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Review Paper

# Pyrogenic organic matter effects on soil bacterial community composition

Jamie Woolet, Thea Whitman *

*Department of Soil Science, University of Wisconsin-Madison, 1525 Observatory Dr., Madison, WI, USA, 53706*

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ABSTRACT

Pyrogenic organic matter (PyOM) is produced by the incomplete combustion of organic matter, and can represent a large portion of total soil organic carbon in both fire-affected systems and managed systems where PyOM is added intentionally as a soil amendment. The effects of PyOM on the structure of soil microbial communities remain a topic of fundamental interest, and a number of studies have begun to identify and characterize the PyOM-associated microbial community. However, it is unclear to what extent the effects of PyOM on soil bacteria are consistent. Our goals were to synthesize current related studies to (1) determine if there is a detectable and consistent "charosphere" community that characterizes PyOM-amended soils, (2) distinguish consistent responders at the phylum level to PyOM amendments, and (3) identify individual PyOM-responsive taxa that increase in relative abundance consistently across different soil types. We re-analyzed publicly available raw 16S Illumina sequencing data from studies that investigated the bacterial communities of PyOM-amended soils. We determined that soil source is more important than PyOM for shaping the trajectory of the community composition. Although we were able to identify a few genera that respond positively and somewhat consistently to PyOM amendments, including *Nocardioides, Micromonospora, Ramlibacter, Noviherbaspirillum,* and *Mesorhizobium*, in general, neither phylum-level nor genus-level responses to PyOM were consistent across soils and PyOM types. We offer suggestions for our future efforts to synthesize the effects PyOM may have on soil microbial communities in an array of different systems. Due to the dual challenges of high functional diversity at fine taxonomic scales in bacteria, and diverse ranges of soil and PyOM properties, researchers conducting future studies should be wary of reaching a premature consensus on PyOM effects on soil bacterial community composition. In addition, we emphasize the importance of focusing on effect sizes, their real-world meanings, and on cross-study effect consistency, as well as making data publicly available to enable syntheses such as this one.

## 1. Introduction

Pyrogenic organic matter (PyOM) is produced during the incomplete combustion of organic matter and is of interest for both natural and managed systems (Czimczik and Masiello, 2007). In fire-affected ecosystems, PyOM can represent large fractions of total soil organic carbon (SOC) (over 50% (Reisser et al., 2016)). In managed systems, PyOM can be produced intentionally and added to soils as an agronomic amendment and/or as a tool for carbon management (in which case it is often referred to as "biochar") (Laird, 2008; Whitman et al., 2010). In both of these systems – wildfire and biochar – the interactive effects of PyOM and the microbial community on SOC stocks and cycling are of critical interest. In order to understand how changing wildfire regimes will affect global SOC or nutrient stocks (Pellegrini et al., 2018), or in order to understand whether biochar additions to soil will result in net SOC increases or decreases over time (Woolf and Lehmann, 2012), we must understand how microbes respond to PyOM inputs (in addition to other effects of fires). For this review, we identified ten Illumina-based high-throughput sequencing (HTS) studies for which sequencing data were publicly available (Table 1). We have re-analyzed these data using standardized approaches in order to characterize the effects of PyOM additions on soil bacterial community composition, with the goal of determining: (1) Is there a detectable and consistent "charosphere" (Quilliam et al., 2013) community that characterizes PyOM-amended soils – *i.e.*, do PyOM additions overwhelm the effects of pre-PyOM soil properties? (2) Are there consistent responses at the phylum or another taxonomic level to PyOM amendments? (3) Can we identify individual PyOM-responsive taxa that increase in relative abundance consistently across different soil types?

Scientific interest in microbial interactions with pyrogenic organic

* Corresponding author. 1525 Observatory Dr., Madison, WI, 53703, USA.
*E-mail address:* twhitman@wisc.edu (T. Whitman).

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matter (PyOM) dates back to at least the 1930s, when researchers noted that the addition of charcoal to culture media could enhance the growth of gonococci (Glass and Kennett, 1939). Glass and Kennett's systematic consideration of the possible mechanisms driving this effect is particularly interesting to read today, as their ideas include many of the mechanisms that we still dwell upon: PyOM as a C source, supply of other soluble or insoluble nutrients, adsorption of inhibitory molecules, adsorption of stimulatory molecules, and a possible catalytic role for the charcoal. This research continued into the 1950s (Ensminger et al., 1953; Gorelick et al., 1951), where the effect was largely attributed to possible sorptive properties of the charcoal. Other early studies investigated the sorption of bacterial cells by charcoal (Krishnamurti and Soman, 1951). However, there are numerous potential mechanisms through which PyOM is known to potentially affect microbial growth, activity, and/or community composition (Lehmann et al., 2011), including direct provision of a carbon or nutrient source (Whitman et al., 2014), interactions with signalling molecules (Masiello et al., 2013), provision of a habitat (DeCiucies et al., 2018; Pietikäinen et al., 2000), changes to soil pH (Luo et al., 2011) or moisture (Chen et al., 2018), among others. While an increasing number of studies has supported useful meta-analyses of PyOM effects on chemical and physical properties (Ding et al., 2017; Maestrini et al., 2014) or plant responses (Jeffery et al., 2011), developing a predictive understanding of these systems has been limited by the wide range of soil properties, PyOM properties, application rates, experimental conditions, and timescales of study (Ameloot et al., 2013; Jeffery et al., 2015; Kammann et al., 2017). This challenge is just as influential when seeking to improve our understanding of the effects of PyOM on the biological properties of soil – in particular, its effect on microbial community composition.

There are at least three reasons one may care whether PyOM alters soil microbial community composition. First, the processes that govern the structure of soil biotic communities, and how perturbations to these systems affect soil microbial community composition, remain questions of fundamental interest in soil ecology (Baldrian, 2019; Fierer, 2017; Fierer et al., 2009). Second, even if one is not interested in microbial diversity *per se* (Shade, 2017), the PyOM-related impacts that land managers would be expected to primarily care about (*e.g.*, changes to greenhouse gas emissions or plant growth) are often strongly influenced by microbial community composition, or, at a minimum, reflected in the microbial community composition. Although we remain a long way from effectively linking high-resolution community composition to most of these functions of interest and there are likely numerous processes that may never be well-predicted by microbial community composition (Hall et al., 2018), there are still processes of interest for which microbial community composition often has predictive value, such as methane fluxes in soils (Judd et al., 2016). Third, as we attempt to predict the biogeochemical implications of changing fire regimes, understanding the effects of wildfire on soil microbes is a critical topic (Holden and Treseder, 2013; Pressler et al., 2018). Studying the addition of PyOM to soils in the absence of fire may help us decompose the multi-faceted effects of fire on microbial communities into its constituent parts (direct killing by heat, rapid recolonization post-fire, plant-mediated effects, or changes to the post-fire soil environment such as the addition of PyOM (Hart et al., 2005)), allowing us to better predict how changing fire regimes will affect soil microbial communities and their response to post-fire soil C.

In this paper, we do not attempt to conduct a new comprehensive review of the broad range of PyOM effects on soil microbes, and refer the reader instead to a number of other recent reviews of PyOM effects on soil biota (Lehmann et al., 2011), on microbially-relevant soil properties, microbial biomass, community structure, enzyme activity, and signalling (Gul et al., 2015; Zhu et al., 2017), and specifically on microbial mobilization of nutrients (Schmalenberger and Fox, 2016) or carbon (Maestrini et al., 2014; Wang et al., 2016; Whitman et al., 2015). Rather, our aim is to focus specifically on soil bacterial responses to PyOM additions. Briefly, we remain far from being able to consistently predict these responses. For example, Gul et al., 2015 suggest that observed increases in microbial biomass with PyOM additions may be greater when the PyOM materials come from low-lignocellulosic biomass and are pyrolyzed at temperatures below 500 °C, but also note that these trends are not consistent across all studies. As with many aspects of PyOM effects on soils, this is not a surprising conclusion – soil properties and PyOM materials can vary widely, as can the environmental conditions and timescales under which they are studied. Zhu et al., 2017report, "[c]hanges in the relative abundances of *Acidobacteria*, *Actinobacteria*, *Gemmatimonadetes*, and *Verrucomicrobia* are frequently detected using high-throughput sequencing, under treatment with biochar (Mackie et al., 2015; Nielsen et al., 2014)." However, these generalizations may be premature: there are still relatively few studies of the effects of PyOM additions to soil on microbial communities at the fine phylogenetic scales afforded by recent advances in HTS.

While all approaches to characterizing the effects of PyOM on soils are challenged by the diversity of both soils and PyOM materials, the vast array of data generated in HTS studies generates additional challenges for understanding soil microbial community response to PyOM. In particular, one of the challenges specific to HTS is the need for the authors to distill coherent stories from the "firehose of data", which requires that, for the paper, they identify and select the trends that are of most interest or are most readily interpretable. In a single study, when dealing with thousands to tens of thousands of different operational taxonomic units (OTUs), which may not respond coherently to the treatment of interest even at the genus level, let alone the phylum level (Whitman et al., 2016), there could be as many stories to be told as there are OTUs. Thus, understandably, authors usually limit themselves to highlighting the responses that are consistent at tractable levels (*e.g.*, increases in the relative abundance of a given phylum) and the finer-scale responses for specific taxa that are either of high abundance or have particularly strong responses to the factor of interest – in our case, PyOM additions. Authors sometimes report the raw results in supplementary data, but this is not always practiced, and, if it is, is still not done in a consistent manner that is readily searchable. While an increased emphasis on open science, reproducible science, and improved data storage capabilities may improve this situation in the future, today, it remains a common limitation. It presents a particularly large hurdle when looking for patterns across studies, such as in a meta-analysis. If we limit ourselves to considering only the effects that the authors chose to highlight, we risk both false positives and false negatives due to (necessarily) selective reporting. Furthermore, it can be difficult to elucidate broader patterns because of authors' choices to report their results at different taxonomic levels. For example, we recorded all the PyOM-responsive taxonomic groups, across phylogenetic levels, that authors in our ten studies mentioned directly or drew attention to in figures (Table 2), but this list is certainly incomplete. An additional challenge in compiling results across HTS studies is that the studies use combinations of different methods, from the steps of DNA extraction, primer choices, sequencing library preparation, and sequencing methods, to data processing and quality control. Because of these limitations, we would argue that in order to comprehensively review these data, it is necessary to re-analyze them from scratch. While we cannot adjust for different pre-sequencing methodological decisions, for our attempt here to synthesize common trends across current studies of PyOM effects on soil microbial communities, we have chosen to re-analyze the original raw sequencing data, processing each dataset in the same way.

We emphasize that we have limited our investigation to bacterial community composition in this study, and do not directly consider changes in function, such as increases or decreases in C mineralization rates, nor do we consider other microbes such as fungi. While community composition is necessarily related to microbial functions in the soil, functional changes can, of course, also occur without substantial changes in community composition, and vice versa. Thus, we underscore that changes in community composition reported in this study may

**Table 1**
Studies included in this paper.

| First_Author_Lastname | Year_Published | Main_Effects_Studied | PyOM_Material | PyOM_Temperature | Field_Lab | PyOM_Amendment_Amount | Treatments | Num_Replicates | Soil_Type | Study_Duration_Weeks | Seq_Technology | Mean_QC_Read_Count | SD_QC_Read_Count | Min_QC_Read_Count | Max_QC_Read_Count |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Nielsen | 2014 | Study the effect of enhanced biochar on soil microbial communities in agricultural fields compared to farmer-practice fertilizer additions | jarrah wood | 600C | Field | 1 and 5 Mg ha-1 | Farmer practice fertilizer, PyOM | 3 replicates per treatment per plot | Ferrosol | 45 | HiSeq 2000 Illlumina | 189750 | 28628 | 110197 | 262080 |
| Whitman | 2015 | Investigate effects of PyOM addition on the microbial community in a field-trial | corn | 350C | Field | 4.1 Mg ha-1 | unamended, corn stover, PyOM | 16 replicates for PyOM, 8 replicates for corn stover additions | Fragiudept | 12 | Illumina Miseq | 25554 | 19206 | 1631 | 82705 |
| Wu | 2016 | Study bacterial community of soil when treated with biochar, compost, and combined ammendments | corn cob | 450C | Lab | 50 g kg-1 | Unamended (control), compost, biochar, compost and biochar, composted biochar, composted biochar and straw | 1 replicate per treatment type | Gleysol | 60 | Illumina Miseq PE 300x2 | 4155 | 1485 | 3145 | 6803 |
| Yao | 2017 | Study the effect PyOM has on bacterial community composition after 3 years at different times during the cropping cycle | commercial powder (corn stalks) | Not reported | Field | 50, 100, 200 Mg ha-1 | unamended, PyOM | 3 replicates per treatment type | Mollisol | 128 | Illumina Miseq | 18147 | 3486 | 8768 | 24048 |
| Dai | 2016 | Identify responders to PyOM, how they respond, and the difference between responders in rhizosphere vs bulk soil | swine manure or straw | 550C | Lab | 30 g kg-1 | unamended, PyOM | 3 replicates per treatment type | Inceptisol | Not specified - after rice seedling maturation | Illumina Miseq | 1846 | 292 | 1465 | 2680 |
| Imparato | 2016 | Compare the effects of straw and GBC on the bacterial community in an agricultural field | straw gasification biochar | 750C | Field | 14.26 and 2.3 Mg ha-1 | unamended, straw, PyOM | 9 replicates per treatment type | Luvisol | 13 | Illumina Miseq | 26530 | 9248 | 10288 | 44376 |
| Song | 2017 | Study the bacterial community in PAH-contaminated soil treated with two levels of PyOM addition and two different PyOM temps | wheat straw | 300C and 600C | Lab | 10 and 20 g kg-1 | unamended, PyOM | 8 replicates per treatment type | Gleysol | 24 | Illumina Miseq | 25938 | 3191 | 18929 | 31834 |
| Dai | 2017 | Study the effect PyOM and PyOM pyrolosis temp has on the bacterial community | manure | 300C and 700C | Lab | 30 g kg-1 | unamended, PyOM | 3 replicates per treatment type | Psammaquent & Argiustoll | 34 | Illumina Miseq PE 300x2 | 50694 | 29995 | 27095 | 191926 |

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**Table 1** (*continued*)

| *FirstAuthorLastname* | *YearPublished* | *MainEffectsStudied* | *PyOMmaterial* | *PyOMtemperature* | *FieldorLab* | *PyOMamendmentamount* | *Treatments* | *NumReplicates* | *SoilType* | *StudyDurationweeks* | *SeqTechnology* | *MeanQCReadCount* | *SDQCReadCount* | *MinQCReadCount* | *MaxQCReadCount* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ye | 2016 | composition and colonization investigate how bacteria and plant properties respond to biochar or organic fertilizer | jarrah wood | 600C | Lab | 1.5 Mg ha-1 | unamended, PyOM, fertilizer | 3 replicates per treatment for initial, 5 replicates per treatment for final | Fluvisol | 60 | Illumina Miseq | 4709 | 5058 | 1054 | 20619 |
| Xu | 2016 | Study the effect biochar has on N-leaching and investigate effect on bacterial community in a leaching condition | corn straw | 500C | Lab | 0, 40, 80, 160 Mg ha-1 | unamended, PyOM | 3 replicates per treatment, which were pooled for DNA analysis | Cambisol | 20 | Illumina Hiseq | 5704 | 819 | 4474 | 6546 |

or may not have been accompanied by changes in function. Furthermore, a lack of change in community composition does not necessarily mean PyOM additions did not affect functions of interest. Still, we believe it is worthwhile to investigate changes in microbial community composition, (i) due to the role of these changes as possible indicators/integrators of PyOM effects on soils, (ii) because of possible accompanying changes in functions, and (iii) because of inherent interest in the mechanisms and processes that underpin microbial community composition and diversity in soils.

## 2. Methods

### 2.1. Study selection

Web of Science was used to search for studies investigating the microbial response to PyOM; keywords used were: "PyOM", "pyrogenic organic matter", "pyrogenic carbon", "black carbon", "biochar", "microbial community", "community", "bacteria", and utilizing the wildcard (*) function for versatility. Each study was reviewed and information about the study was compiled. From this list, the studies chosen for this meta-analysis were selected based on the following requirements: (1) the study system contained a soil-only and PyOM-only treatment, (2) 16*S* sequencing was performed on an Illumina Hiseq or Miseq platform, (3) the raw reads were accessible online or from the author directly, and (4) the study was available before 2018. This left us with ten studies (and eleven soils) (Dai et al., 2017a, 2016; Imparato et al., 2016; Nielsen et al., 2014; Song et al., 2017; Whitman et al., 2016; Wu et al., 2016; Xu et al., 2016; Yao et al., 2017; Ye et al., 2016): (Table 1). In addition, there were six studies that did not make their data publicly available and were not able to provide us with their raw data after we contacted them, and four studies that used 454 sequencing, which we did not include here. We chose to focus on PyOM additions only (rather than formally including fire-affected soils) largely because there were very few studies of soil microbial communities before and after fire that also characterized PyOM production rates and conditions. In addition, it would be difficult to control for the confounding factors of fire (*e.g.*, heating effects on soil microbes or post-fire water repellency (Certini, 2005)). However, we do qualitatively compare our findings to studies of natural fires as well as to other analogous systems such as polyaromatic hydrocarbon (PAH)-contaminated soils.

### 2.2. Sequence processing

All sequences were either downloaded from the NCBI or the EMBL databases using accession numbers provided by the authors, or received directly from the author. The QIIME2 pipeline (QIIME2, v. 2018.2, Bolyen et al., 2018) was used to process the sequences for each study, processing each study individually, but using a consistent approach across studies. FASTQ files were separated into forward and reverse reads if necessary using demuxbyname.sh from bbmap v. 35.95 (Bushnell et al., 2017) (Supplementary Information). The dada2 (Callahan et al., 2016) denoise-paired or denoise-single command as implemented within QIIME2 was used to determine amplicon sequence variant-level OTUs, using default parameters unless specified here. Trimming and truncating parameters were tested to determine values for optimal sequence retention and quality control for each dataset. For most datasets, sequences were truncated where mean quality scores dropped below 35, except Whitman et al. (2016), Xu et al., 2016, and Ye et al. (2016), for which reads were truncated where quality scores dropped below 25. Sequences were trimmed at the first 5–26 base pairs to either remove primer bases that were included or to remove sequences that contained poor quality scores. Taxonomy was assigned using the Silva132 database (Quast et al., 2013) at 99% similarity at the majority taxonomy 7 levels using the QIIME2 feature-classifier classify-sklearn (a naïve Bayes classifier; Pedregosa et al., 2011). Taxonomy classifiers were trained on the regions specific to the primers used for each study.

**Table 2**
Phyla, classes, orders, families, genera, and species that were named and identified as PyOM-responsive or generally increased in abundance with PyOM additions.

| Study | Named Phyla | Named Classes | Named Orders | Named Families | Named Genera | Named Species |
|---|---|---|---|---|---|---|
| Dai et al. (2016) | Acidobacteria decreased in bulk and rhizosphere soil<br>Bacteroidetes increased in bulk and rhizosphere soil<br>Actinobacteria and Alphaproteobacteria increased in rhizosphere soil<br>Betaproteobacteria decreased in bulk soil and increased in rhizosphere soil | | | | | |
| Ye et al. (2016) | | | | | Phytohabitans<br>Rhodoplanes<br>Hyphomicrobium<br>unclassified from Rhodospirillacae<br>unclassified from PRR-10<br>unclassified from Cyrophagacae<br>unclassified from Acidobacteria-5<br>unclassified from Chitinophagaceae<br>unclassified from Rhizobiales<br>unclassified from Cerasicoccaceae | |
| Song et al. (2017) | Proteobacteria<br>Bacterioidetes<br>Chloroflexi<br>Verrucomicrobia with 1%BC300 at 24 weeks<br>Firmicutes mostly increased at 12 weeks, decreased at 24<br>Actinobacteria<br>Saccharibacteria with BC300 at 12 weeks and 24 weeks<br>Parcubacteria with 2%BC300 at 12 weeks and 24 weeks<br>Elusimicrobia with 1%BC300 at 24 weeks<br>Gemmatimonadetes<br>Nitrospirae with BC600 at 24 weeks | | | | | |
| Wu et al. (2016) | Actinobacteria (biochar and compost)<br>Bacteroidetes (biochar + compost and composted biochar + biomass)<br>Acidobacteria (composted biochar)<br>Gemmatimonadetes (composted biochar) | | | | Patulibacter<br>Marmoricola<br>Gemmatimonadaceae<br>Acidimicrobiales<br>Nitrosomonadaceae<br>Intrasporangiaceae<br>Ramlibacter<br>Arthrobacter<br>Nocardioides | |
| Yao et al. (2017) | Chloroflexi<br>Chlorobi | Alphaproteobacteria<br>Deltaproteobacteria | | | Nitrosomonadaceae<br>Nitrospira<br>Lysobacter<br>Piscinibacter<br>Bradyrhizobium<br>Gemmatimonas<br>Pedomicrobium<br>Bacillus | Nitrosococcus sp.<br>Rhodoplanes sp.<br>Acidobacteria - Subgroup 7<br>Uncultured Burkholderiacae<br>Thiobacillus sp. |
| Dai et al., 2017a,b | Actinobacteria<br>Proteobacteria (Psammaquent soil, both biochar) | | Actinomycetales | | | |

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**Table 2** (*continued*)

| Study | Named Phyla | Named Classes | Named Orders | Named Families | Named Genera | Named Species |
|---|---|---|---|---|---|---|
| | Acidobacteria (Psammaquent soil with 700 biochar)<br>Firmicutes (Argiustoll soil, both biochar) | | | | | |
| Imparato et al. (2016) | | Acidobacteria_Gp16 | | | Pseudolabrys | |
| | | | | | Bradyrhizobium | |
| | | | | | Acidobacteria_Gp11 | |
| Nielsen et al. (2014) | | Acidobacteria Gp1 | Acidimicrobiales | Burholderiaceae | Ktedonobacter | |
| | | Acidobacteria Gp3 | Solirubrobacterales | | Subdivision 3 genus incertaesedis | |
| | | | Bacillales | | | |
| Whitman et al. (2015) | Bacteroidetes | | Gemmatimonadales | | | Arthrobacter spp. |
| | Actinobacteria (day 12, not significant) | | Ellin5290 | | | Roseomonas aquatica |
| | Gemmatimonadetes (not significant) | | Rhizobiales | | | Thermomonas dokdonensis |
| | | | Burkholderiales | | | Oxalicibacterium flavum |
| | | | Sphingomonadales | | | Beijerinckia derxii subsp. Venexuelae |
| | | | Rhodospirillales | | | Flavobacterium beibuense |
| | | | Caulobacterales | | | Achromobacter spanius |
| | | | Bdellovibrionales | | | Comamonas thiooxydans |
| | | | Legionellales | | | Niastella sp. JCN-23 |
| | | | Myxococcales | | | Adhaeribacter terreus |
| | | | Rhodobacterales | | | Bosea sp. R-46060 |
| | | | Rhodocyclales | | | Flavisolibacter ginsengisoli |
| | | | Cytophagales | | | Caulobacter henricii |
| | | | Saprospirales | | | Brevundimonas halotolerans |
| | | | Sphingobacteriales | | | Ochrobactrum pseudogrignonense |
| | | | Verrucomicrobiales | | | Flavisolibacter ginsengisoli |
| | | | Chthoniobacterales | | | Brevundimonas vesicularis |
| | | | Pedosphaerales | | | Rhodococcus wratislaviensis |
| | | | WD2101 | | | Brevundimonas alba |
| | | | Gemmatales | | | Nocardioides hwasunensis |
| | | | Pirellulales | | | Methylobacterium rhodesianum |
| | | | Acidimicrobiales | | | Prosthecobacter fluviatilis |
| | | | RB41 | | | Methylobacterium aquaticum |
| | | | Solibacterales | | | Cupriavidus necator |
| | | | JG30-KF-CM45 | | | Gemmatimonas aurantiaca |
| | | | Actinomycetales | | | Sphingomonadaceae bacterium KMM 6042 |
| | | | | | | Rhodococcus jostii |
| | | | | | | Shinella granuli |
| | | | | | | Luteolibacter sp. CCTCC AB 2010415 |
| | | | | | | Sediminibacterium salmoneum |
| | | | | | | Pedobacter sp. N7d-4 |
| | | | | | | Hymenobacter algoicola |
| | | | | | | Dongia mobilis |
| | | | | | | Flavisolibacter ginsengisoli |
| | | | | | | Georgfuchsia toluolica |
| | | | | | | Dyadobacter beijingensis |
| | | | | | | Pedobacter glucosidilyticus |
| | | | | | | Rhodanobacter sp. DCY45 |
| | | | | | | Ohtaekwangia koreensis |
| | | | | | | Azospirillum rugosum |
| | | | | | | Lysobacter sp. DCY21T |
| | | | | | | Devosia crocina |
| | | | | | | Pedobacter insulae |
| | | | | | | Chitinophaga niabensis |
| | | | | | | Rhodococcus triatomae |
| | | | | | | Segetibacter koreensis |
| | | | | | | Bacteriovorax stolpii |
| | | | | | | Nocardiopsis alba |
| | | | | | | Leifsonia poae |

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**Table 2** (*continued*)

| Study | Named Phyla | Named Classes | Named Orders | Named Families | Named Genera | Named Species |
|---|---|---|---|---|---|---|
| | | | | | | Burkholderia sp. ATSB16 |
| | | | | | | Sphingomonas japonica |
| | | | | | | Hymenobacter ocellatus |
| | | | | | | Dyella marensis |
| | | | | | | Sphingomonas trueperi |
| | | | | | | Sphingopyxis panaciterae |
| | | | | | | Arthobacter crystallopoietes |
| | | | | | | Chitinophaga niabensis |
| | | | | | | Rhodococcus yunnanensis |
| | | | | | | Dyella koreensis |
| | | | | | | Lacibacter cauensis |
| | | | | | | Bryobacter aggregatus |
| | | | | | | Armantimonas rosea |
| | | | | | | Roseomonas ludipueritiae |
| | | | | | | Panacagrimonas perspica |
| | | | | | | Delftia tsuruhatensis |
| | | | | | | Pseudomonas alcaligenes |
| | | | | | | Hymenobacter gelipupurascens |
| | | | | | | Ferrimicrobium acidiphilum |
| | | | | | | Wenxinia marina |
| | | | | | | Bdellovibrio bacteriovorus |
| | | | | | | Anaeromyxobacter dehalogenans |
| | | | | | | Solibius ginsengiterrae |
| | | | | | | Halioglobus pacificus |
| | | | | | | Fluviicola taffensis |
| | | | | | | Conexibacter arvalis |
| | | | | | | Prosthecobacter dejongeii |
| | | | | | | Undibacterium pigrum |
| | | | | | | Magnetospira thiophila |
| | | | | | | Aurantimonas sp. L9-753 |
| | | | | | | Aquicella siphonis |
| | | | | | | Fimbriimonas ginsengisoli Gsoil 348 |
| | | | | | | Ferruginibacter alkanlilentus |
| | | | | | | Amaricoccus macauensis |
| | | | | | | Catellibacterium nectariphilum |
| | | | | | | Adhaeribacter aquaticus |
| | | | | | | Pseudoxanthomonas mexicana |
| | | | | | | Niastella yeongjuensis |
| | | | | | | Byssovorax cruenta |
| | | | | | | Luteolibacter sp. E100 |
| | | | | | | Thioprofundum hispidum |
| | | | | | | Roseomonas sp. Enrichment culture clone 03SU |
| | | | | | | Solitalea canadensis |
| | | | | | | Hoefles phototrophica DFL-43 |
| | | | | | | Chitinophaga ginsengisegetis |
| | | | | | | Chitinophaga sancti |
| | | | | | | Verrucomicrobiaceae bacterium DC2a-G7 |
| | | | | | | Sediminibacterium salmoneum |
| | | | | | | Afipia massiliensis |
| | | | | | | Variovorax paradoxus |
| | | | | | | Pigmentiphaga litoralis |
| | | | | | | Belnapia moabensis |
| | | | | | | Nocardioides plantarum |
| | | | | | | Chelatococcus daeguensis |
| | | | | | | Pirellula staleyi DSM 6068 |
| | | | | | | Skermanella aerolata |
| | | | | | | Frankia sp. S9-650 |
| | | | | | | Rubellimicrobium mesophilim DSM 19309 |
| | | | | | | Legionella cincinnatiensis |
| | | | | | | Cystobacter badius |
| | | | | | | Paucimonas lemoignei |
| | | | | | | Devosia subaequoris |
| | | | | | | Afipia felis |

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**Table 2** (*continued*)

| Study | Named Phyla | Named Classes | Named Orders | Named Families | Named Genera | Named Species |
|---|---|---|---|---|---|---|
| | | | | | | Bdellovibrio bacteriovorus |
| | | | | | | Sphingomonas jaspsi |
| | | | | | | Amycolatopsis pigmentata |
| | | | | | | Ohtaekwangia kribbensis |
| | | | | | | Skermanella xinjiangensis |
| | | | | | | Sphingomonas sp. YC6722 |
| | | | | | | Oxalicibacterium horti |
| | | | | | | Desulfomonile tiedjei |
| | | | | | | Diaphorobacter nitroreducens |
| | | | | | | Comamonas terrigena |
| | | | | | | Acidovorax caeni |
| | | | | | | Delftia lacustris |
| | | | | | | Glaciimonas sp. A2-57 |
| | | | | | | Glaciimonas immobilis |
| | | | | | | Oxalicibacterium faecigallinarum |
| | | | | | | Rhizobiales bacterium WSM3557 |
| | | | | | | Terrimonas sp. M-8 |
| | | | | | | Adhaeribacter terreus |
| | | | | | | Brevundimonas staleyi |
| | | | | | | Sphingomonas changbaiensis |
| | | | | | | Rhizobium skierniewicense |
| | | | | | | Caulobacter vibrioides |
| | | | | | | Caulobacter segnis |
| | | | | | | Arenimonas malthae |
| | | | | | | Altereryhrobacter sp. H32 |
| | | | | | | Altereryhrobacter sp. MSW-14 |
| | | | | | | Rhocoplanes piscinae |
| | | | | | | Novosphingobium hassiacum |
| | | | | | | Yonghaparkia alkaliphila |
| | | | | | | Rhodococcus qingshengii |
| | | | | | | Rhodococcus erythropolis |
| | | | | | | Rhodococcus sp. Djl-6-2 |
| | | | | | | Nodocardia coeliaca |
| | | | | | | Luteimonas marina |
| | | | | | | Luteimonas litimaris |
| | | | | | | Pedobacter boryungensis |
| | | | | | | Sphingomonas yunnanensis |
| | | | | | | Filimonas lacunae |
| | | | | | | Luteimonas sp. KMM 9005 |
| | | | | | | Stenotrophomonas rhizophila |
| | | | | | | Bacillus patagoniensis |
| | | | | | | Rhodoplanes roseus |
| | | | | | | Gordonia neofelifaecis NRRL B-59395 |
| | | | | | | Gordonia cholesterolivorans |
| | | | | | | Gordonia malaquae |
| | | | | | | Acidovorax temperans |
| | | | | | | Pedobacter koreensis |
| | | | | | | Limnobacter thiooxidans |
| | | | | | | Beijerinckia derxii subsp. derxii |
| | | | | | | Beijerinckia indica subsp. Indica ATCC 9039 |
| | | | | | | Beijerinckia indica subsp. Lacticogenes |
| | | | | | | Flavobacterium sp. FCS-5 |
| | | | | | | Achromobacter insolitus |
| | | | | | | Comamonas testosteroni |
| | | | | | | Brevundimonas nasdae |
| | | | | | | Methylobacterium populi |
| | | | | | | Methylobacterium zatmanii |
| | | | | | | Wautersia numazuensis |
| | | | | | | Cupriavidus basilensis |
| | | | | | | Sediminibacterium salmoneum |
| | | | | | | Niastella yeongjuensis |
| | | | | | | Rhodanobacter fulvus |
| | | | | | | Skermanella xinjiandensis |
| | | | | | | Fulvimonas soli |

(*continued on next page*)

**Table 2** (*continued*)

| Study | Named Phyla | Named Classes | Named Orders | Named Families | Named Genera | Named Species |
|---|---|---|---|---|---|---|
| | | | | | | Dyella terrae<br>Dokdonella sp. LM 2-5<br>Sphingomonas pituitosa<br>Sphingomonas chilensis<br>Rhodococcus fascians<br>Rhodococcus kyotonensis<br>Rhodococcus cercidiphylli<br>Rhodococcus sp. C5(2010)<br>Prosthecobacter debontii<br>Roseomonas stagni<br>Hoeflea alexandrii<br>Niastella koreensis<br>Rhizobium sp. HT4<br>Legionella longbeachae<br>Cystobacter velatus<br>Cystobacter miniatus<br>Oligotropha carboxidovorans |
| Xu et al., 2016 | Proteobacteria<br>Bacteriodtes<br>Actinobacteria | | | | Nitrosospira | |

Sequences for each study were then classified using the classifier trained on the appropriate 16S region for that study.

All data analysis was performed in R (R Core Team, 2019), relying extensively on R packages phyloseq (McMurdie and Holmes, 2013), dplyr (Wickham et al., 2019), vegan (Oksanen et al., 2019), ggplot2 (Wickham, 2016), and deseq2 (Love et al., 2014). We entered corresponding sample data for each study, noting parameters including soil type, amendment (with or without PyOM), PyOM production temperature, PyOM feedstock, pH of PyOM, pH of PyOM-amended soil, and others, where present within the corresponding papers or archived sequence data (Supplementary Table S1). Where sample data were not explicitly matched to the sequencing data files (Dai et al. (2016, 2017a, b) and Song et al. (2017)), we used ordination plots and relative abundances to manually match sequencing data to their corresponding sample data and treatment type based on the original paper. If soil type was not explicitly stated in the paper, soil type was speculated by locating the study site on the FAO/UNESCO Soil Map of the World (FAO, 1947).

In order to evaluate trends at different phylogenetic resolutions, we also created merged OTU tables at the genus, family, and order levels using the tax_glom function in phyloseq (McMurdie and Holmes, 2013). Merged OTUs were removed if they included ambiguous names, such as "uncultured", "metagenome", "ambiguous_taxa", or "unknown".

### 2.3. Statistical analyses

To compare community composition across soil types and with and without PyOM additions, we calculated Bray-Curtis dissimilarity

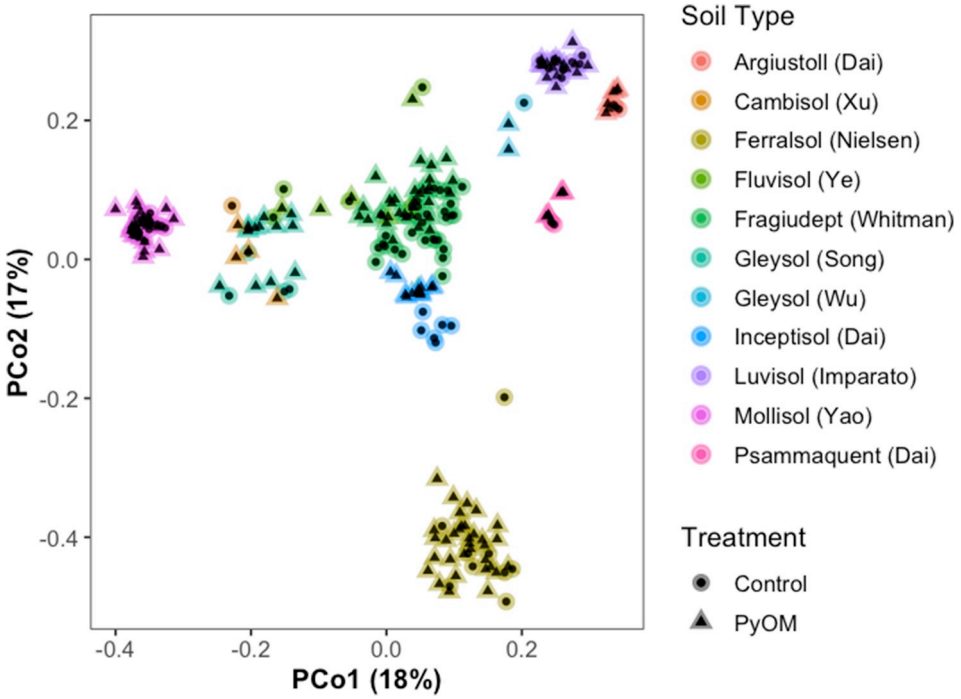


**Fig. 1.** Principle Co-ordinates axes 1 (18%) and 2 (17%) for Bray-Curtis dissimilarities between samples with read abundances merged by taxonomy at the genus level to allow for cross-sample comparisons. Points are coloured by soil type/study (p = 0.001, PERMANOVA). Circles indicate no addition controls, while triangles indicate PyOM-amended soils (p = 0.001, PERMANOVA), excluding samples taken at time = 0.

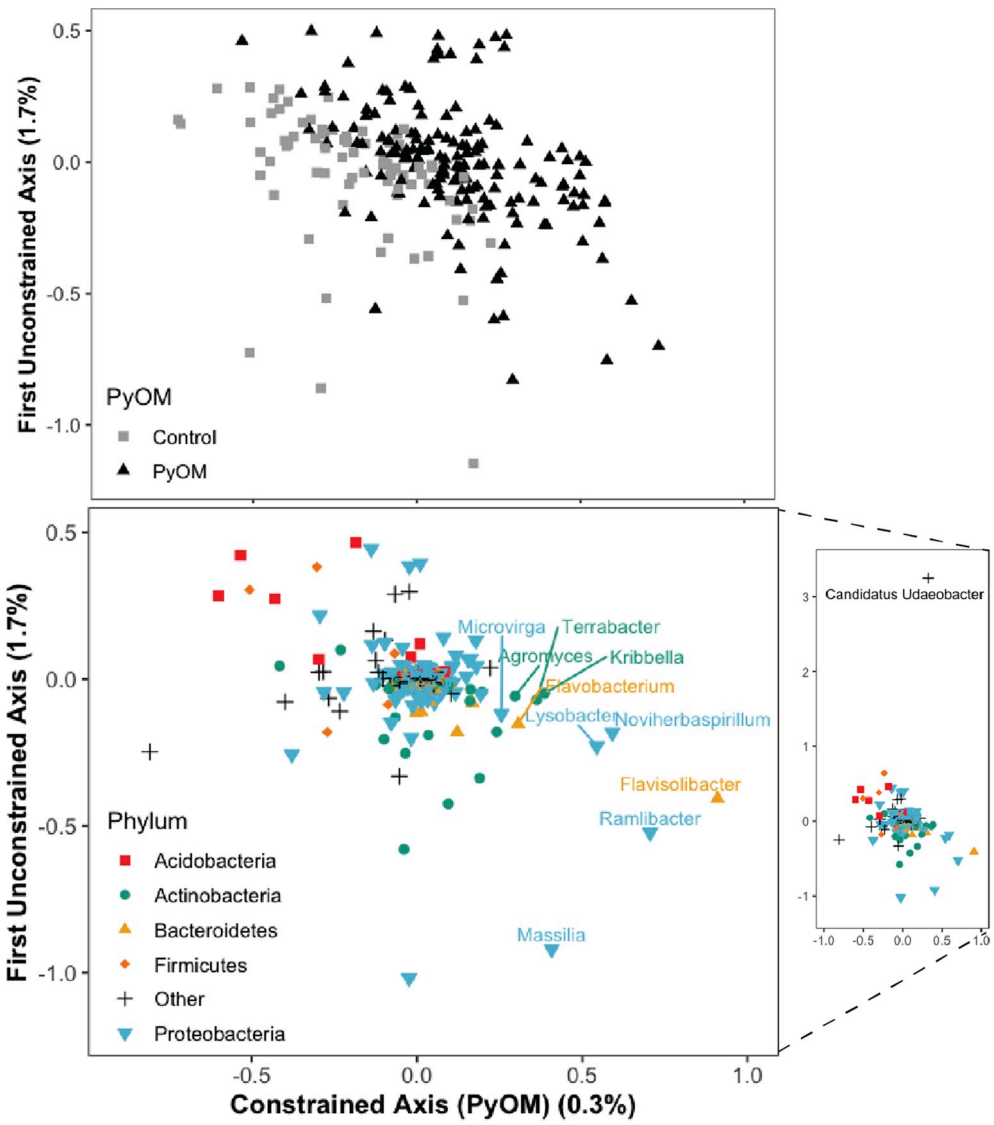


**Fig. 2.** Partial dbRDA for Bray-Curtis dissimilarities between samples with read abundances merged by taxonomy at the genus level to allow for cross-sample comparisons, controlling for soil type/study and constraining by PyOM additions for samples (top) and genera (bottom and inset). For top panel (samples), points represent samples are coloured by whether or not PyOM was added (black triangles = PyOM, grey circles = Control). For bottom panel (genera), points represent genera and are coloured by the most abundant phyla, and the 10 most divergent genera along the constrained axis are labelled. Note inset with different scale to show *Candidatus Udaeobacter* datapoint (+).

between samples (Bray and Curtis, 1957), normalized by relative abundance, and testing for effects of PyOM additions and soil type/study using permutational multivariate ANOVA on Bray-Curtis dissimilarities as implemented in vegan as the "adonis" function (Oksanen et al., 2019). In order to examine the effect of PyOM on the bacterial community after controlling for soil type/study, we used partial distance-based redundancy analysis (dbRDA) on Bray-Curtis dissimilarities as implemented in vegan under the capscale function (Oksanen et al., 2019).

In order to try to identify whether other factors such as effect of PyOM over time, PyOM application rate, PyOM production temperature, and PyOM-induced pH shifts determined the degree of effect of PyOM on bacterial community composition, we used ANOVAs to test whether dissimilarities between PyOM-amended plots and their corresponding (time-matched) control plots and the variables of interest were different across the variable of interest (for the relevant studies) as implemented in vegan (Oksanen et al., 2019).

To look for consistent PyOM-responsive taxa across studies, we calculated $\log_2$-fold change values in taxon abundances with *vs.* without PyOM additions, testing each different study, soil type, char type (separating feedstocks and temperatures but combining rates), and timepoint separately using deseq2 (Love et al., 2014). Because of the obvious risk of multiple comparisons across this many sub-datasets yielding many spurious "significant" responders, we combined the results from all datasets and adjusted p-values across the combined datasets using a Benjamini-Hochberg correction ($p < 0.05$), thus attempting to limit false discoveries. We used this approach at the OTU, genus, family, and order level. We then identified taxa that consistently increased in relative abundance with PyOM additions across studies. In order to merit reporting a response, we required that the taxonomic grouping (OTU, genus, family, or order) be designated as a positive responder in at least three different soils/studies and that it be present in at least five different studies. We then consider on a qualitative basis the extent to which this response is consistent across studies.

To compare the responses of individual OTUs to PyOM produced at different temperatures, or to the same PyOM at different timepoints, we tested for a linear relationship between the $\log_2$-fold change of all OTUs that responded in at least one timepoint or temperature using an ANOVA as implemented in vegan (Oksanen et al., 2019). All code used for analyses in this paper can be found at github.com/whitmanlab/Meta-analysis.

## 3. Results

### 3.1. Whole community responses to PyOM additions

Soil bacterial communities were structured by study/soil type

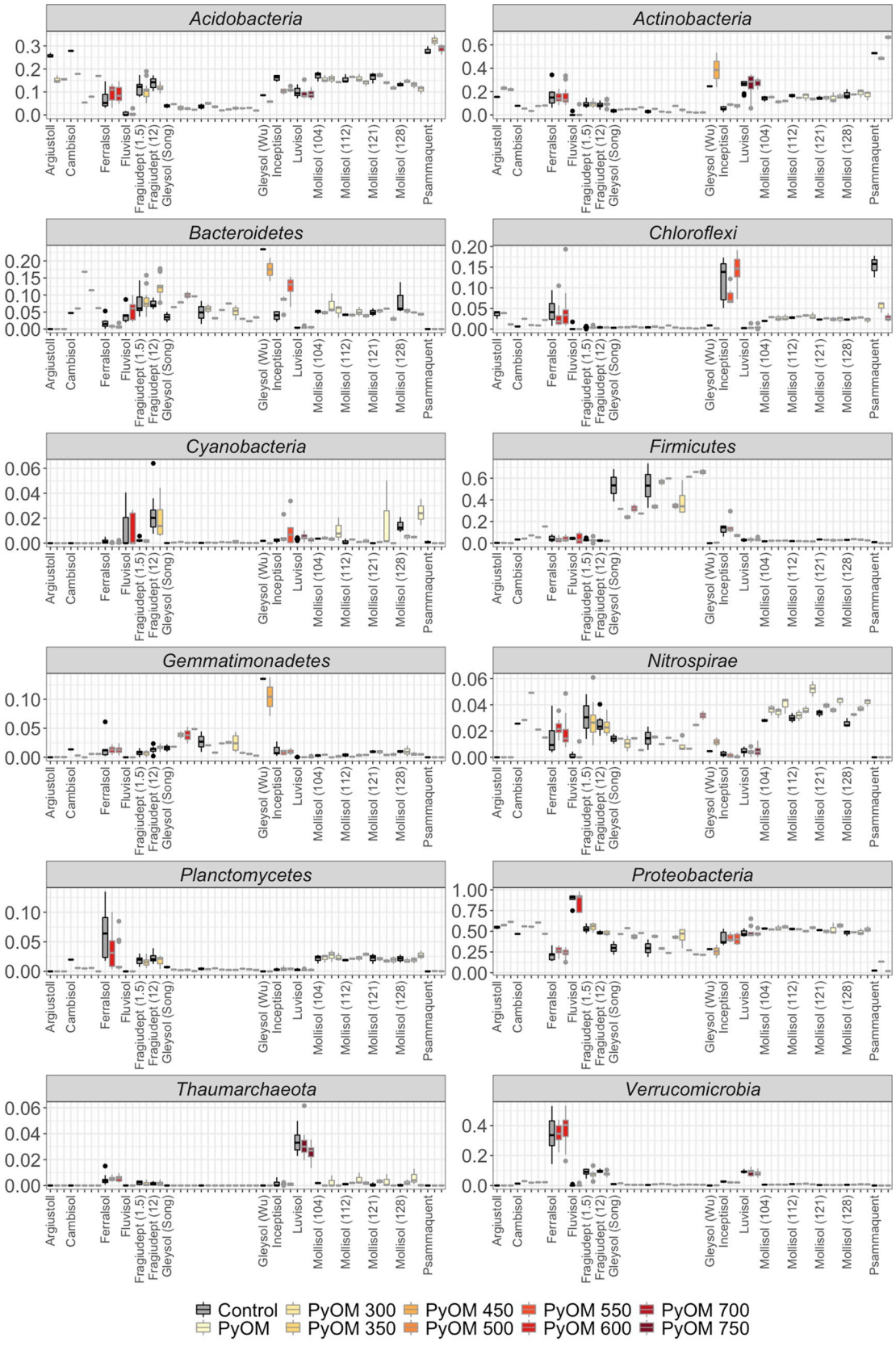


**Fig. 3.** Relative abundance of 12 most abundant phyla across studies and treatments. Black outlines with grey fill represent control (no addition) samples, while grey outlines with coloured fill represent samples with PyOM additions. PyOM samples are coloured by increasing (yellow to red) low to high production temperatures. Samples within a given study are ordered from left to right by increasing incubation time (including corresponding control) and then within incubation time by increasing application rates. The x-axis is labelled by soil type, and, then, in cases of duplication, distinguished by author or weeks of PyOM exposure in parentheses. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

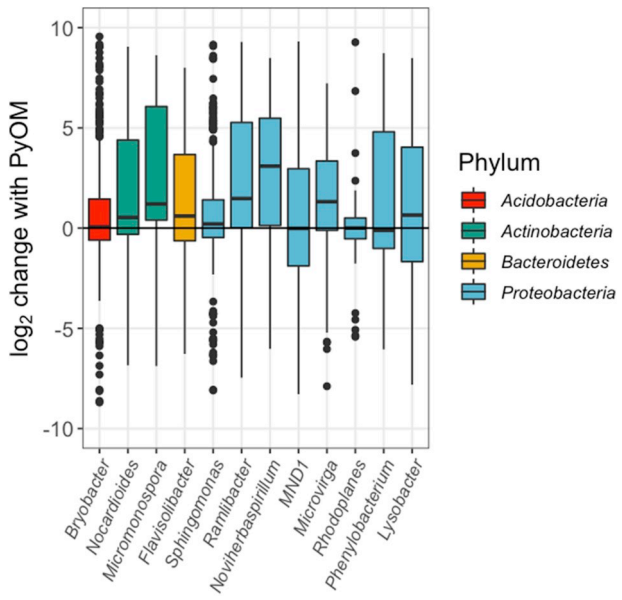


**Fig. 4.** $Log_2$-fold change with PyOM additions *vs.* control for OTUs across all studies within responsive genera. Only genera that were identified as responders in at least three studies/soil types and were present in at least five studies are shown. Boxplots are coloured by phylum. If there were multiple timepoints or multiple types of PyOM in a single study, the response of the genus is counted for each. Individual responses of each OTU for each study are shown in Supplementary Fig. S2.

(PERMANOVA, $p = 0.001$), and by whether PyOM had been added (PERMANOVA, $p = 0.004$). The explanatory power of study/soil type ($R^2 = 0.81$) was many times greater than the explanatory power of PyOM additions ($R^2 = 0.003$) (Fig. 1).

The explanatory power of PyOM additions alone, after controlling for soil type/study using a partial dbRDA, was minimal, and only explained 0.3% of the total variation in the dataset ($p = 0.001$) (Fig. 2).

Bacterial communities in PyOM-amended plots became more similar to their corresponding control plots over time in one study (Song et al. (2017); ANOVA, Tukey's HSD, Bray-Curtis dissimilarity to control at 12 weeks was 0.11 units lower than at 4 weeks ($p = 0.04$)), but did not differ in their similarity to control plots over time in another (Whitman et al. (2016); ANOVA, Tukey's HSD, 1.5 weeks *vs.* 12 weeks ($p = 0.99$)). In the study where char had been present for two years before the study's initiation, PyOM *vs.* control plots became slightly more similar in the two later timepoints within the study (Yao et al. (2017); ANOVA, Tukey's HSD, Bray-Curtis dissimilarity to control at 104 weeks was 0.06 higher than at 121weeks ($p = 0.004$), and 0.05 higher at 104 weeks than at 128 weeks ($p = 0.03$)).

PyOM application rates ranged dramatically, from 1.1 to 100 t $ha^{-1}$ (and from 10 to 30 g $kg^{-1}$, where reported on a mass basis) (Table 1), and the effect of increasing application rates on the PyOM-amended *vs.* control plots varied across studies and over time. For example, with the exception of the 112 week timepoint, controlling for incubation time, increasing application rates between 50 and 200 t $ha^{-1}$ resulted in slightly more dissimilar communities (Yao et al., 2017; ANOVA, Tukey's HSD, Bray-Curtis dissimilarity to control at 200 t PyOM $ha^{-1}$ was 0.07 higher than at 50 t PyOM $ha^{-1}$ ($p = 2 \times 10^{-16}$)). For Song et al. (2017), controlling for PyOM production temperature, increasing applications from 10.1 to 20.4 g PyOM $kg^{-1}$ soil produced increasingly dissimilar communities at 4 weeks (ANOVA, Tukey's HSD, Bray-Curtis dissimilarity to control at 20.4 g PyOM $kg^{-1}$ soil was 0.12 higher than at 10.1 g PyOM $kg^{-1}$ soil ($p = 0.02$)), but no differences by 12 weeks (ANOVA, $p = 0.57$). Conversely, dissimilarities between PyOM-amended plots and control plots decreased when application rate increased from 2.3 to 14.3 t $ha^{-1}$, (Imparato et al., 2016; ANOVA, Tukey's HSD, Bray-Curtis dissimilarity to control at 14.3 t $ha^{-1}$ was 0.07 lower than at 2.3 t $ha^{-1}$ ($p = 2 \times 10^{-5}$)).

Two studies considered different PyOM production temperatures. Despite having the same char types, application rates, and incubation times in the two Dai et al. (2017a,b) soils, the 700 °C PyOM community was much more dissimilar to the control than the 300 °C community in the Argiustoll (ANOVA, Tukey's HSD, Bray-Curtis dissimilarity to control at 300 °C was 0.28 higher than at 600 °C ($p = 2 \times 10^{-16}$)), but no trend was seen in the Psammaquent (ANOVA, $p = 0.22$). In the one other study with different PyOM types (Song et al., 2017), the two different PyOM temperatures (300 °C vs. 600 °C) did not have different dissimilarities from the control (ANOVA, $p_{4\ weeks} = 0.74$; $p_{12\ weeks} = 0.57$, controlling for application rate).

For the studies that reported post-amendment pH values, we did not find a consistent relationship between change in pH with PyOM additions and dissimilarity between PyOM-amended and unamended soils (Supplementary Fig. S1).

### 3.2. Phylum-level responses to PyOM

There were few consistent patterns at the phylum level (Fig. 3), making it almost impossible to make generalizations about responses to PyOM at the phylum level.

### 3.3. OTU-, genus-, family-, and order-level responses to PyOM additions

We identified 12 genera that contained OTUs that were enriched with PyOM additions in at least three studies/soil types and were present in at least five different studies/soil types. However, all of these genera also contained OTUs with no or negative responses to PyOM (Fig. 4 and Supplementary Fig. S2). Of these responsive genera, the strongest responses (unweighted mean across all OTUs) were found in *Noviherbaspirillum* (6.6x mean increase with PyOM, present in 6 studies), *Micromonospora* (5.9x mean increase with PyOM, present in 5 studies), and *Ramlibacter* (5.8x mean increase with PyOM, present in 6 studies).

When we performed the same analysis using OTU tables merged at the genus level, we identified only 3 responder genera (Supplementary Figs. S3 and S4). These were *Nocardioides* (5.7x mean increase with PyOM, present in 9 studies), *Mesorhizobium* (3.1x mean increase with PyOM, present in 9 studies), and *Noviherbaspirillum* (2.8x mean increase with PyOM, present in 5 studies).

When we performed the same analysis using OTU tables merged at the family level, we identified 7 families that were significantly enriched with PyOM additions in at least three studies/soil types and were detected in at least five different studies/soil types: *Microbacteriaceae, Micromonosporaceae,* and *Micrococcaceae* in the *Actinobacteria* phylum, and *Rhizobiaceae, Beijerinckiaceae, Acetobacteraceae*, and *Sphingomonadaceae* in the *Proteobacteria* phylum (Supplementary Figs. S5 and S6). Similarly to the OTU and genus levels, when analyzing the data merged at the family level, response to PyOM at the family level was not necessarily consistent across studies, and the highest mean response was only 2.7x (for *Rhizobiaceae*, which also had the widest range of responses).

Finally, when we performed the same analysis using OTU tables merged at the order level, we identified 4 orders that were significantly enriched with PyOM additions in at least three studies/soil types and were detected in at least five different studies/soil types: *Propionibcteriales, Micromonosporales* and *Micrococcales*, in the *Actinobacteria* phylum, and *Acetobacterales* and *Rhizobiales* in the *Proteobacteria* phylum (Supplementary Figs. S7 and S8). Still, the mean response for all orders was less than 2x.

Numerous taxa that were identified as PyOM responders in the original studies were not detected as being positive responders in this study. This is likely due in no small part to the increased number of comparisons that were performed in this meta-analysis, which resulted in greater stringency for a p-value to indicate a strong effect.

Additionally, in the analyses where we pooled taxa at the genus level or higher, responses of individual species or strains within that group might have been obscured by the non-responsive members of that group that were pooled together.

For the three studies that considered different temperatures of PyOM (two of which used the same PyOM materials), there was a positive relationship between the responses of the same OTUs at the two different temperatures in each case (ANOVA, $p = 0.001$; Supplementary Figs. S9–S11). For two of the three studies that considered the same material at different timepoints, there was a positive relationship in OTUs' response between the two different timepoints (Whitman et al. (2016), ANOVA, $p = 0.02$; Song et al. (2017), ANOVA, $p = 0.001$; Supplementary Figs. S12–S14), but there is substantial variability within the responses.

## 4. Discussion

### 4.1. Soil type determines microbial community composition much more than PyOM additions

Although the addition of PyOM to soils altered the total bacterial community composition across studies individually, this effect was very small at the whole community level (Figs. 1 and 2). *I.e.*, the soils analyzed here are sufficiently different to begin with that there is not a "typical" PyOM-induced community that overpowers the effects of the original soils. This is not necessarily surprising – the soils in this paper span diverse regions of the globe, and numerous other factors, such as pH or moisture, are likely structuring the communities (Delgado-Baquerizo et al., 2018; Rousk et al., 2010). Furthermore, for the studies that included different timepoints, the effects of PyOM on microbial community composition did not increase with time. This relative resilience of bacterial communities after PyOM amendments suggest that, although PyOM additions can cause a subset of the community to increase in abundance, it is unlikely that PyOM amendments would change the core composition, and, hence, long-term functional potential of a soil microbial community. The samples analyzed here were collected after a range of PyOM exposure durations, from less than two weeks (Whitman et al., 2016) to over two years (Yao et al., 2017), and a range of application rates, from 1.1 Mg PyOM $ha^{-1}$ (Nielsen et al., 2014) to 200 Mg PyOM $ha^{-1}$ (Yao et al., 2017). Despite this extreme range, the bacterial communities of PyOM-amended soils still broadly resembled their unamended counterparts, much more than they resembled different soils that had been amended with PyOM. Thus, the utility of examining the soil bacterial community response to PyOM likely lies primarily in understanding which microbes may be responsible for PyOM-induced changes in processes of interest, such as C mineralization.

### 4.2. Neither phylum-level nor genus-level responses to PyOM are consistent across soils and PyOM types

Each study included in this review reported some generalizations at the phylum level in their discussions of bacterial community response to PyOM (Table 2). However, we did not observe consistent phylum-level responses to PyOM amendments across studies (Fig. 2). There are numerous reasons this is not surprising. Phyla are extremely diverse groupings of bacteria, and encompass numerous taxa that are genetically and functionally very different. However, we also did not find strongly consistent PyOM responses across OTUs within a single genus, which, of course, is a much finer taxonomic scale (Fig. 4; Supplemental Fig. S2). This highlights that even genus-level groupings likely contain important functional diversity. However, it also underscores the fact that ten studies are clearly inadequate to span the full range of possible combinations of soil types, PyOM materials, application rates, study durations, and environmental conditions. Thus, if a given phylum or genus was observed to have a consistent response within one study, it is perhaps not surprising if it had the opposite response in another. For example, there are consistent small increases in *Chloroflexi* with corn PyOM additions in the Mollisol of Yao et al. (2017), but substantial decreases in *Chloroflexi* with manure PyOM additions in the Psammaquent of Dai et al. (2017a,b) (Fig. 3). Just as one would not likely write a paper describing the effects of "organic matter" additions on *Chloroflexi*, without distinguishing between corn and manure, one should not expect consistent responses from PyOM that is made from such different materials. While PyOM materials do share or converge upon numerous common characteristics (particularly at high production temperatures), they can still differ substantially in fundamental properties such as nutrient availability, pH, or easily-mineralizable C (Enders et al., 2012; Whitman et al., 2013). Furthermore, their effects will interact with the properties of the soil to which they are applied. Thus, it is likely that we will gain the most predictive understanding by considering the effects of PyOM on bacteria in the context of the materials' differing properties, and not expecting a consistent "PyOM effect".

### 4.3. Common PyOM responders include putative aromatic C degraders

Given the wide range of bacterial community responses that is to be expected from studies as divergent as those considered here, it is perhaps most surprising that we were able to identify a number of genera that do seem to have consistent responses across multiple soils. This suggests that, despite wide-ranging community-level responses to PyOM, there are taxa that often increase in relative abundance after PyOM additions. The responsive genera (Fig. 4 and Supplemental Fig. S3) mostly come from phyla that have previously been identified as being more abundant post-fire: *Actinobacteria* and *Proteobacteria* (Cobo-Díaz et al., 2015; Mikita-Barbato et al., 2015; Smith et al., 2008; Weber et al., 2014). More importantly, specific genera we identified as PyOM-responders here have been previously identified as being fire-responders in other studies, including *Nocardioides, Noviherbaspirillum, Phenylobacterium* (Whitman et al., 2019), *Sphingomonas* (Sun et al., 2016; Weber et al., 2014; Whitman et al., 2019), and *Microvirga* (Fernández-González et al., 2017). This suggests that part of bacterial response to fires might be common to their response to PyOM additions.

Similarly, many of these PyOM-responders have also been identified as being from genera that also contain polyaromatic hydrocarbon (PAH) degraders, as recently reviewed by (Ghosal et al., 2016). *Sphingomonas* was one of the genera that was found to be enriched with PyOM additions in two different studies, while *Noviherbaspirillium* and *Nocardioides* were found to be enriched in three. Members of *Nocardioides* have previously been identified as putative degraders of PAHs, such as phenanthrene by *Nocardioides sp.* strain KP7 (Iwabuchi and Harayama, 1998). Additionally (Zhao et al., 2018), observed *Nocardioides sp*. to be associated with the presence of numerous PAH types in a 12-m deep borehole near a coking chemical plant in Beijing, China. Similarly (Manucharova et al., 2017), observed increases in *Nocardioides sp*. in haplic abruptic Luvisols in Russia that had been treated with gasoline and diesel fuel. For genus *Noviherbaspirillum*, one of its first isolates (*N. malthae*) was cultured under oil enrichment from an oil-contaminated soil in Taiwan (Lin et al., 2013). *Sphingomonas sp.* are also commonly-identified as being able to degrade a wide range of PAHs (Ghosal et al., 2016), possibly through genes carried on large plasmids (Basta et al., 2005). Thus, one possible explanation for the significantly increased abundance of *Nocardioides*, *Noviherbaspirillum,* or *Sphingomonas* with PyOM additions could be that the organisms are able to degrade the aromatic carbon associated with PyOM additions.

The identification of *Mesorhizobium* as a PyOM-responsive genus in three studies (Supplemetnal Fig. S3) is interesting, because *Mesorhizobium spp.* are perhaps most readily known for their ability to fix nitrogen (Zehr et al., 2003). However, the 16S sequence of the most abundant *Mesorhizobium* genus in the Dai et al. (2017b) study is a 100% match to a *Mesorhizobium sp.* that was isolated from a "bacterial consortium degrading a mixture of hydrocarbons, gasoline, and diesel oil"

(GenBank ID: KM047474.1). On its own, that *Mesorhizobium* isolate had a marked ability to degrade numerous complex carbon compounds, including benzene, toluene, ethylbenzene, and xylene compounds, naphthalene, and cyclohexane (Auffret et al., 2015). We did not identify *Mesorhizobium* as a PyOM-responsive genus for the Ye et al. (2016) soil (perhaps due to the stringent statistical cutoffs in this paper to define a responder), but the original paper did note it as being associated with PyOM (when combined with compost additions). However, the interpretation in the original paper focuses on its possible role as a putative $N_2$-fixer, and it did not seem to increase in relative abundance with PyOM amendments alone. None of the other papers analyzed here focused on this genus as a core PyOM-responder during their discussion. As described in the introduction, with numerous responsive OTUs, it is usually impractical to discuss all the individual OTUs in a given paper. However, by comparing across studies, we have identified the enrichment of *Mesorhizobium* with PyOM additions – and suggested an interesting putative reason – its potential ability to degrade aromatic C compounds.

### 4.4. Recommendations and future areas of study

The processes that determine the structure of soil biotic communities and how changes to these systems affect the soil microbial community remain questions of fundamental interest. There are many reasons why it is important to understand the effects of wildfire or biochar additions on soil microbes. These include predicting biogeochemical implications of changing fire regimes and recognizing how the microbial community composition influences how PyOM impacts the changes in greenhouse gas emissions or plant growth. In our study, we showed that across the studies analyzed, the addition of PyOM caused only a very small consistent shift in community composition, and, ultimately, pre-addition soil characteristics were much more important than PyOM additions for predicting community composition. However, although the whole-community response to PyOM was not consistent, we did identify some genera that contained positive responders in at least three studies, some of which are known PAH degraders or have previously been identified as being enriched after wildfires: *Nocardioides, Noviherbaspirillum, Phenylobacterium, Sphingomonas, Mesorhizobium,* and *Microvirga*. However, the overarching findings of this study suggest that our current ability to consistently predict the response of specific bacteria to PyOM additions is severely limited, likely both by the diversity of the microorganisms as well as the diversity of soil types, PyOM materials, and study designs.

Thus, we propose some considerations to help design future studies, aimed at improving our ability to collectively advance our understanding of these systems. To start, the review by Jeffery et al. (2015) offers valuable suggestions on robust experimental design, appropriate controls, and standardized reporting across PyOM-addition studies, while Fierer (2017), Shade (2017), and Baldrian (2019) all offer useful suggestions for improving our understanding of microbial communities and processes. In addition to emphasizing these recommendations, we draw from principles discussed in The ASA Statement on P-Values and Statistical Significance (Wasserstein and Lazar, 2016), and, particularly, some of the subsequent recommendations for moving beyond p-values from a follow-up special issue (including the editorial by Wasserstein et al., 2019 and Ziliak, 2019). Reporting p-values as exact numbers and avoiding the term "statistically significant" will help us avoid putting undue weight on the p-value as a binary indicator of the intrinsic value of a given finding. Instead, we should remember to focus on the effect sizes (how much is the variable affected?) and their real-world meanings (why does this effect size matter?), and on cross-study or cross-system effect consistency (how generalizable is this effect?). In the case of the effects of PyOM additions to soil on soil bacteria, we might approach this by establishing, before beginning the study, how abundant a taxon needs to be in order to be of interest (*e.g.,* likely at least present in all samples) and what levels of increases in the numbers of a given taxon would be of interest (*e.g.,* perhaps we decide we are interested in taxa that double or more in abundance). Then, we would need to discuss our findings in these terms, focusing on the effect size, and reporting exact p-values (if another statistical approach is not used). Furthermore, we would refrain from suggesting our observations will be broadly upheld across systems until sufficient other studies in similar and in different systems have been conducted.

In order to be able to deepen our understanding by comparing results across studies, it is essential that data be accessible, ideally in their raw form and with appropriate and clear metadata. If this had been standard practice, we would have had 6 more studies to analyze in this current paper, increasing our total to 16. The availability of raw data is particularly important for soil microbial community analyses, because of the extreme diversity that we deal with in such datasets. One important recommendation from Wasserstein et al. (2019) is that we report the results of all tests that we do – not just those with p values $< 0.05$. For tests that are applied to thousands of OTUs, this suggestion would be impractical and make for unreadable papers. However, by focusing on and reporting only significant responders, we run the risk of bias when scanning the text of other papers looking for examples of other studies that found the same effects as ourselves. We might easily conclude that a given organism responds in a certain way consistently, without knowing whether it was not responsive or even not present in studies where it is not mentioned. Making raw data available is at least one step in the direction of allowing for consistent inter-study comparability. This would also help with finer-scale analyses in general: making inter-study comparisons of microbial community responses from stacked bar charts is nearly impossible.

We appreciate the recommendations from (Wasserstein et al., 2019) that we follow the ATOM model: "**A**ccept uncertainty. Be **t**houghtful, **o**pen, and **m**odest" when designing, conducting, and reporting research. We also note that a predictive understanding of the responses of microbial communities to PyOM additions remains limited by the wide range of soil properties, PyOM properties, application rates, experimental conditions, and timescales that current studies span. Because of this, it is imperative that we do not readily extrapolate findings from one system to another system without rigorous cross-study comparisons. Finally, we underscore that in order to advance our understanding of these systems, it is essential that researchers make their data available. Similarly, we thank the authors from all the studies considered here for doing so, without which, we would have been limited to synthesizing and re-reporting only the author-reported findings.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2019.107678.

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