Supplementary Information

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

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1. Details on isotopic partitioning

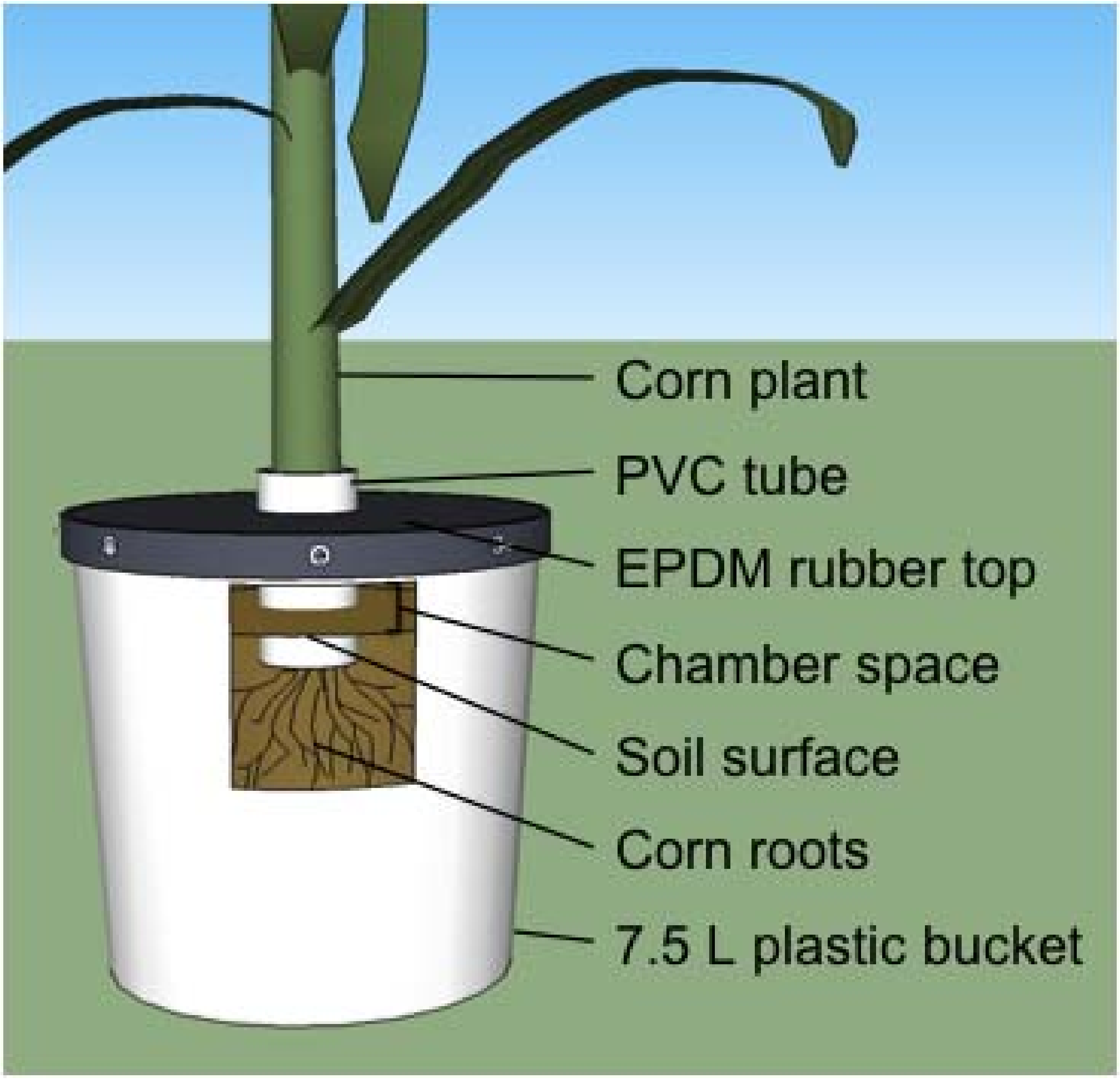
A key tool for priming research is the use of stable C isotopes, ^{12}C and ^{13}C, to differentiate the original sources of a common product in a two-part system. Briefly, the use of ^{13}C isotopic tracers for SOC studies derives from the contrasting metabolic pathways of C_3 and C_4 plants. During photosynthetic uptake of CO_2, C_3 plants discriminate more against the rare ^{13}C stable C isotope than C_4 plants (Farquhar *et al.*, 1989; O'Leary, 1988). Terrestrial plants with the C_3 pathway have $\delta^{13}C$ values ("$\delta^{13}C$" ties the measured $^{13}C/^{12}C$ to a standard $^{13}C/^{12}C$ ratio) in the range of -32‰ to -22‰. Plants with C_4 pathway have higher $\delta^{13}C$ values, ranging from -17‰ to -9‰ (Boutton, 1991). Furthermore, over time, the isotopic composition of SOC grows to closely resemble the isotopic composition of the vegetation from which it has been derived (Ågren *et al.*, 1996). Thus, given a pool of C, such as soil CO_2 emissions, and knowing the $\delta^{13}C$ values of its two C sources, one can mathematically derive what fraction each source contributed to the whole (Werth and Kuzyakov, 2010). In an experiment where a C_4 plant is grown on a soil developed under C_3 vegetation, we could derive the fraction of total soil CO_2 emissions that are from this plant as compared to those from the C_3 soil using the equation:

$$f_{C_4veg} = \frac{\delta_T - \delta_{C_3soil}}{\delta_{C_4veg} - \delta_{C_3soil}},$$

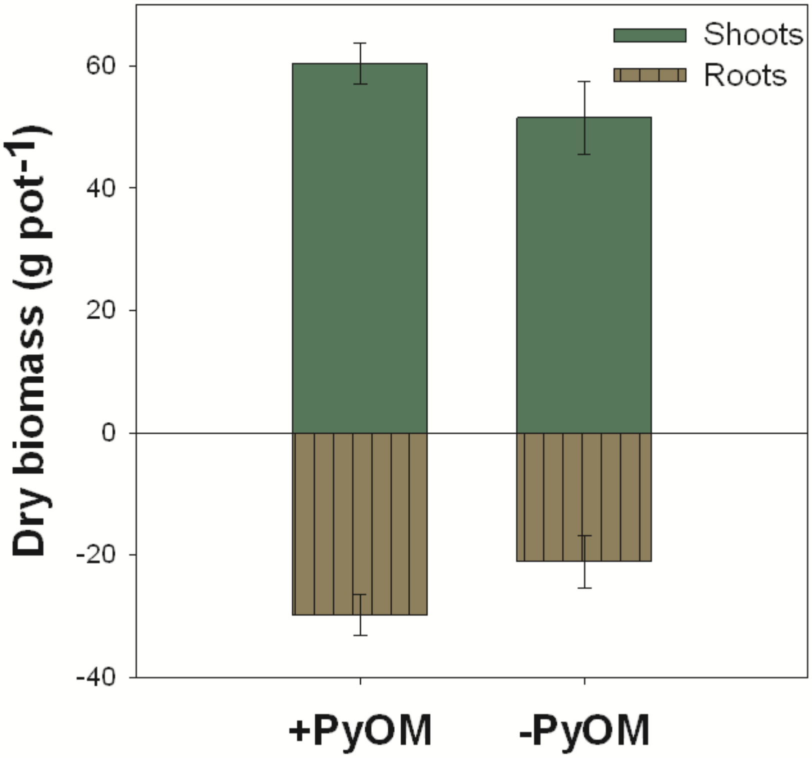
where f_{C4veg} is the fraction of CO_2 contributed by the C_4 plant, δ is the $\delta^{13}C$ signature of the total CO_2 (δ_T), the C_3 soil (δ_{C3soil}), and the C_4 vegetation (δ_{C4veg}) (Werth and Kuzyakov, 2010).

2. Notes on the challenges of applying isotopic partitioning

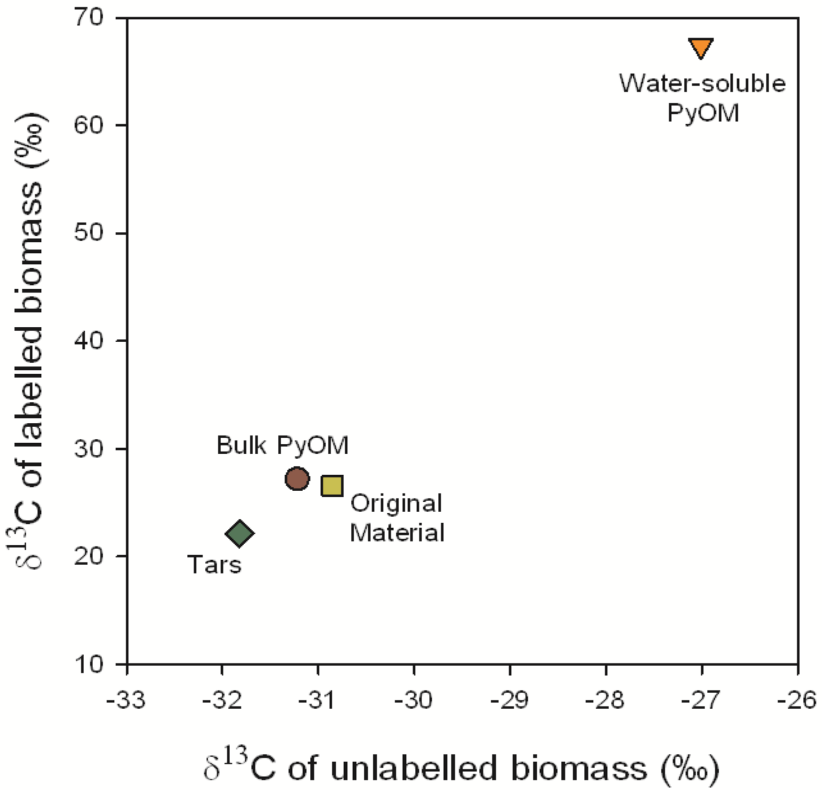
Although isotopic partitioning is an elegant concept, it can be challenging to apply, because a consistent approach does not exist for choosing what biomass (shoots, roots, or sugars in roots) or soil C (dissolved organic C [DOC], SOC, or microbial biomass) component is the best proxy for the $\delta^{13}C$ of the CO_2 emitted from the plant or the soil. If they all shared the same $\delta^{13}C$, this would not be a problem, but important isotopic fractionation can happen at coarse (roots *vs*. shoots) to fine (carbohydrates *vs*. lignin) levels. For example, the $\delta^{13}C$ of roots and the CO_2 they emit can differ by over 5‰ (Werth and Kuzyakov, 2010). We expect that PyOM also suffers from these issues. Czimczik *et al*. (2002) found that PyOM produced at lower charring temperatures was enriched in ^{13}C relative to the initial biomass, while higher temperatures resulted in a ^{13}C depletion. The volatiles released at each charring step ranged widely (by as much as 10‰ in softwood), likely due to the varied temperature ranges over which different compounds (characterized by different $\delta^{13}C$ values) undergo thermal decomposition. Furthermore, Zimmerman *et al*. (2011) showed that the $\delta^{13}C$ of CO_2 evolved from a PyOM incubation varied substantially over the course of a >500-day incubation. Thus, it is clearly important to identify whether the $\delta^{13}C$ of sub-components of PyOM serve as a better proxy for the $\delta^{13}C$ of the CO_2 derived from it than its bulk initial $\delta^{13}C$ value.



Supplementary Fig. S1. Pot and chamber design inspired by Yang and Cai (2006). Chamber is shown in closed (sampling) position. Sampling occurs through a rubber septum (not shown) and chamber includes a tube vent to prevent pressure changes (not shown).



Supplementary Fig. S2. Biomass production with and without PyOM additions. Error bars represent ±1SE (n_{+PyOM}=5,n_{-PyOM}=6).



Supplementary Fig. S3. Comparison of ∂^{13}C values for labelled and unlabelled sugar maple PyOM and sub-components, including original materials. Water-soluble PyOM is consistently enriched in ^{13}C, while tars are consistently depleted.

Supplementary Table S1

Total elemental analysis of Mehlich III extraction (mg kg^{-1}).

Element	Initial soil	Wood feedstock	PyOM
B	19.3	6.6	6.3
Ca	104.8	3280.3	2344.6
Cu	6.9	3.2	0.8
Fe	257.8	20.6	9.1
K	40.4	2482.3	2434.1
Mg	27.8	500.1	230.9
Mn	88.1	502.3	265.1
P	1.0	479.7	360.1
S	21.3	59.6	33.1
Zn	52.1	23.3	8.5

Supplementary Table S2

Modified Hoagland's solution.

Stock	**Chemical**	**Concentration**		**Final additions per pot**	
				- plants	**+ plants**
Macronutrients	KNO_3	0.7755	M	54.5	91.8
	$MgSO_4$	0.3	M	21.1	35.5
	$NH_4H_2PO_4$	0.255	M	17.9	30.2
	NH_4NO_3	0.33	M	23.2	39.1
Ca	$Ca(NO_3)2$	3.75	M	52.3	88.0
Micronutrients	H_3BO_3	1.875	mM	0.132	0.222
	$MnSO_4$	0.15	mM	0.011	0.018
	$ZnSO_4$	0.0375	mM	0.003	0.004
	$CuSO_4$	0.0375	mM	0.003	0.004
	Na_2MoO_4	0.0375	mM	0.003	0.004
	$NiSO_4$	0.06	mM	0.004	0.007
Fe	FeEDTA	93.75	mM	1.306	2.200

Supplementary Table S3

Measured ^{13}C proxies (∂^{13}C relative to PDB standard ±SE (‰)) for PyOM.

Sub-component	**Unlabelled PyOM**	**Labelled PyOM**
Bulk	-31.22 ± 0.01 (n=3)	+27.21 ± 0.19 (n=7)
Dissolved PyOM	-27.01 (n=1)	+67.37 ± 1.67(n=5)
Tars or volatiles	-31.82 ± 0.04 (n=4)	+22.15 ± 0.19 (n=5)
Original wood	-30.85 ± 0.03 (n=3)	+26.53 ± 1.04 (n=3)
Respired PyOM	n.d.	+27.04 ± 0.64 (Keeling plot intercept, n=6)

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