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Emission of CO_2 from biochar-amended soils and implications for soil organic carbon

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Abstract

Soil amendment with pyrogenic organic matter (PyOM), also named biochar, is claimed to sequester carbon (C). However, possible interactions between PyOM and native soil organic carbon (SOC) may accelerate the loss of SOC, thus reducing PyOM's C sequestration potential. We combined the results of 46 studies in a meta-analysis to investigate changes in CO_2 emission of PyOM-amended soils and to identify the causes of these changes and the possible factors involved. Our results showed a statistically significant increase of 28% in CO_2 emission from PyOM-amended soils. When grouped by PyOM C (PyC):SOC ratios, the group of studies with a ratio >2 showed a significant increase in CO_2 emissions, but those with a ratio <2 showed no significant effect of PyOM application on CO_2 emission. Our data are consistent with the hypothesis that increased CO_2 emission after PyOM addition is additive and mainly derived from PyOM's labile C fractions. The PyC:SOC ratio provided the best predictor of increases in CO_2 production after PyOM addition to soil. This meta-analysis highlights the importance of taking into account the amount of applied PyC in relation to SOC for designing future decomposition experiments.

Keywords: additive effects, carbon sequestration, decomposition, priming, pyrogenic organic matter, recalcitrance

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Introduction

Soil organic carbon (SOC) plays vital roles in important soil ecosystem services such as soil fertility, carbon (C) sequestration and mitigation of greenhouse gas emission (Paustian *et al.*, 1997). Application of pyrogenic organic matter (PyOM; also named biochar) to agricultural soils has the potential to sequester C in the long-term because PyOM is assumed to be highly recalcitrant in soil (Goldberg, 1985). Pyrogenic organic matter is composed of a range of different forms of C, with predominance of fused aromatic ring structures (Shindo *et al.*, 1986; Glaser *et al.*, 1998; Schmidt & Noack, 2000; Novotny *et al.*, 2009). When formed under natural conditions, such as in forest fires, this material is known as 'black carbon' (BC). Black carbon has been found to be the oldest fraction of C in soils, even compared to the most protected C in soil aggregates and organo-mineral complexes (Pessenda *et al.*, 2001); it can persist in the soil for millennia (Kuzyakov *et al.*, 2009). The production of PyOM and its application to soil is, therefore, a potential strategy to sequester C in soils (Lehmann, 2007).

Although PyOM is considered to be highly recalcitrant, it is not completely biologically inert (Jones *et al.*, 2012; Farrell *et al.*, 2013). Application of PyOM to soil can increase CO_2 emission as PyOM contains a proportion of relatively labile aliphatic C structures (Cheng *et al.*, 2006; Liang *et al.*, 2008). Such increases may be additive if the fluxes from SOC and PyOM behave independently and the decomposition rate of SOC is not affected by PyOM addition. However, it has been proposed that PyOM can interact with native SOC turnover, either accelerating its decomposition (Farrell *et al.*, 2013) or decelerating it (Keith *et al.*, 2011). These interactions are summarized under the term 'priming effect' (PE). Priming effect is defined as strong short-term changes in the turnover of soil organic matter caused by comparatively moderate treatments of the soil (Kuzyakov *et al.*, 2000).

Wardle *et al.* (2008) suggested that PyOM accelerates decomposition of nonpyrogenic carbon as they found greater CO_2 production from mixtures of PyOM and forest humus than predicted from the sum of these components considered separately. However, the origin of the (additionally) respired CO_2 (i.e. whether from SOC or PyOM) was not identified. In a recent meta-analysis investigating priming effects of PyOM addition, Maestrini *et al.* (2014b) showed that labile C fractions of applied PyOM induce a short-term positive priming effect on native SOC, especially when PyOM with low C content is used. They also showed that priming

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becomes negative over time (after 200 days), possibly as a result of sorption of dissolved organic carbon on PyOM surface.

Irrespective of the mechanisms involved, these results suggest that PyOM addition to soil could undermine its C sequestration potential (Cross & Sohi, 2011). Several studies have investigated this topic using isotope analysis. Findings suggested acceleration of SOC decomposition (Luo *et al*., 2011; Farrell *et al*., 2013), deceleration (Keith *et al*., 2011; Knicker *et al*., 2013), both effects (Zimmerman *et al*., 2011; Bamminger *et al*., 2013) or no effects on SOC decomposition (Jones *et al*., 2012; Santos *et al*., 2012; Díaz-Rojas *et al*., 2014), without indicating the probable cause(s) of this variation in effects (Maestrini *et al*., 2014b).

When soils are amended with PyOM, changes in CO_2 emission rates can occur as a consequence of several factors, either associated with environmental conditions such as temperature (Hilscher & Knicker, 2011) or soil (type or disturbance intensity), or with PyOM (type, application rate) (Singh *et al*., 2010). Pyrolysis process parameters (e.g. pyrolysis temperature, residence time) also play a role (Bamminger *et al*., 2013; Farrell *et al*., 2013) as they affect the characteristics of the resulting PyOM, including the amount of labile C remaining after production. While there are now a number of studies in the primary literature reporting changes in CO_2 emission following PyOM application to soil, individual studies necessarily utilize a limited number of soils and PyOM types. As such, they do not allow the drawing of robust conclusions regarding the nature of changes in CO_2 emission for the wide range of PyOM and soil types outside of the experimental conditions used. Therefore, a joint analysis, utilizing the entire body of studies currently available, is required to better formulate hypotheses regarding the mechanisms behind observed effects.

Meta-analysis is a powerful technique that provides a quantitative statistical means of integrating the results of independent studies, allowing for general conclusions to be drawn (Gurevitch & Hedges, 2001; Borenstein *et al*., 2009). Currently, a few meta-analyses on PyOM research have been published in the fields of crop yield (Jeffery *et al*., 2011; Liu *et al*., 2013; Biederman & Harpole, 2013 – but see Jeffery *et al*., 2014), N_2O emission (Cayuela *et al*., 2013) and priming (Maestrini *et al*., 2014b), with relevant contributions for enhancing our understanding of PyOM effects on soil processes and functions.

Here, we build and expand on work reported by Maestrini *et al.* (2014b) and integrate results from 46 independent studies, including those which did not use stable isotopes, to gain a better understanding of the effects of soil amendments with PyOM on CO_2 emissions. Such analysis will allow the identification of factors associated with soil, PyOM properties and experimental conditions that can affect CO_2 emission from PyOM-amended soils. These data are vital to allow the effective guidance of policy, for example, to determine whether PyOM is eligible for future C-trading schemes (Lehmann *et al*., 2006).

Materials and methods

Data sources and compilation

We performed a systematic literature search of peer-reviewed publications on the effects of PyOM addition to soils on soil CO_2 fluxes using Scopus and Web of Science databases. Different combinations of keywords were used ('biochar' OR 'PyOM' OR 'charcoal' OR 'black carbon' AND 'priming' OR 'CO_2'), selecting also 'Abstract, Title, Keywords' for search field with data range '2000 to present'. We limited our search to 2000 because as far as we are aware, there are no relevant studies published in this topic before that date. The cut-off date was 23 April 2014.

Studies performed under laboratory, greenhouse and field conditions were included. Experiments were grouped according to the provenance of the soils used, whether from (sub-) tropical or temperate regions. Data are reported as rates of CO_2 emission on the basis of soil mass or area. If CO_2 emission had been measured several times in the same study, only the last sampling date was used. Our approach intended to avoid introducing bias into the analysis because some studies included considerably more data points than others. Cumulative CO_2 emissions were far more commonly reported in studies than daily fluxes. Therefore, cumulative values were preferred over daily or individual measurements when both types were available. In both cases, only studies that reported CO_2 emission from bulk soil samples after a clearly defined experimental period were included.

A minimum of three replicates per treatment was required for the study to be included in the meta-analysis. When PyOM was produced from the same feedstock and pyrolysis type, but under a range of temperatures, data from the highest and lowest temperatures were recorded. This reduced the potential bias of introducing many nonindependent data points from a single study. When pyrolysis temperature was given as a range (e.g. 400–500°C), the highest value was chosen (i.e. 500°C). Only studies that used PyOM in combination with soil were included in the meta-analysis; we excluded studies where washed sand or humus was used instead of soil. Studies of CO_2 emission from anthropogenic dark earths were also excluded as information is not available on the original amounts of PyOM applied to or present in the soil, its age, production conditions nor the environmental factors to which PyOM has been subjected (Pereira *et al*., 2014).

We collected data comparing CO_2 emission between a control and a PyOM treatment. One major assumption of meta-analysis is that studies and data points are independent (Gurevitch & Hedges, 2001; Borenstein *et al*., 2009). When particular publications reported data from more than one study

system (e.g. different PyOM feedstock, pyrolysis type, pH and experimental type; lab or field), those systems were considered independent and were treated as such. The control was chosen to be identical to the treatment for all variables but without the addition of PyOM. For each observation within every study, we collected the means of the control treatment (soil without PyOM) and the experimental treatment (PyOM-amended soil), as well as their standard deviation (SD) and replicate numbers (n). We acknowledge that in some instances, this procedure means that more than one experimental treatment may be compared to the same control. In these instances, this approach artificially increases the number of replicates that the statistic is based on and as such may bias the results towards overconfidence (i.e. confidence intervals may be too narrow). However, utilizing the more conservative approach of using only a single average measurement for all potentially dependent measures (e.g. Borenstein *et al*., 2009) sacrifices too much information, as discussed in Guo & Gifford (2002).

For studies that did not report SD or a measure of variance that could be used to calculate SD, such as standard error (SE), efforts were made to obtain these from the corresponding authors. In some cases, this was successful (see Acknowledgements). If not, those studies were excluded from the analysis. When data were only provided in graphs, Plot Digitizer 2.6.2 was used to extract data points. Unidentified error bars were present in three studies and were conservatively assumed to denote SE rather than SD.

The search resulted in 46 peer-reviewed papers published from 2009 to 2014 that were suitable for being included in the meta-analysis (Spokas & Reicosky, 2009; Spokas *et al*., 2009; Steinbeiss *et al*., 2009; Novak *et al*., 2010; Smith *et al*., 2010; Bruun *et al*., 2011; Jones *et al*., 2011; Kharu *et al*., 2011; Knoblauch *et al*., 2011; Luo *et al*., 2011; Streubel *et al*., 2011; Zavalloni *et al*., 2011; Zimmerman *et al*., 2011; Aguilar-Chávez *et al*., 2012; Awad *et al*., 2012; Bruun *et al*., 2012; Case *et al*., 2012; Dempster *et al*., 2012; Galvez *et al*., 2012; Jones *et al*., 2012; Kuzyakov *et al*., 2009; Quilliam *et al*., 2012; Santos *et al*., 2012; Singh *et al*., 2012; Zhang *et al*., 2012; Ameloot *et al*., 2013; Bamminger *et al*., 2013; Knicker *et al*., 2013; Lai *et al*., 2013; Major *et al*., 2010; Malghani *et al*., 2013; Mukome *et al*., 2013; Saarnio *et al*., 2013; Stewart *et al*., 2013; van Zwieten *et al*., 2013; Troy *et al*., 2013; Yu *et al*., 2013; Zhang *et al*., 2013; Díaz-Rojas *et al*., 2014 ; Fernández *et al*., 2014 ; Junna *et al*., 2014; Maestrini *et al*., 2014a; Mukherjee & Zimmerman, 2014; Schimmelpfennig *et al*., 2014; Sigua *et al*., 2014; Whitman *et al*., 2014). The database covered 288 side-by-side comparisons and is integrally available as an excel file at supporting information (Dataset_1_SuppInfo.xls).

Data grouping and treatment

Besides the data on measured response variables, details of experimental conditions also needed to be specified as categories for inclusion in the analysis. Studies were categorized by soil properties and environmental conditions (C content, pH, C:N ratio, fertilization background, texture and provenance), PyOM properties (C content, C:N ratio, pH, ash content, surface area, pyrolysis type and residence time, pyrolysis temperature and feedstock) and experimental conditions (duration, temperature of incubation, moisture content and type). The proportion of applied PyOM C (PyC) in relation to original SOC content (PyC:SOC ratio) was also calculated and included. Because initial analyses demonstrated a highly significant difference between studies with higher and lower PyC:SOC ratios (i.e. >2 and <2, respectively), the data were split into two subgroups for ratios >2 and <2 within each category to assess the impact of this factor on other potentially relevant factors (categories).

Data from the different categories were subjected to a standardization process to allow for comparisons. For instance, soil or PyOM pH values measured with $CaCl_2$ were found in six studies and were made comparable with pH measured with distilled water using the formula $pH(H_2O) = 1.65 + 0.86 * pH(CaCl_2)$ (Augusto *et al*., 2008). When no information on pH measurement method was provided, data were assumed to denote pH measured in distilled water. Data reported on a continuous scale (such as pH) were placed into categories which covered a range of scales, such as 1 pH unit (i.e. pH 6–7), resulting in an adequate number of data points in each subgroup. When the authors did not explicitly provide soil texture, it was defined based on soil contents of sand, clay and silt, according to FAO/UNESCO (2003). It is important to note that not all studies are included in all subcategories. This may be due to the study not having reported that metric as it was not of interest to that particular study. The number of studies included in each subcategory is included in the figures and tables under the label 'n'.

Statistics

A quantitative index of the effect size in each comparison was calculated as the natural log of the response ratio using the following formula (Rosenberg *et al*., 2000):

$$\ln RR = \ln\left(\frac{X^E}{X^C}\right)$$

where RR = response ratio, X^E = mean of experimental group and X^C = mean of control group. The effect size of each grouping was calculated using a categorical random effects model, where the effect size was weighted in inverse proportion to its variance (Adams *et al*., 1997). Publication bias (Rothstein *et al*., 2005) is unlikely in our meta-analysis. Not only increases and decreases, but also lack of significant effects in CO_2 emission in PyOM-amended soils are equally publishable. Nevertheless, we tested the effects of publication bias using the Fail-safe N technique (Orwin, 1983; Rosenthal & Rosnow, 1991). This technique involved computing the combined P value for all of the studies included and calculating the number of additional studies showing no effect (i.e. average Z value of 0) that would be needed to change the P value from significant to nonsignificant at $P = 0.05$.

The mean of a response variable was considered significant if the lower limit of the 95% CI was >1 or the upper limit of the 95% CI <1. The latter case can be considered evidence for negative priming; however, significantly positive values could be the result of additive effects and/or priming. The means of different subcategories were tested for significant differences

based on the model heterogeneity test (*Q*-test), which is tested against a χ^2 distribution with 1 degree of freedom (df) (Rosenberg *et al.*, 2000). Calculations were performed using METAWIN version 2 Statistical software (Rosenberg *et al.*, 2000) and in Microsoft Excel worksheets. The response ratio (RR) and CI of treatments presented in Tables and Figures were back-transformed from ln RR.

Results

Main effect

On average, CO_2 emission increased significantly by 28% (RR = 1.28; CI = 1.15–1.41) following the addition of PyOM to soil. Rosenthal's Fail-safe *N* was 1738, indicating that the statistical significance of the reported increase of CO_2 emission after PyOM addition is unlikely due to publication bias. Studies with high PyOM C additions relative to SOC (PyC:SOC ratio >2) had a significantly higher CO_2 flux than studies with a PyC:SOC ratio <2 (RR = 1.99 and 1.04, respectively; $P < 0.01$; Fail-safe *N* = 1589 for the relatively high application; Fig. 1). For the relatively low application, the effect on CO_2 emission was not significant. For this reason, we analysed all further factors for both categories separately.

Influence of soil characteristics

There were significant differences in CO_2 emission ($P \leq 0.05$) between subgroups of different soil-associated categories for PyC:SOC ratios >2, for soil fertilization background and soil provenance (Table 1). For PyC:SOC ratios <2, there were significant differences between subgroups for soil C content and soil texture.

Soils with C content ≤10 g kg^{-1} showed a significantly increased CO_2 emission (Table 1) at both PyC:SOC ratios. Soils with C content >30.0 g kg^{-1} were not affected by PyOM additions in terms of CO_2 emissions, regardless of the PyC:SOC ratios. Soils with C:N ratios ≤30.0 showed increased CO_2 emission only at PyC:SOC ratios >2. Soils with C:N ratio >10.0 had emissions of CO_2 significantly increased independently of the PyC:SOC ratios. Additions of PyOM to soils with pH values >6.0 resulted in increased CO_2 emission only when PyC:SOC ratio was >2. Additions of PyOM to soils with pH values ≤6.0 resulted in significant increases in CO_2 emission regardless of the PyC:SOC ratio. Soils with a history of N fertilization and those without any fertilizer input increased CO_2 emission only at PyC:SOC ratios >2. Contrarily, the CO_2 emission from soils with a history of NPK fertilization was unaffected by PyOM addition.

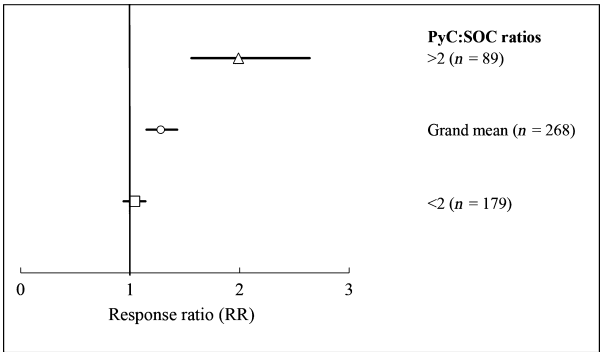

Fig. 1 Influence of different PyC:SOC ratios on CO_2 emission from PyOM-amended soils. Symbols show response ratio (RR), and bars show 95% confidence intervals. The numbers shown in parentheses correspond to the number of observations upon which the statistical analysis is based.

Emission of CO_2 increased in medium-textured soils after addition of PyOM, regardless of the PyC:SOC ratio. Coarse-textured soils increased CO_2 emissions only at high PyC:SOC ratios (>2) and fine-textured soils only when PyC:SOC ratio was <2. Soils originating from temperate regions increased their CO_2 emission following PyOM addition only at PyC:SOC ratios >2. However, experiments using soils from (sub-)tropical regions did not show significant effects in terms of CO_2 emission irrespective of the PyC:SOC ratio.

Influence of PyOM characteristics

For subgroups of PyOM-associated characteristics, there were significant ($P \leq 0.05$) differences in CO_2 emission at PyC:SOC ratios >2, for PyOM surface area, pyrolysis residence time and PyOM feedstock (Table 2). At PyC:SOC ratios <2, there were significant differences in CO_2 emission among subgroups of different categories for PyOM C content and PyOM pH.

We observed a significant increase in CO_2 emission at both PyC:SOC ratios when PyOM with C content varying from 50.1 to 80.0% was used. However, the response ratio was significantly larger ($P < 0.01$) at PyC:SOC ratios >2 (RR = 1.88) than <2 (RR = 1.16). When PyOM with C content ≤50.0% and >80.0% was applied to the soil, only PyC:SOC ratios >2 resulted in increased CO_2 emission. Similarly, only PyC:SOC ratios >2 resulted in increased CO_2 emission both at PyOM C:N ratios ≤50 and >50 (RR = 2.40 and 2.54, respectively). Pyrogenic organic matter with pH values ≤8.0 resulted in increased CO_2 emission from the soil independently of PyC:SOC ratios. When PyOM with pH values >8.0 was used, only PyC:SOC ratios >2 significantly increased CO_2 emission.

Only PyC:SOC ratios >2 resulted in significant increases of CO_2 emission using PyOM with ash contents ≤10.0 and >10.0% (RR = 1.98 and 1.79, respectively). When PyOM with surface area ≤50.0 m^2 g^{-1}

Table 1 Meta-analysis of the effects of PyC:SOC ratios on CO_2 emission, within soil-associated characteristics. PyC:SOC ratios are divided as >2 and <2

Categories	PyC:SOC ratio	RR	95% CI	*n*	*P**	*P***
Soil C content (g kg^{-1})						
≤10.0	>2	2.55	1.57–4.53	23	<0.01	0.22
	<2	1.16	1.05–1.27	29		<0.01
10.1–30.0	>2	1.94	1.42–2.63	61	<0.01	
	<2	1.13	1.01–1.25	85		
>30.0	>2	1.05	0.13–3.20	3	0.63	
	<2	0.97	0.82–1.14	65		
Soil C:N ratio						
≤10.0	>2	2.85	1.65–5.17	22	<0.01	0.08
	<2	1.17	0.99–1.40	25		0.97
>10.0	>2	1.38	1.04–1.86	6	0.08	
	<2	1.14	1.06–1.23	83		
Soil pH						
≤6.0	>2	1.48	1.01–2.11	35	0.19	<0.01
	<2	1.14	1.04–1.24	58		0.04
>6.0	>2	2.64	1.83–4.00	46	<0.01	
	<2	0.98	0.85–1.12	109		
Fertilization background						
N	>2	12.01	6.75–20.99	8	<0.01	<0.01
	<2	1.04	0.88–1.22	12		0.86
None	>2	1.92	1.40–2.58	65	<0.01	
	<2	1.00	0.85–1.17	97		
NPK	>2	1.07	0.86–1.42	14	0.36	
	<2	0.97	0.88–1.06	32		
Soil texture						
Medium	>2	3.01	1.95–4.57	32	<0.01	0.11
	<2	1.14	1.02–1.30	54		<0.01
Coarse	>2	3.01	1.69–5.30	24	<0.01	
	<2	0.85	0.65–1.09	53		
Fine	>2	1.20	0.78–2.19	5	0.46	
	<2	1.33	1.19–1.49	25		
Soil provenance						
Temperate	>2	2.16	1.67–2.90	79	<0.01	0.03
	<2	1.05	0.94–1.17	140		0.50
(Sub-) tropical	>2	1.04	0.80–1.50	10	0.75	
	<2	1.00	0.91–1.09	37		

RR, response ratio; CI, 95% confidence interval for RR; *n*, number of observations within each category; *P**, probability of the model heterogeneity test between the two relative application ratios (PyC:SOC ratio >2 and <2, respectively) within the various groupings; *P***, probability of the model heterogeneity test between the various groupings within the categories of relative application ratios.

was applied to the soil, there was increased CO_2 emission at PyC:SOC ratios >2. However, when PyOM with surface area >50.0 was used, there were no changes in CO_2 emission at both PyC:SOC ratios.

Emissions of CO_2 from soils amended with pyrogenic organic matter produced under pyrolysis residence times ≤0.5 h were significantly increased at PyC:SOC ratios >2 (RR = 12.01), but not at PyC:SOC ratios <2. No significant increases in CO_2 emission were observed when PyOM produced under pyrolysis residence times >0.5 h was applied. At PyC:SOC ratios >2, there was a significant increase in CO_2 emission independently of feedstock used for PyOM production. Emission of CO_2 from soils amended with PyOM produced from lignocellulosic waste and herbaceous materials was greater (RR = 3.29 and 2.97, respectively) than from wood materials (RR = 1.52) at PyC:SOC ratios >2.

Pyrogenic organic matter produced at temperatures ≤350°C increased CO_2 emission independently of the PyC:SOC ratio (RR = 2.22 and RR = 1.50 for PyC:SOC ratio >2 and <2, respectively) (Fig. 2). When PyOM was produced at temperatures ranging from 351 to 550°C, there was increased CO_2 emission only at PyC:SOC ratio >2 (RR = 1.97). For PyOM produced at

Table 2 Meta-analysis of the effects of PyC:SOC ratios on CO_2 emission, within PyOM-associated characteristics. PyC:SOC ratios are divided as >2 and <2

Categories	PyC:SOC ratio	RR	95% CI	*n*	*P**	*P***
PyOM C content (%)						
≤50.0	>2	2.92	1.40–4.89	6	<0.01	0.70
	<2	0.77	0.63–0.95	37		<0.01
50.1–80.0	>2	1.88	1.43–2.46	64	<0.01	
	<2	1.16	1.03–1.31	110		
>80.0	>2	2.27	1.19–4.68	19	0.04	
	<2	0.98	0.85–1.13	28		
PyOM C:N ratio						
≤50.0	>2	2.54	1.50–4.31	21	<0.01	0.85
	<2	1.01	0.88–1.15	80		0.67
>50.0	>2	2.40	1.68–3.56	48	<0.01	
	<2	1.04	0.86–1.24	62		
PyOM pH						
≤8.0	>2	3.23	1.36–7.90	10	<0.01	0.11
	<2	1.36	1.18–1.58	44		<0.01
>8.0	>2	1.67	1.16–2.57	24	0.13	
	<2	1.02	0.95–1.09	75		
PyOM ash content (%)						
≤10.0	>2	1.98	1.21–3.42	28	<0.01	0.74
	<2	1.00	0.84–1.15	45		0.61
>10.0	>2	1.79	1.22–2.61	31	<0.01	
	<2	0.95	0.82–1.12	82		
PyOM surface area ($m^2\ g^{-1}$)						
≤50.0	>2	4.02	2.53–6.58	26	<0.01	<0.01
	<2	0.82	0.62–1.07	47		0.11
>50.0	>2	1.15	0.79–1.58	29	0.26	
	<2	1.01	0.88–1.15	32		
Pyrolysis residence time (h)						
≤0.5	>2	12.01	6.77–20.75	8	<0.01	<0.01
	<2	1.05	0.96–1.14	39		0.86
>0.5	>2	1.10	0.84–1.42	34	0.57	
	<2	1.05	0.96–1.14	65		
PyOM feedstock						
Lignocellulosic waste	>2	3.29	1.11–9.15	10	0.01	<0.01
	<2	0.93	0.74–1.14	17		0.63
Herbaceous	>2	2.97	1.82–4.88	27	<0.01	
	<2	1.05	0.90–1.21	74		
Wood	>2	1.52	1.21–2.01	52	<0.01	
	<2	1.05	0.91–1.22	62		

RR, response ratio; CI, 95% confidence interval for RR; *n*, number of observations within each category; *P**, probability of the model heterogeneity test between the two relative application ratios (PyC:SOC ratio >2 and <2, respectively) within the various groupings; *P***, probability of the model heterogeneity test between the various groupings within the categories of relative application ratios.

temperatures >550°C, no significant changes in CO_2 emission were observed at PyC:SOC ratio >2, but a significant decrease in CO_2 emission at PyC:SOC ratio <2 (RR = 0.86; CI = 0.72–0.99).

Influence of experimental conditions

Significant differences among subgroups at PyC:SOC ratios >2 were observed in most instances, except for experiment type. At PyC:SOC ratios <2, these differences were significant only for incubation temperature and soil moisture (Table 3).

Experiments performed for periods shorter than 200 days showed increased CO_2 emission only when the PyC:SOC ratio was >2 (RR = 2.48). For experiments performed for periods longer than 200 days, no significant effects on CO_2 emission were observed (Table 3). Soils incubated at temperatures of ≤30.0°C had their CO_2 emissions significantly increased independently of the PyC:SOC ratio. However, CO_2 emission from soils

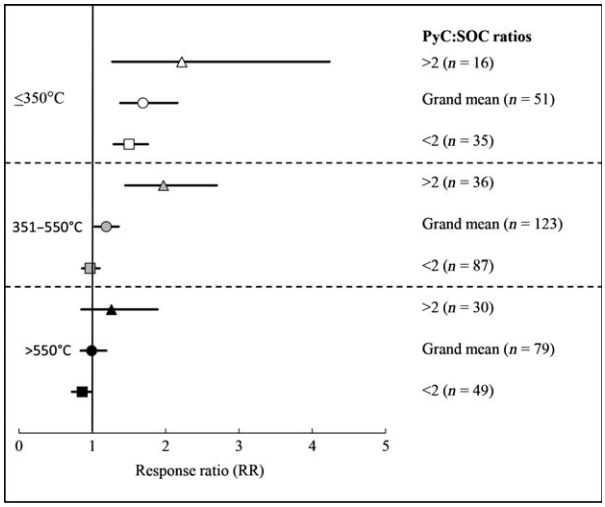

Fig. 2 Influence of different PyC:SOC ratios, within different PyOM pyrolysis temperatures on CO_2 emission from PyOM-amended soils. Symbols show response ratio (RR), and bars show 95% confidence intervals. The numbers shown in parentheses correspond to the number of observations upon which the statistical analysis is based.

incubated at temperatures >30.0°C or under variable temperatures was not influenced by PyOM additions.

There was a significant increase in CO_2 emission from soils incubated at moistures ≤40% and >80% of water holding capacity (WHC) at PyC:SOC ratios >2 (RR = 3.03 and 3.32, respectively). Conversely, for soils incubated under moisture ranging from 40.1 to 80.0% WHC, there was a significant increase in CO_2 emission when the PyC:SOC ratio was <2 (RR = 1.14). Soils amended with PyOM in laboratory and field experiments had their CO_2 increased only at PyC:SOC ratios >2 (RR = 2.14 and 1.17, respectively). Emissions of CO_2 from greenhouse experiments were not influenced by PyOM additions.

Discussion

Our meta-analysis provided different outcomes depending on the level it was performed. On the most general level, it showed an overall statistically significant increase in CO_2 emission from PyOM-amended soils compared to the control. By refining the analysis into a deeper level, we have shown that significant increases in CO_2 emission were only evident at PyC:SOC ratios >2. These findings are consistent with the hypothesis that the main source of increased CO_2 emission from PyOM-amended soils is the labile C fraction of PyOM. For the first time, we demonstrate that the PyC:SOC ratio is the best predictor for increases in CO_2 production in PyOM-amended soils.

In an even more detailed level, our analysis made explicit particularities within categories, for instance a higher CO_2 emission from soils with low C contents and low C:N ratios at PyC:SOC ratios >2 than from soils with high C contents and high C:N ratios (Table 1). Soils with low C contents are reported as being more responsive to PyOM additions in terms of CO_2 emission (Stewart *et al.*, 2013; Yu *et al.*, 2013). In these relatively low-SOC soils, labile fractions of PyOM may provide an important source of C that is used as selective substrate for microbial activity (Cross & Sohi, 2011). Coarse-textured (i.e. sandy) soils normally have lower amounts of SOC as they offer less protection against decomposition than fine-textured (i.e. clayey) soils (Roscoe *et al.*, 2001). This lower protective effect may also enhance PyOM exposure and hence its decomposition (Brodowski *et al.*, 2005), resulting in increased CO_2 emission. Such a hypothesis is supported by our findings of higher CO_2 emissions from soils with medium and coarse texture at PyC:SOC ratios >2, compared to fine-textured soils (Table 1). It further suggests that the soil environment influences the persistence of PyOM, in contrast to suggestions by Schmidt *et al.* (2011).

Results from PyOM pyrolysis temperature subgroups (Fig. 2) further support the hypothesis that increases in CO_2 emission following soil–PyOM additions may derive mainly from PyOM-labile fractions. Pyrogenic organic materials produced at ≤350°C, which usually have a higher labile fraction of C (Sun *et al.*, 2014), significantly increased CO_2 emission irrespective of PyC:SOC ratio, but PyOM produced at >550°C (i.e. with a lower labile fraction) did not, even at high application rates. For the studies included in our meta-analysis, high pyrolysis temperatures were generally associated with PyOM with higher C contents than those produced at low pyrolysis temperatures, as confirmed by Sun *et al.* (2014). However, PyOM C content was not a significant factor controlling CO_2 emission, as relevant increases occurred only at PyC:SOC ratios >2, irrespective of PyOM C content (Table 2).

Although our analysis included data from studies where isotopic analyses were not employed, our results are consistent with the assumption that if any positive priming occurs after PyOM additions, it is not the main driver of increased CO_2 emission. While the original definition of priming (Bingeman *et al.*, 1953) referred to increased decomposition of SOC after the addition of organic sources, later definitions are more restrictive (e.g. Kuzyakov *et al.*, 2000). The studies covered in this meta-analysis do not fit with the definition by Kuzyakov *et al.* (2000) in two respects. Amounts added were usually large (and significant increases in respiration only occurred at PyC:SOC ratios >2). Furthermore, the material added was supposedly recalcitrant rather than

Table 3 Meta-analysis of the effects of PyC:SOC ratios on CO_2 emission, within experimental-associated characteristics. PyC:SOC ratios are divided as >2 and <2

Categories	PyC:SOC ratio	RR	95% CI	*n*	P^*	P^{**}
Experiment duration						
≤200 days	>2	2.48	1.84–3.31	72	<0.01	<0.01
	<2	1.04	0.92–1.18	117		0.94
>200 days	>2	0.86	0.55–1.20	17	0.01	
	<2	1.06	0.99–1.13	62		
Incubation temperature (T°C)						
≤20.0	>2	1.66	1.03–2.64	2	0.21	0.03
	<2	1.25	1.06–1.51	28		0.01
20.1–30.0	>2	2.22	1.44–3.38	30	<0.01	
	<2	1.18	1.10–1.26	76		
>30.0	>2	1.08	0.72–1.59	20	0.77	
	<2	1.04	0.89–1.22	20		
Variable	>2	1.02	0.73–1.67	7	0.60	
	<2	0.95	0.86–1.04	31		
Soil moisture (%)						
≤40.0	>2	3.03	1.70–5.31	20	0.02	<0.01
	<2	0.92	0.71–1.14	5		<0.01
40.1–80.0	>2	1.21	0.92–1.56	28	<0.43	
	<2	1.14	1.06–1.23	87		
>80.0	>2	3.32	2.03–5.45	30	<0.01	
	<2	0.76	0.59–1.01	44		
Experiment type						
Laboratory	>2	2.14	1.63–2.85	80	<0.01	0.11
	<2	1.06	0.96–1.19	144		0.47
Greenhouse	>2	1.02	0.74–1.66	7	0.54	
	<2	0.92	0.76–1.12	10		
Field	>2	1.17	1.16–1.18	2	0.45	
	<2	1.01	0.93–1.10	25		

RR, response ratio; CI, 95% confidence interval for RR; *n*, number of observations within each category; *P**, probability of the model heterogeneity test between the two relative application ratios (PyC:SOC ratio >2 and <2 respectively) within the various groupings; *P***, probability of the model heterogeneity test between the various groupings within the categories of relative application ratios.

labile. However, labile C fractions can also be present on the surface of PyOM following pyrolysis, for example in the form of sugars and aldehydes (Painter, 2001). The total amount of these labile C fractions increases with increasing PyOM application rates. Therefore, the fact that CO_2 emission increased significantly only at PyC:SOC rates >2 (Fig. 1) suggests that this CO_2 originated to a large extent from the decomposition of labile C fractions of the PyOM (cf. Smith *et al*., 2010; Cross & Sohi, 2011; Hilscher & Knicker, 2011; Luo *et al*., 2011; Méndez *et al*., 2013).

Maestrini *et al.* (2014b) recently analysed priming effects by biochar on SOC decomposition, based on studies with stable C isotope-labelled substrates. They concluded that positive priming occurs shortly after soil-PyOM incubations (especially within periods <20 days) and negative priming in incubations lasting for >200 days. While their data are not directly comparable with ours (their data were not expressed as a response ratio, and they did not separate studies with high and low relative biochar addition rates), they noted that a major cause for positive priming was the occurrence of a labile fraction in PyOM. In cases where negative priming was observed, they proposed sorption of SOC onto the PyOM surface as a major mechanism.

The surface area of PyOM is likely to increase over time as particles weather and break up. Furthermore, evidence suggests that the surface of PyOM may become more reactive over time, increasing in properties such as cation exchange capacity (Cheng *et al*., 2006). As such, it is also possible that the CO_2 adsorption capacity of PyOM in soil will increase over time. Indeed, our meta-analysis shows that CO_2 emission significantly decreased after periods >200 days compared to the first 200 days, especially at PyC:SOC ratios >2 (Table 3). However, while time-dependent changes in PyOM surface area properties should not be excluded as a mechanism, it is also possible that exhaustion of

labile C contributed for decreased CO_2 emission after periods >200 days.

We noted significant reductions in CO_2 emission when PyOM with low C content (<50%; Table 2) and when PyOM produced at high temperatures (>550°C; Fig. 2) was applied, both at relatively low addition ratios. Data from our meta-analysis partially agree with those from Maestrini *et al.* (2014b), who showed that negative priming was strongest with more stable PyOM. However, the authors suggested a strong decrease in CO_2 emission with PyOM containing high C content, which is not supported by our data. Further research is needed regarding the importance of pyrolysis processes (especially temperature) and PyOM C content, as well as sorption processes as mechanisms for negative priming. There is also a need for standardization in determining data on PyOM C content, as it has not likely always been consistently reported with potential corrections (e.g. for ash content).

An increase in CO_2 emission (almost a doubling; Fig. 1) at high PyC:SOC ratios (> 2) and the lack of responses at low PyC:SOC rations (<2) indicate that only a small part of the char is relatively labile; otherwise, significant increases in CO_2 emission would also be evident at low PyC:SOC ratios. Support for a major recalcitrant fraction of the biochar comes from an alternative calculation where we expressed our CO_2 flux data per unit of C, from both SOC and PyOM. Conversion of the data to respired CO_2 per unit of C results in a significant relative decrease in the response ratios, irrespective of PyC:SOC ratios (Tables S1, S2 and S3 at Supporting information). This decrease is due to the higher recalcitrance and therefore lower decomposition rate of PyOM compared to that of SOC, as also observed by Cross & Sohi (2011). However, such information must be interpreted with caution as it could easily lead to the wrong suggestion that PyOM additions result in strong negative priming.

The way forward in research on PyOM and SOC decomposition

The studies used in our meta-analysis were predominantly from laboratory experiments (82%), compared to field (10%) and greenhouse experiments (8%). Moreover, laboratory experiments accounted for 90% of data points with PyC:SOC ratio >2. In fact, there was a significant difference in relative PyC addition rates between laboratory and field studies (Fisher's exact test, two-sided; $P = 0.001$). Laboratory experiments with high PyC:SOC ratios are useful for identifying potential mechanisms driving changes in CO_2 emission. However, such ratios likely result in overestimation of the effect size. Furthermore, they are unrealistic representations of expected results under field conditions. It is necessary therefore that future research utilizes experimental designs with realistic PyOM treatments rather than the large amounts often used in laboratory experiments. Such experiments should include multiple controls. This implies that not only treatments without addition of PyOM should be used, but also additional treatments that are designed to test non-PyOM mechanisms (effects of ash, pH increases, nutrient additions if charred manure is used, etc.) that are due to co-variation of these factors with PyOM (Jeffery *et al.*, 2013). This approach would allow for unequivocal conclusions to be drawn regarding the effects exclusively inherent to PyOM.

Experimental details were often incompletely reported. Therefore, adequately reporting experimental details is a necessary aspect for (i) a standardization process for future research aimed at quantifying (interactive) effects of PyOM on SOC dynamics; (ii) a straightforward way of avoiding confounding results; and (iii) the repeatability of results under similar experimental conditions.

Research on PyOM has recently emphasized areas that deserve particular attention. Effects of PyOM additions on the decomposition rate of SOC are among these research priorities (Verheijen *et al.*, 2014). Most data in our meta-analysis are from short-term studies. But some mechanisms responsible for physical and chemical protection of PyOM and SOC take place only in the long term, for example as PyOM ages in the soil and its surface gains charge. This process of oxidation and charging of PyOM surface may occur over long periods of time (Brodowski *et al.*, 2005). Therefore, there is a need for long-term experiments, especially under field conditions. Such experiments, associated with stable isotope techniques, would permit an effective testing of the potential mechanisms controlling the decomposition and potential interactions between PyOM and native SOC.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Meta-analysis of the effects of PyOM on soil CO_2 emissions relative to SOC units, grouped by soil and environment characteristics.

Table S2. Meta-analysis of the effects of PyOM on soil CO_2 emissions relative to SOC units, grouped by PyOM characteristics.

Table S3. Meta-analysis of the effects of PyOM on soil CO_2 emissions relative to SOC units, grouped by experimental conditions.

Data S1. Matrix showing studies and data for each categorical grouping on which the meta-analyses were run.