b	73.29 b	158.81 c	261.74 d
1-day pre-incubated soil	High DPyOM	44.54 a	73.71 a	131.34 a	198.39 a	312.87 b	420.53 c
	Med DPyOM	45.40 a	74.70 a	132.63 a	198.43 a	316.51 b	428.96 b
	Low DPyOM	44.95 a	72.72 a	128.91 a	196.02 a	311.15 b	422.86 bc
	No PyOM	43.93 a	71.09 a	128.83 a	196.37 a	318.57 a	461.32 a

Table S6. Cumulative CO_2 fluxes from PyOM (mg CO_2-C kg^{-1} soil). Different letters indicate significant differences between soil-PyOM combinations for a given day (mixed effects model, $p<0.05$, Tukey correction).

PyOM	Soil	Day					
		1	2	5	10	25	47
High DPyOM	6 month pre-incubated soil	17.23 a	28.85 a	41.38 a	52.80 a	65.44 a	78.79 a
	1 day pre-incubated soil	11.06 b	18.63 bc	35.69 b	47.48 b	57.84 b	70.91 b
Med DPyOM	6 month pre-incubated soil	11.70 b	19.86 b	31.33 b	41.17 c	55.02 b	67.67 b
	1 day pre-incubated soil	7.15 c	12.25 d	24.87 c	35.59 d	45.85 cd	57.77 cd
Low DPyOM	6 month pre-incubated soil	8.96 bc	16.03 c	26.69 c	36.64 d	49.29 c	60.70 c
	1 day pre-incubated soil	4.93 c	10.03 d	23.70 c	34.16 d	45.02 d	56.99 d

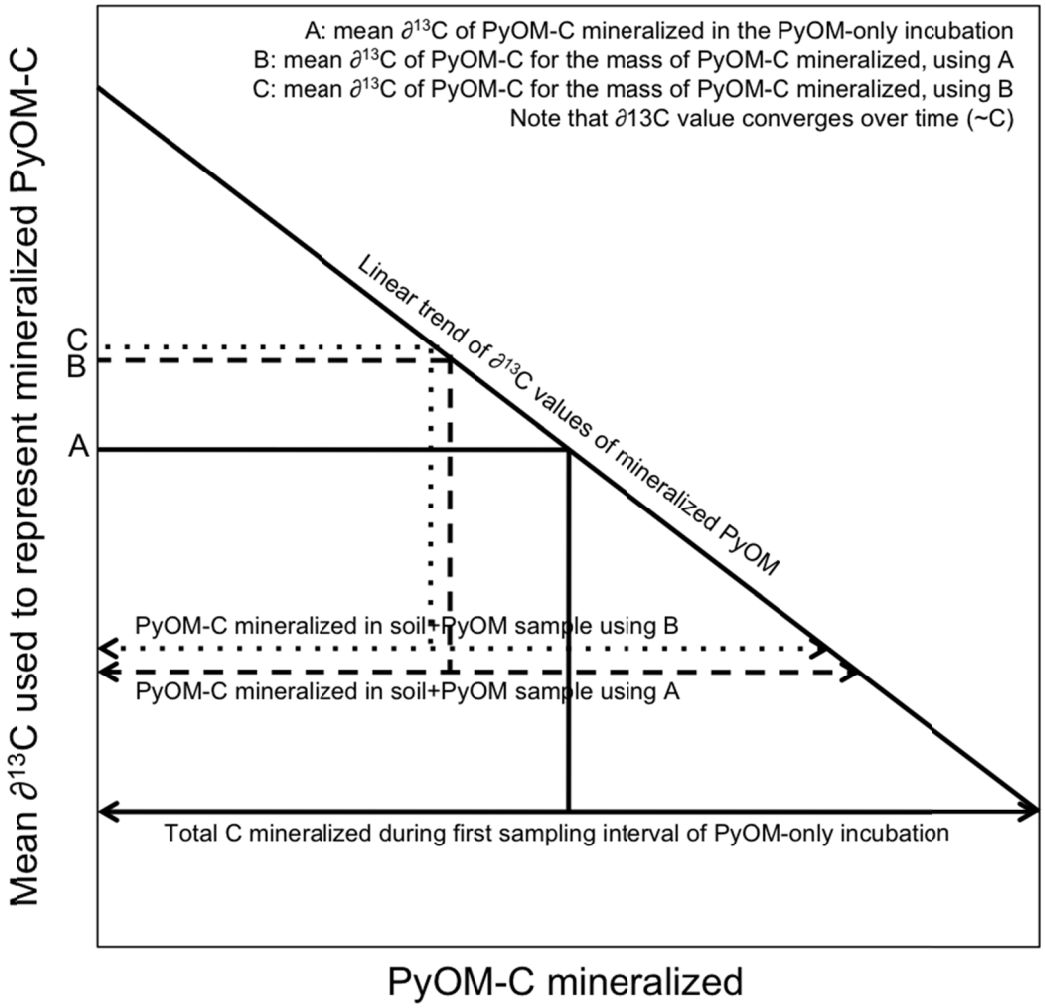

Figure S1. Iterative adjustments of $\delta^{13}C$ of mineralized PyOM to calculate the correct $\delta^{13}C$ value for a mineralized mass of PyOM in the soil+PyOM incubation that is less than that released in the first stage of the PyOM-only incubation.

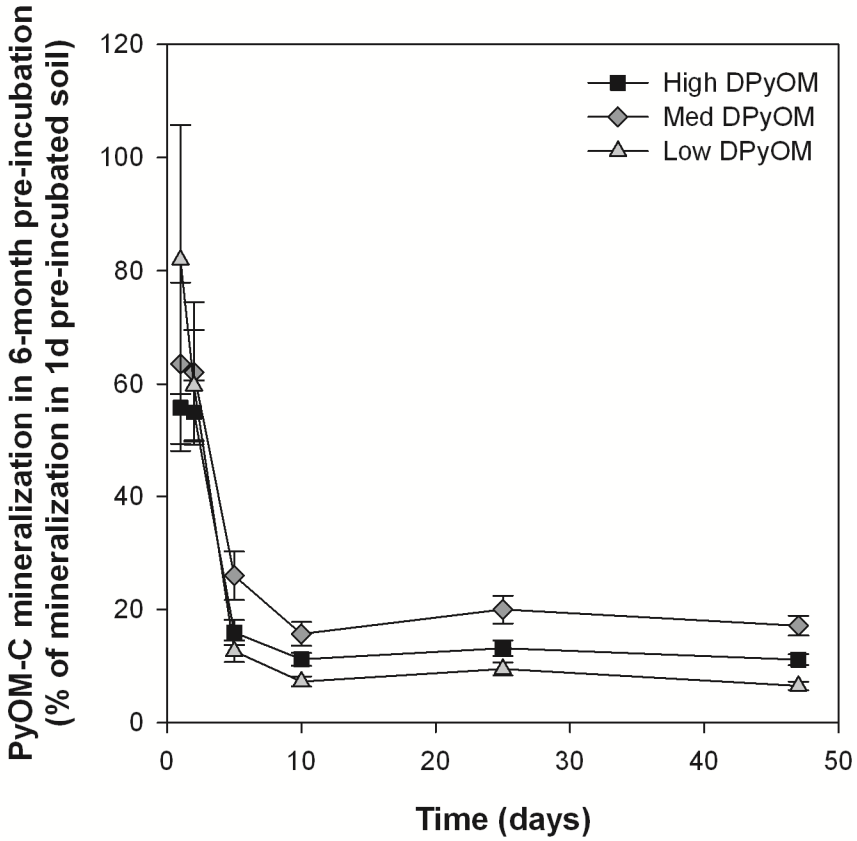

Figure S2. Cumulative mean relative effect of SOC on PyOM-C mineralization ((PyOM-C mineralized in the 6-month pre-incubated soil - PyOM-C mineralized in the 1-day pre-incubated soil)/(PyOM-C mineralized in the 6-month pre-incubated soil) x 100) over time. Error bars ±1SE, n=6-8.

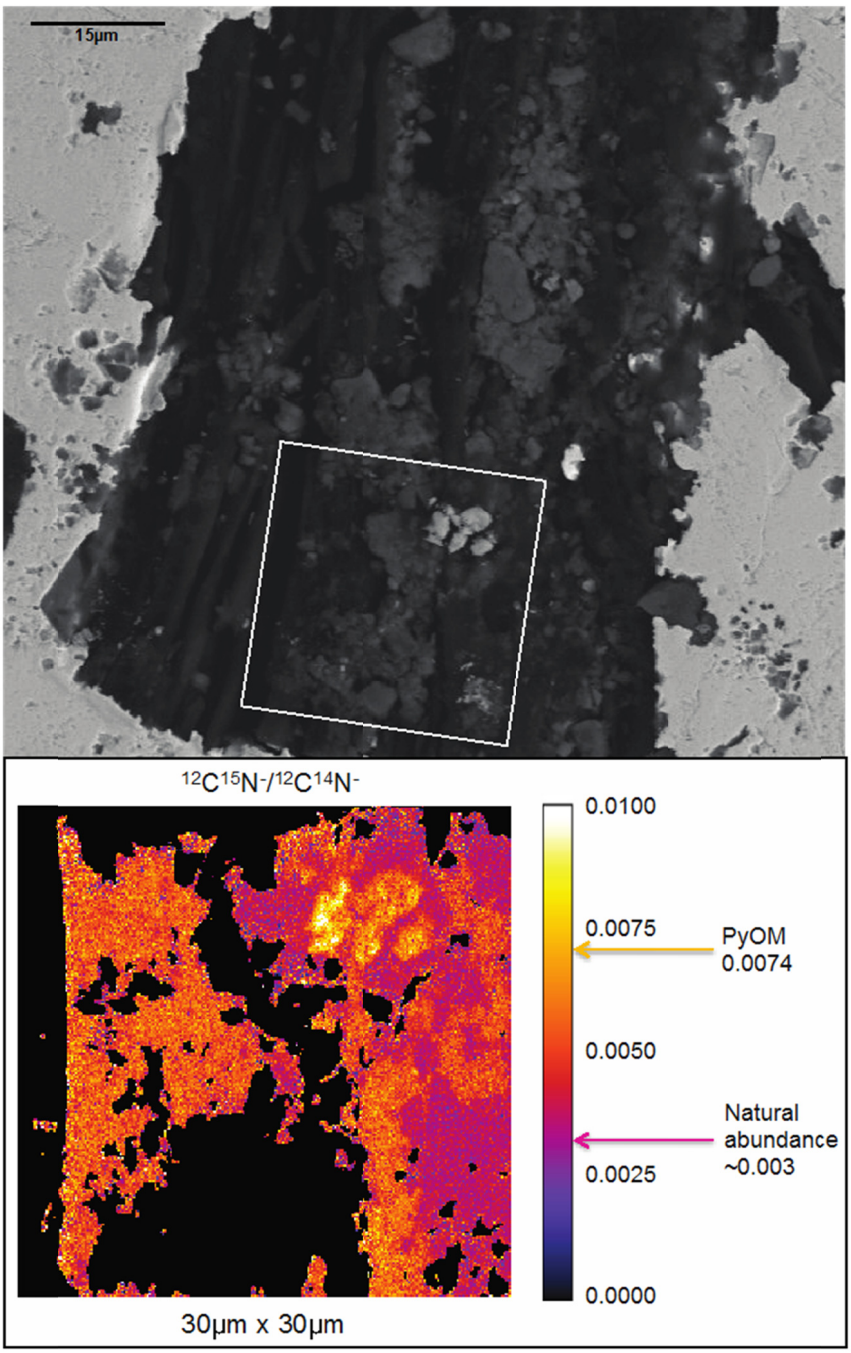

Figure S3. Top: SEM of PyOM particle with the white box indicating the region analysed with nanoSIMS. Bottom: calculated image of $^{15}N/^{14}N$ ratio (as $^{12}C^{15}N^-$ and $^{12}C^{14}N^-$ ions detected on 200 scans of nanoSIMS) across a 30 µm x 30 µm region of selected incubated PyOM particle. Note that the $^{15}N/^{14}N$ ratio of naturally-occurring organic matter is about 0.0037, while labelled sample was 0.0074. Thus, orange (light) areas indicate PyOM, while pink (dark) areas indicate sorbed SOM. Masked black regions indicate non-OM regions (soil minerals) or regions with low-resolution data due to image shifting during scanning.

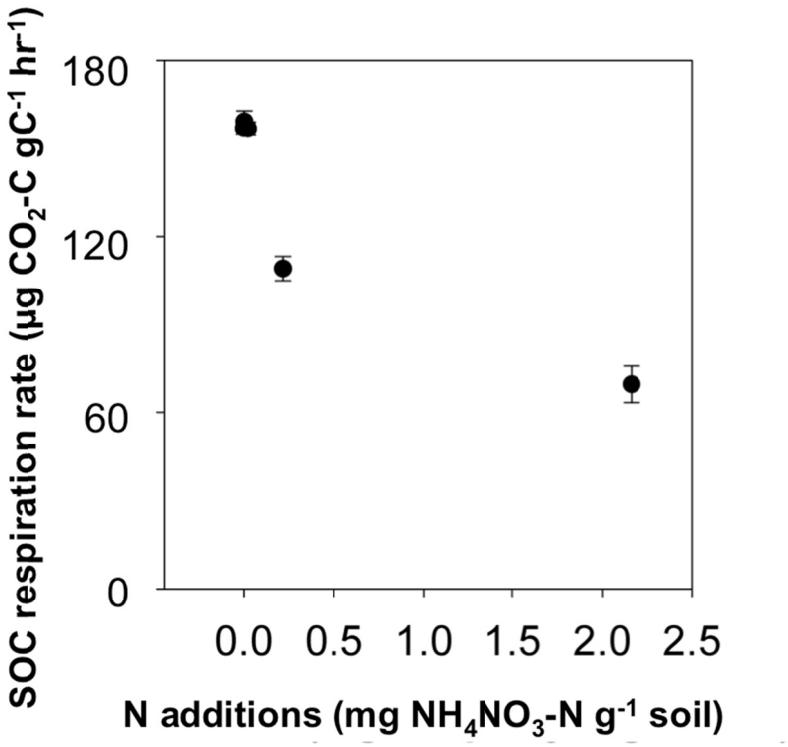

Figure S4. Effect of mineral N additions on 24-hour SOC mineralization. Between 0-2.2 mg NH_4NO_3-N g^{-1} soil were added to 10 g < 2 mm sieved soil, incubated at 30°C at 55% WFPS. Error bars ±SE, n=3.

The addition of nutrients equivalent to those added with the high-water-extractable PyOM treatment resulted in a significant decrease (*t*-test, $p<0.05$) in 48-hour SOC mineralization for all durations of pre-incubation (Supporting Figure S4).

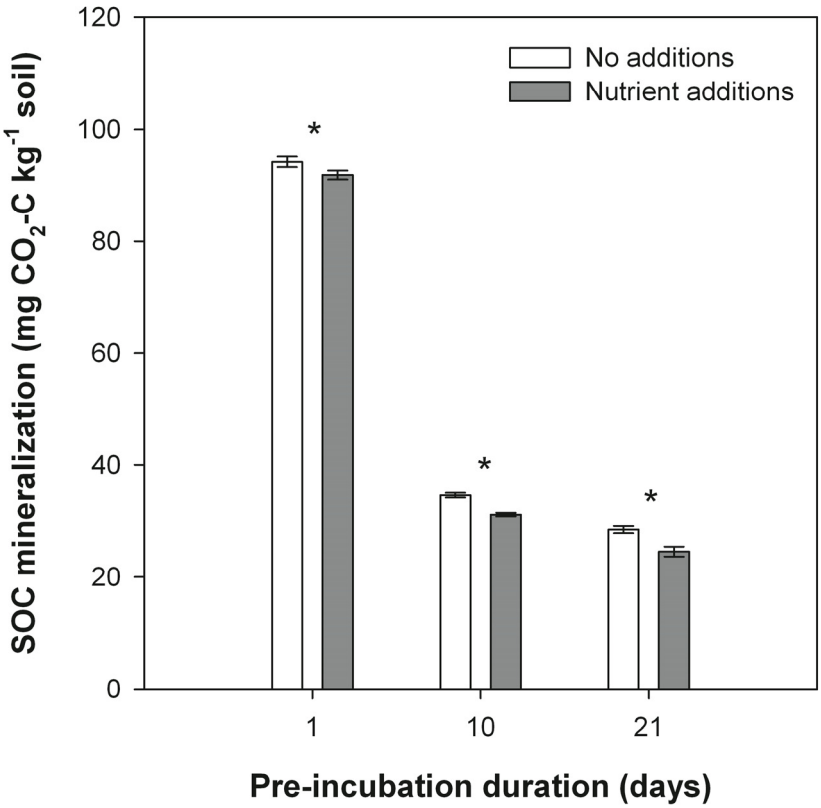

Figure S5. Mean 48-hour SOC mineralization in soils with (grey bars) and without (white bars) nutrient additions equivalent to those added with the PyOM. A * indicates significantly lower mineralization in the soils which received nutrients. Error bars ±1 SE, n=4-5.

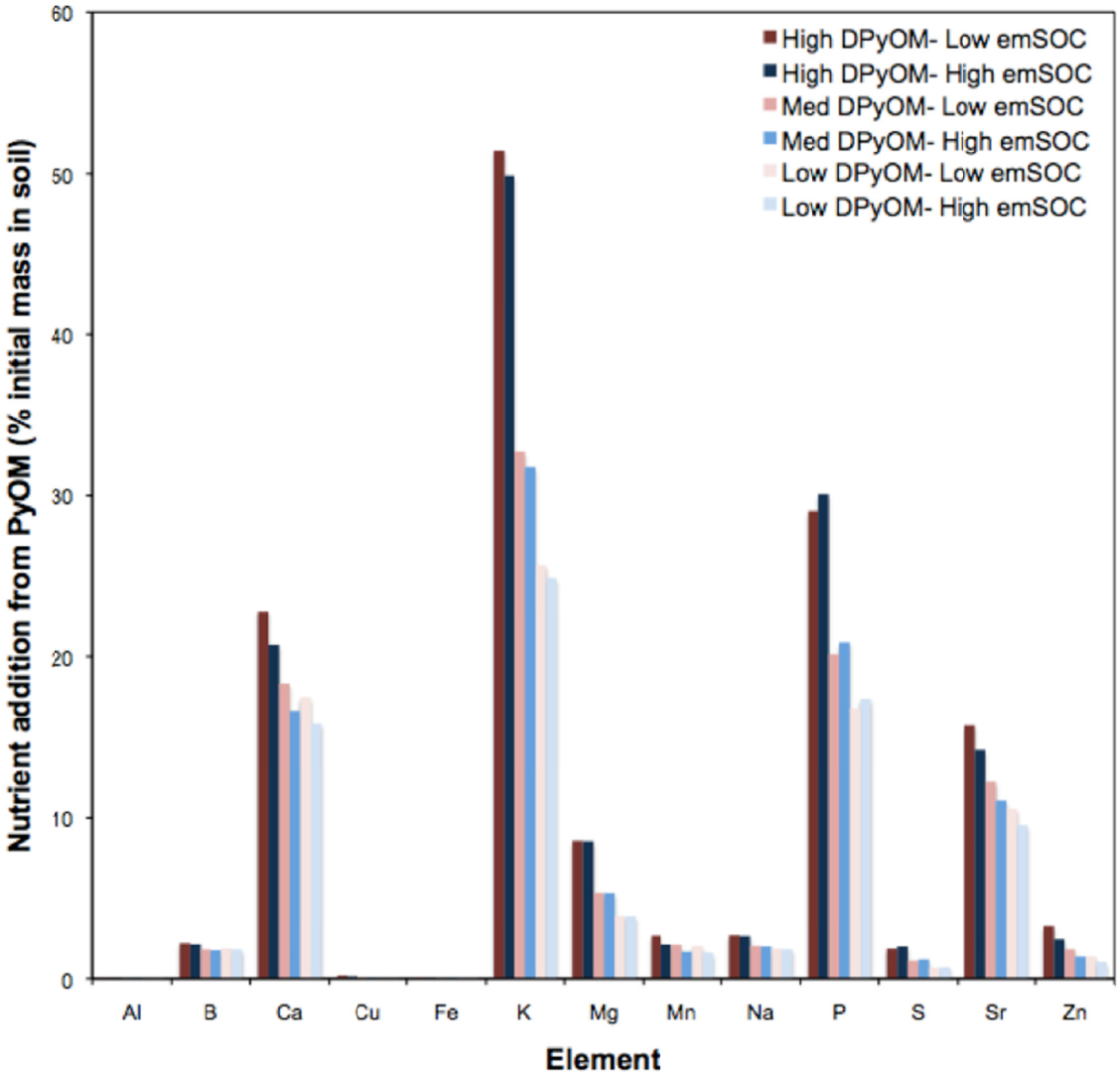

Figure S6. Increase in Mehlich-III-extractable nutrients with PyOM additions in relation to mass already present in soil.

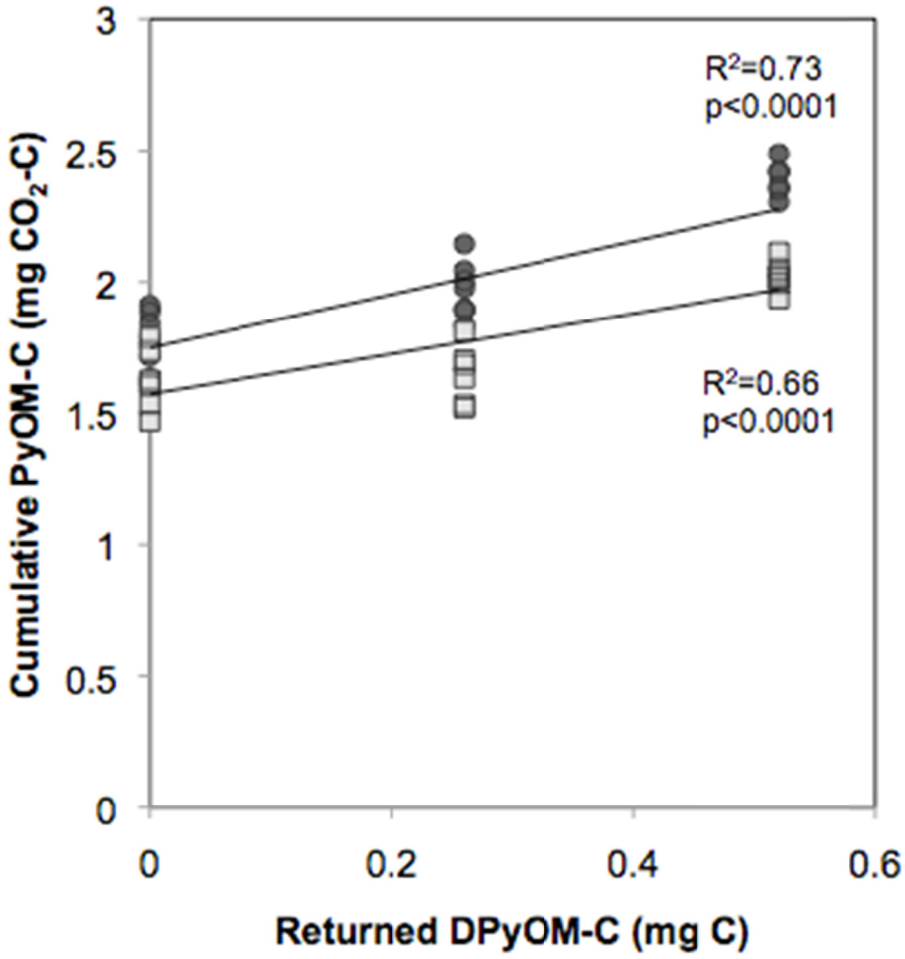

Figure S7. Final cumulative PyOM-C respired vs. amount of initial DPyOM-C returned after extraction. Dark grey circles indicate 6-month pre-incubated soils, while light grey squares indicate 1-day pre-incubated soils. Lines represent linear regressions. 6-month pre-incubation (top): $y=1.01x+1.75$, $p<0.0001$, $R^2=0.73$; 1-day pre-incubation (bottom): $y=0.76x+1.57$, $p<0.0001$, $R^2=0.66$.

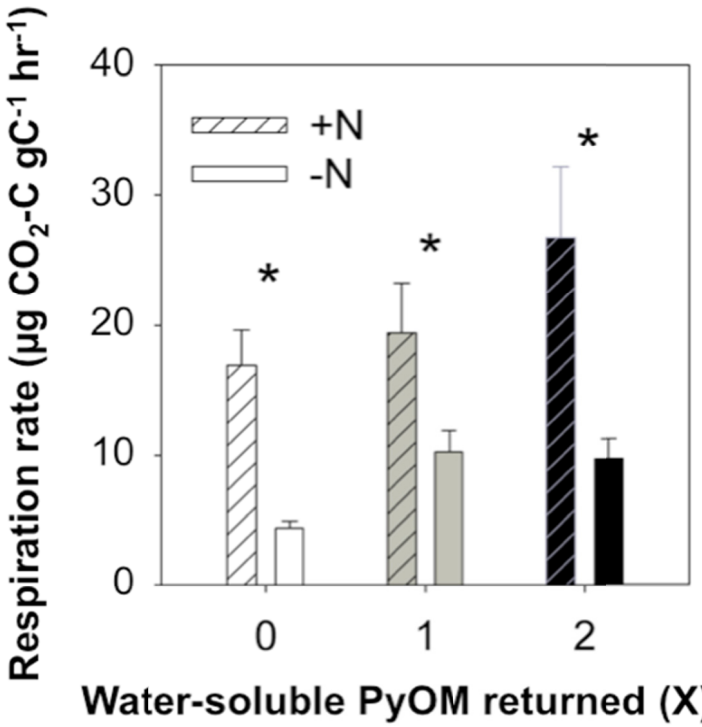

Figure S8. Mean impact of NH_4NO_3 additions on PyOM-C mineralization rates. 9.3 mg NH_4NO_3-N g^{-1} PyOM were added to 10 g ashed sand + 100 mg PyOM and maintained at 55% WFPS, and incubated at 30°C for 24 hours. * indicates significant differences between treatments with and with N additions ($p<0.05$), n=12.