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New insights into the role of microbial community composition in driving soil respiration rates

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Abstract

Microbial community plays critical roles in driving soil carbon (C) cycling in terrestrial ecosystems. However, we lack empirical evidence to demonstrate the role of microbial community in driving soil respiration -a key ecosystem process for global sustainability and climate regulation. Here, we used a long-term field experiment including multiple management practices, to identify, via statistical modeling, the role of microbial community composition in influencing soil respiration. We analyzed major soil properties and microbial (both bacterial and fungal) abundance, diversity and community composition. We found that different management regimes led to different soil respiration rates. Most importantly, microbial community composition explained a unique portion of the variation in soil respiration, which cannot be accounted for by key respiration drivers such as soil properties and other microbial attributes (richness and total abundance). Microbial biomass and fungal richness were also identified as key drivers of soil respiration. Our results indicate that inclusions of microbial compositional data in Earth system models can be potentially used to improve our capacity to predict changes in soil C balance under changing environments.

Key words

Bacteria, fungi, carbon cycling, ecosystem processes, microbial community, global change

1. Introduction

Soils store four times as much carbon (C) as plant and atmospheric pools (Singh et al., 2010; Karhu et al., 2014), and soil respiration releases about 60 Pg C annually from the land surface (Shao et al., 2013). Both C sequestration and soil respiration are critical processes controlling key ecosystem functions such as climate regulation, nutrient cycling and plant productivity (Singh et al., 2010; Victoria et al., 2012; Trivedi et al., 2017). Global climate change and human disturbances including intensive agricultural practices are increasing the amount of C emitted to the atmosphere with important implications for the climate regulation of Earth (Nazaries et al., 2015; Spohn et al., 2016). Because of this, predictions of soil C balance in terrestrial ecosystems have become a global priority during the last decades with the development of Earth system models as primary tools (Luo et al., 2016).

Soil respiration is driven by both biotic and abiotic factors (Walker et al., 2004; Monson et al., 2006; Orwin et al., 2015). Previous studies have demonstrated the importance of geographic location (Campbell et al., 2004; Whitaker et al., 2014), climate (temperature and rainfall) (García-Palacios et al., 2012; Karhu et al., 2014), soil properties (Delgado-Baquerizo et al., 2016a) and plant features (Raich and Tufekciogul, 2000; Knowles et al., 2015) as key predictors of soil respiration. However, current models are not able to accurately predict the variation in soil stocks and respirations, leading to a high level of uncertainty for these predictions. Identifying new major predictors of soil respiration that allow the improvement of predictive models is one of the major challenges that we are facing today. Most recently, the inclusion of microbial processes (reflected by microbial biomass and enzyme activities) has been reported to improve the prediction of soil C fluxes at the global scales (Allison et al., 2010; Wieder et al., 2013). Similarly, microbial diversity has been reported to drive multiple soil functions including soil respiration (Delgado-Baquerizo et al., 2016b; Liu et al., 2017). Further, in addition to microbial biomass and diversity, other microbial parameters such as community composition (relative abundance of phylotypes) may greatly improve our prediction for soil respiration (Whitaker et al., 2014), given the strong positive relationships between microbial composition and the functional genes that regulate soil respiration (Trivedi et al., 2016). However, the importance of soil microbial community composition in driving soil respiration remains poorly understood and largely unexplored; and no experimental

approach has been used to address this important gap of knowledge.

A growing number of studies had emphasized the significance of microbial community composition in driving soil processes and functions including gaseous emission, decomposition, nutrient cycling and plant production (Fierer et al., 2007; Peter et al., 2011; Trivedi et al., 2013; Trivedi et al., 2016). We argue that current knowledge on microbial life strategies and functional attributes can be used to markedly improve our prediction of soil respiration rates. For example, previous studies suggested that ecological functional categories of copiotrophs and oligotrophs have specific roles in utilizing soil organic C (SOC) for respiration (Fierer et al., 2007; Ramirez et al., 2012; Trivedi et al., 2013). Thus, copiotrophs such as Bacteroidetes, Alphaproteobacteria and Gammaproteobacteria are expected to have higher respiration rates compared to oligotrophs including Actinobacteria, Acidobacteria and Deltaproteobacteria (Fierer et al., 2007; Bastian et al., 2009; Singh et al., 2010; Trivedi et al., 2013). In addition, bacteria are considered to have lower C use efficiency compared to fungi (Austin et al., 2004; Waring et al., 2013). All these suggest that differences in microbial community composition could potentially explain a unique portion of the variation in soil respiration.

Herein, we posit that community composition of fungi and bacteria can potentially help explain unique portions of the variation in soil respiration which cannot be accounted for by other key drivers of soil respiration including soil properties, land management practices (i.e. inorganic and organic fertilization) and other key microbial attributes such as microbial biomass or community richness. To test our hypothesis, we collected soils from a long-term field experiment including multiple combined applications of inorganic and organic fertilizers including nitrogen (N), phosphorus (P) and potassium (K) (NPK), livestock manure, wheat straw, and commercial organic fertilizers. These agricultural practices are well-known to simultaneously modify soil abiotic properties, microbial biomass, and community richness and composition (Sun et al., 2015; 2016), which in turn provides a unique opportunity to empirically identify the role of relative importance of soil microbial community in driving soil respiration responses to the combined fertilization after accounting for key soil properties.

2. Materials and Methods

2.1 Experimental design and soil sampling

The long-term experiment was established in Linquan county, Anhui province, China (33°04′58N, 115°13′42E), in October 2010. Mean annual temperature in this region is 15.3°C and mean annual precipitation is 892 mm. The experimental plots (10 × 5 m in size) were subject to wheat-corn rotation, and the locations were selected using a randomization approach. The soil in this site belongs to a lime concretion black soil (Eutric Acrisols) (Zhang et al., 2016), with 23% clay and 48% silt content. The initial pH of the soil was 5.72, which had 0.73% organic C, 80.16 mg kg^{-1} available N, 16.92 mg kg^{-1} available P and 116.7 mg kg^{-1} available K. This experiment included nine treatments with three replicate plots for each: (1) control, no fertilization; (2) chemical NPK fertilizers application (NPK); (3) 50% NPK fertilizers plus 6000 kg fresh cow manure ha^{-1} y^{-1} (NPK+CM); (4) 50% NPK fertilizers plus 6000 kg fresh pig manure ha^{-1} y^{-1} (NPK+PM); (5) NPK fertilizers plus all of preceding crop wheat straw (NPK+ST); (6) 50% NPK fertilizers plus 6000 kg pig manure and wheat straw from all of preceding crop (NPK+PM+ST); (7) 50% NPK fertilizers plus 6000 kg cow manure and wheat straw from all of preceding crop (NPK+CM+ST); (8) 30% NPK fertilizers plus 3600 kg commercial organic fertilizer ha^{-1} y^{-1}(NPK+OCM), which is made of cow manure; (9) 30% NPK fertilizers plus 3600 kg commercial organic fertilizer (NPK+OPM), which is made of pig manure. The NPK fertilizer comprised urea (300 kg N ha^{-1} y^{-1}), superphosphate (120 kg P_2O_5 ha^{-1} y^{-1}) and potassium chloride (100 kg K_2O ha^{-1} y^{-1}). These different proportions of NPK fertilizers with corresponding organic matter were applied to manipulate the relative balance of nutrients in the soil for crop growing. All chemical fertilizers and organic matter were applied once before sowing of the winter wheat in October, and the quantities of nutrient yearly added to the plots is showed in Table S1. The wheat straws were cut into small pieces of ~10 cm in length before use. Surface soil (0-15 cm) from each plot was collected in June 2016 after the harvest of wheat (*Triticum* spp.). Soil samples were sealed in plastic bags, and shipped back to the laboratory in an iced cooler. All the soil samples were mixed homogenously, passed through a 2.0 mm sieve, followed by dividing into two sub-samples. One sub-sample was stored at -20 °C for microbial analysis, and

another sub-sample was stored at 4°C for the analysis of soil properties.

2.2 Soil properties and respiration rate analysis

Soil water content was determined by oven-drying the samples at 105°C, and soil texture was analyzed using the pipette method (Gee and Bauder,1986). Soil pH was measured using a fresh soil to water ratio of 1: 2.5 with a Delta pH-meter, and soil organic carbon (SOC) was determined using the K_2CrO_7 oxidation titration method (Walkley & Black 1934). Total carbon (TC) and total nitrogen (TN) were measured on a LECO macro-CN analyzer (LECO, St. Joseph, MI, USA). Inorganic N and labile carbon in the soils were extracted with 0.5 M K_2SO_4 in a ratio of 1:5 by shaking at 200 rpm for 1 h and filtered through 0.45-μm Millipore filter paper. Total C and N concentrations in the extracts were analyzed by TOC analyzer with total nitrogen unit (TOC-L Analyzer, Shimadzu, Japan). In parallel, the carbon in microbial biomass (MBC) was determined using the fumigation-extraction method (Vance et al., 1987). For each measurement of respiration rate, approximately 10 g of fresh soil (within 48 h after sampling) was incubated in a 120 ml container at 25 °C for 24 h. At the end of this period, CO_2 concentrations in headspace were measured using an Agilent-7890a gas chromatograph equipped with a flame ionization detector (FID) and an electron capture detector (ECD) (Agilent Technologies, Wilmington, DE, USA). Soil respiration rates were calculated from the net accumulation of CO_2 over time.

2.3 Soil microbial community characterization

The total genomic DNA was extracted from 0.30 g of soil using the MoBio PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) following the manufacturer instructions. The concentration and quality of isolated DNA was checked using a NanoDrop® ND-2000c UV-Vis spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The abundance of bacteria and fungi were evaluated by quantifying *16S rRNA* and *ITS* gene copy number on an iCycler iQ5 thermocycler (Biorad, USA) using the primer pairs Eub338F/Eub518R (Cregger et al., 2012) and ITS1-5.8S (Fierer et al., 2005), respectively.

To evaluate the microbial community composition, the V4 region of the bacterial *16S rRNA* gene and *ITS* of fungi were amplified using the primer pairs of 338F/806R (Liu et al.,

2016) and ITS1F /2043R (Zhao et al., 2016b), respectively. The 50 µl PCR reaction mixtures consisted of 25 µl PremixTaq™ (Takara Biotechnology, Dalian, China), 1 µl of each primer (10 µM), 3 µl of template DNA, and 20 µl of sterilized ddH_2O. The resultant PCR products were purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, San Luis Obispo, USA). The purified amplicons were equimolarly mixed, and 2 × 250 bp paired-end sequencing was carried out on an Illumina Miseq sequencer (Illumina Inc., San Diego, USA). Raw reads generated from the MiSeq paired-end sequencing were merged together using the Fast Length Adjustment of Short reads (FLASH). A chimera filtering approach UPARSE was employed as the Operational Taxonomic Unit (OTU) picking strategy at 97% sequence similarity. Representative sequences from individual OTUs generated in UPARSE were processed using the Quantitative Insights into Microbial Ecology (QIIME) pipeline. The resultant OTU map file was converted into a biom file for diversity calculation in QIIME. A resampling procedure was conducted at a depth of 51,438 sequences for bacteria and 52,561 for fungi per sample before diversity calculation. Bacterial and fungal diversity index were calculated based on 97 % OTU similarity of obtained bacterial and fungal sequences, respectively. The taxonomic identity of each phylotype was determined using the SILVA ribosomal RNA gene database project (Quast et al., 2013).

2.4 Statistical analyses

ANOVA was used to evaluate the effect of different fertilization treatments on the respiration rate. Spearman's correlation analyses were performed to assess the relationships between soil properties, respiration and bacterial and fungal community including abundance, diversity and composition. The *16S rRNA* and *ITS* gene copy numbers were log-transformed prior to statistical analysis to meet normality assumptions.

We conducted a classification random forest analysis (Breiman, 2001), as done in Trivedi et al. (2016), to identify the major statistically significant microbial predictors of the composition (relative abundance: number of sequences of major phyla/class level) of bacteria and fungi. These analyses were conducted using the rfPermute package (Archer, 2016) of the R statistical software (http://cran.r-project.org/). The random forest model determined the importance of each predictor variable via evaluating the decrease in prediction accuracy (i.e.

increase in the mean square error between observations and OOB predictions) when the data for that predictor are randomly permuted, as previously described (Delgado-Baquerizo et al., 2015).

To examine the importance of microbial community attributes for soil respiration compared with soil properties, we used a multi-model inference approach based on information theory and non-parametric distance based linear regressions (DISTLM; (McArdle and Anderson, 2001). In particular, this analysis provided insights on whether the microbial community composition in our models provides additional predictive strength for soil respiration after accounting for other important soil factors. The phyla/classes included in the models were statistically significant predictors of the respiration rate from classification random forest analysis (Table 1). The Euclidean distance was used as the measure of dissimilarity in soil respiration between pairs of samples. We ranked best-fitting models that could be generated with our independent variables according to the second-order Akaike information criterion (AICc). The lower the AICc index the better the model. Here, we consider a ΔAICc >2 threshold to differentiate between two substantially different models and then select the best of those models (Burnham and Anderson, 2003). Then, we compared the AICc of the best model which presumably includes soil properties and microbial community to that of the corresponding models without microbial community composition. Differences < 2.0 in AICc between alternative models indicate that they are approximately equivalent in explanatory power (Burnham and Anderson, 2003). We conducted distance-based multimodel inference with the PRIMER V6 statistical package for Windows (PRIMER-E Ltd., Plymouth Marine Laboratory, UK).

We used structural equation model (SEM) to evaluate the direct and indirect effect of the microbial community and soil properties on soil respiration in response to different fertilization treatments. The SEM allows us to partition causal influences among multiple variables, and to separate the direct and indirect effects of the predictors included in the model; ultimately providing mechanistic information on the drivers of soil respiration (Grace, 2006). We established an *a priori* model according to our current knowledge of abiotic and biotic impact on soil respiration (Fig. S1). We only included those variables that were identified as statistically significant predictors of the respiration rate from random forest

194 analysis and distance-based best-fitting model. The data matrix was fitted to the model using
195 the maximum-likelihood estimation method. There is no single universally accepted test of
196 overall goodness of fit for SEM. Thus, we used the Chi-square test (χ^2; the model has a good
197 fit when $0 \leq \chi^2/\text{d.o.f} \leq 2$ and $0.05 < P \leq 1.00$) and the root mean square error of
198 approximation (RMSEA; the model has a good fit when RMSEA $0 \leq \text{RMSEA} \leq 0.05$ and
199 $0.10 < P \leq 1.00$ (Schermelleh-Engel et al., 2003). We also calculated the standardized total
200 effects of microbial predictors, soil properties, and fertilization treatments on the soil
201 respiration. The net influence that one variable has upon another is calculated by summing all
202 direct and indirect pathways between the two variables. If the SEM fits the data well, the total
203 effect should approximate to the bivariate correlation coefficient for that pair of variables
204 (Grace 2006). The SEM analyses were performed using AMOS 21.0 (SPSS Inc., Chicago, IL,
205 USA).

206 **3. Results**

207 *3.1 Soil respiration rates and its relationship with soil properties*

208 As expected, different fertilization regimes led to different levels of soil respiration rate (Fig.
209 1, $P = 0.001$). The highest soil respiration rate was found in the treatment with
210 NPK+PM+STR (Fig. 1). Across all treatments, soil respiration rate was related to soil pH and
211 SOC (Table 2, $P < 0.05$). These soil properties also varied in response to the different
212 fertilization treatments (Table 3).

213 *3.2 Unique role of soil microbial community composition in predicting soil respiration rates*

214 Using random forest modeling, we identified the major bacterial and fungal phyla/classes for
215 predicting soil respiration (Fig. 2, $P < 0.05$). These taxa include several bacterial phyla such
216 as Alphaproteobacteria, WPS.2 and Deltaproteobacteria. Soil respiration rate was highly
217 correlated to other microbial attributes too (Table 4, $P < 0.05$). Particularly, there were
218 significant relationships between the respiration and microbial biomass, bacterial abundance,
219 bacterial community and richness, and fungal community and richness.

220 The best-fitting distance-based model accounted for 78% of the variation in soil
221 respiration and included both soil properties (C and C: N ratio) and microbial attributes
222 (community composition and fungal richness, Table 5). Most importantly, our model

provided evidence that microbial community composition accounted for a unique portion of the variation in soil respiration that cannot be accounted for by soil properties, or other key microbial attributes such as microbial biomass, abundance, and diversity. Thus, all models excluding microbial composition showed higher AICc values (AIC > 6.26). The best-fitting model first identified Alphaproteobacteria and Bacteroidetes as major microbial taxa predicting soil respiration (Table 5). Our models further suggest that, besides microbial composition, other microbial attributes such as microbial biomass carbon and fungal richness were also important factors determining soil respiration, as they were included in the best-fitting models.

3.3 Mechanistic understanding on the role of microbial composition in driving soil respiration rates

We used the SEM to account for direct and indirect effects of management practices, soil properties and microbial attributes on driving soil respiration, and therefore, to obtain a system-level mechanistic understanding on the drivers of soil respiration. Our SEM explained 92% of the variance in the soil respiration, parameterized using predictors from the best-fitting distance-based model. Soil properties, microbial composition, and fungal richness have strong direct effects on soil respiration (Fig. 3a). However, fertilization treatment mostly had indirect impacts on soil respiration through soil properties, microbial community composition (i.e. Alphaproteobacteria and Bacteroidetes) and fungal richness. Overall, the most important microbial attributes controlling soil respiration rates were the relative abundances of Alphaproteobacteria and Bacteroidetes, and fungal richness (Fig. 3b).

4. Discussion

Our study provides direct experimental evidence that soil microbial community composition plays a unique role in predicting soil respiration rates after accounting for key soil properties, multiple fertilization treatments and other key microbial attributes such as microbial biomass and richness. In particular, taxa from proposed copiotrophic Alphaproteobacteria and Bacteroidetes (Fierer et al., 2007; Trivedi et al., 2013) were selected as the major microbial predictors of soil respiration according to the best-fitting models. These fast-growing copiotrophic organisms had been suggested to have higher C use efficiency than other taxa belonging to slow-growing oligotrophic organisms (Fierer et al., 2007; Ramirez et al., 2012).

We thus identified their relative importance in driving soil respiration compared to other key drivers of respiration. Our findings also provide evidence from an experimental approach that the inclusion of microbial community composition in Earth system model could potentially improve our capacity to predict C feedbacks (e.g soil C respiration) in terrestrial ecosystems.

Here, we demonstrated that changes in microbial community composition can help explain a unique proportion of the variation in soil respiration. This interesting result implies that major soil properties such as pH, C and C: N ratio, which are well-known to drive both soil respiration rates and microbial community composition (Table 2, Table 6) (Fierer and Jackson, 2006; Lauber et al., 2009; Maestre et al., 2015), were not able to account for the variation in soil respiration explained by microbial community composition. The major microbial predictors of soil respiration were identified through the distance-based multi-model inference, a statistical approach which is specially recommended to identify key predictors explaining a unique portion of the variation in a particular response variable that cannot be explained by other predictors (DISTLM; McArdle & Anderson 2001). Our results suggest a direct link between microbial community composition and soil respiration rates potentially linked to particular microbial life strategies and functional capabilities (Trivedi et al., 2016).

We identified major microbial taxa predicting soil respiration including several bacterial phyla and fungal Ascomycota phylum based on the random forest model analysis (Fig. 2). Further, both proposed taxa (Alphaproteobacteria and Bacteroidetes) from the best-fitting model belong to fast-growing copiotrophs, which have been reported to be important drivers of soil respiration rates (Fierer et al., 2007). We also used SEM to achieve a system-level understanding on the role of microbial community composition in driving soil respiration when considering human management, soil properties and other key microbial drivers selected by our best-fitting distance-based models. Our SEM provided further evidence that class Alphaproteobacteria had the strongest direct effect on soil respiration, which was also modulated directly or indirectly via changes in fungal richness and land management. These results suggest that drawing upon life-strategies, the relative abundance of key microbial taxa at the highest taxonomic rank can be used to improve predictions in soil C respiration and balance in terrestrial ecosystems. These results suggest that these taxa could potentially

influence soil C respiration rates under climate change and land use intensification scenarios through shifting soil microbial community composition. In addition, significant positive relationship of fungal richness with soil respiration rates (Fig. 3b) further suggests fungal role in driving C cycling. These results support the growing literature that demonstrates the important roles of fungal diversity in driving soil functions (Wagg et al., 2014; Delgado-Baquerizo et al., 2016b). Our observations are also in accord with previous studies suggesting that fungi have stronger potential in degrading available and complex forms of C and N, attributing to their relatively strong hyphal growth form and enzymatic capacities (de Boer et al., 2005). All of these observations are in line with recent studies suggesting the significance of soil microbial community for controlling ecosystem sustainability and multiple functions (Delgado-Baquerizo et al., 2016b; Liu et al., 2017).

Finally, our SEM demonstrates that fertilization treatments can indirectly drive soil respiration through changing microbial community composition. Thus, any alteration of dominant microbial taxa may result in subsequent changes in soil respiration, with implications for the balance of terrestrial ecosystems. For example in this study, as one of important microbial drivers of soil respiration, Bacteroidetes were suppressed by NPK fertilization (Fig. 3a), which is a traditional agricultural practice for improving crop productivity. Recently, application of organic matter has become a prevailing strategy for improving soil fertility and mitigating soil degradation resulting from single chemical fertilization (Reeves, 1997; Yadvinder-Singh et al., 2004; Zhao et al., 2016a). Chemical NPK combined with commercial organic fertilizers (NPK+OCM) were also found to strongly influence the richness of soil fungal communities, another major drivers of soil respiration in our study (Fig. 3a). Overall, our results provide important information for land managers to maintain C balance in terrestrial ecosystems.

Taken together, our study provides experimental evidence that microbial community composition explained a unique portion of the variation in soil respiration which cannot be accounted for by soil properties and human land management - the major drivers of soil respiration. We further provide new insights into the role of key microbial taxa in driving soil respiration, with implications for the prediction of this key soil process under global change scenarios. These taxa could be potentially used to modulate soil respiration directly via soil

inoculum or indirectly via land management. Our findings have important implications for improving our ability to predict soil C balance using Earth system models, and future studies will need to test our hypothesis in observational datasets at the global scale.

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Table 1. Complete list of predictors used for the distance-based multi-modeling approach.

Predictor	Group	Variable	Abbreviature	Units
1	Soil properties	Soil water content	SWC	%
2		pH	pH	Unitless
3		Soil total carbon	TC	%
4		ratio soil C:N	C:N	Unitless
5		Soil organic carbon	SOC	g kg^{-1}
6		Labile carbon	LC	mg kg^{-1}
7	Biomass and diversity	Microbial biomass carbon	MBC	mg kg^{-1}
8		Abundance of fungi (qPCR)	Fungi abundance	gene copies g^{-1}
9		Abundance of bacteria (qPCR)	Bacteria abundance	gene copies g^{-1}
10		Richness of fungi	Richness fungi	Number of OTUs
11		Richness of bacteria	Richness bacteria	Number of OTUs
12	Community composition	Fibrobacteres	Fibrobacteres	%
13		Bacteroidetes	Bacteroidetes	%
14		Acidobacteria	Acidobacteria	%
15		Cyanobacteria	Cyanobacteria	%
16		Actinobacteria	Actinobacteria	%
17		Betaproteobacteria	Betaproteobacteria	%
18		WS6	WS6	%
19		Latescibacteria (WS3)	Latescibacteria (WS3)	%
20		Deltaproteobacteria	Deltaproteobacteria	%
21		WPS.2	WPS.2	%
22		Alphaproteobacteria	Alphaproteobacteria	%

Table 2 Correlation coefficients (Spearman's ρ) between soil properties and soil respiration. SWC, soil water content; SOC, soil organic carbon; LC, labile organic carbon; *P* values below 0.05 are in bold.

Soil properties	ρ	*P*
SWC	0.083	0.681
pH	**0.567**	**0.002**
TC	-0.050	0.804
C:N	-0.026	0.899
SOC	**0.388**	**0.046**
Inorganic N	0.279	0.158
LC	0.166	0.408

Table 3 Soil basic properties from the different fertilization treatments. TC and TN, total carbon and total nitrogen, respectively; SOC, soil organic carbon; LC, labile carbon. Control, no fertilization; NPK, chemical NPK fertilizers; NPK+PM, NPK fertilizers plus fresh pig manure; NPK+CM, NPK fertilizers plus fresh cow manure; NPK+ST, NPK fertilizers plus wheat straw; NPK+PM+ST, NPK fertilizers plus fresh pig manure and wheat straw; NPK+CM+ST, NPK fertilizers plus fresh cow manure and wheat straw; NPK+OPM, NPK fertilizers plus commercial organic fertilizer that is made of pig manure; NPK+OCM, NPK fertilizers plus commercial organic fertilizer that is made of cow manure. Variables do not share the same letter are significantly different ($P < 0.05$)

Treatments	pH	TN (%)	TC (%)	C:N	SOC (g kg^{-1})	Inorganic N (mg kg^{-1})	LC (mg kg^{-1})
Control	5.86 ± 0.03a	0.13 ± 0.01a	1.23 ± 0.03a	9.73 ± 0.02a	7.31 ± 0.65a	1.99 ± 2.21a	65.63 ± 3.46a
NPK	5.03 ± 0.02b	0.16 ± 0.01b	1.68 ± 0.02b	10.65 ± 0.06b	7.30 ± 0.40a	30.98 ± 1.67b	78.21 ± 2.10b
NPK+PM	5.74± 0.04c	0.17 ± 0.02b	1.79 ± 0.01c	10.38 ± 0.07b	10.20 ± 1.20b	57.69 ± 17.90c	73.80 ± 3.82b
NPK+CM	5.85± 0.02a	0.16 ± 0.03b	1.46 ± 0.02d	9.16 ± 0.05c	14.17 ± 2.21b	25.35 ± 7.50b	82.42 ± 7.31b
NPK+STR	5.07± 0.01d	0.15 ± 0.03b	1.58 ± 0.01e	10.36 ± 0.10b	10.97 ± 1.30b	17.00 ± 2.38d	87.74 ± 5.68b
NPK+PM+STR	5.31± 0.03e	0.16 ± 0.02b	2.22 ± 0.02f	14.31 ± 0.09d	12.40 ± 1.31b	27.44 ± 3.00bc	87.59 ± 2.42b
NPK+CM+STR	5.21± 0.05e	0.19 ± 0.01bc	1.74 ± 0.03c	9.22 ± 0.08c	10.78 ± 1.03ab	25.63 ± 1.09bc	87.28 ± 6.74b
NPK+OPM	4.75± 0.02f	0.24 ± 0.02c	2.78 ± 0.02g	11.77 ± 0.12e	8.43 ± 5.29ab	9.17 ± 1.41e	81.20 ± 3.99b
NPK+OCM	4.74± 0.02f	0.16 ± 0.03b	1.50 ± 0.03d	9.61 ± 0.04a	8.57 ± 3.46ab	14.13 ± 0.22d	86.09 ± 2.86b

Table 4 Correlation coefficients (Spearman's ρ) between soil microbial traits and soil respiration. MBC, microbial biomass carbon (µg g^{-1}). Bacterial and fungal abundance were calculated from 16S rRNA and ITS gene copies g^{-1} soil, respectively. NMDS, non-metric multidimensional scaling ordination derived from the on Bray-Curtis similarities depicting the bacterial and fungal community compositions. *P* values below 0.05 are in bold.

Microbial community	ρ	*P*
MBC	**0.710**	**<0.001**
Bacterial abundance	**0.400**	**0.039**
Fungal abundance	0.163	0.417
Bacterial NMDS 1	**-0.543**	**0.003**
Bacterial NMDS 2	-0.030	0.881
Fungal NMDS 1	**-0.556**	**0.003**
Fungal NMDS 2	-0.311	0.115
Bacterial richness	**0.521**	**0.005**
Fungal richness	**0.470**	**0.013**

Table 5 Best-fitting models including/excluding microbial community composition as predictors of soil respiration. Model A is the best-fitting model including microbial community composition. Model B is the same model as the best model A, but excludes microbial composition. Model C is the best model when microbial community is taken out of the equation, but microbial diversity and soil properties are freely included. Models are ranked by AICc. AICc measures the relative goodness of fit of a given model; the lower its value, the more likely the model to be correct. ΔAICc are difference between the AICc of each model and that of the best model, and ΔAICc >2 threshold indicates two different models.

Models	Microbial Composition	Microbial diversity	Soil properties	R^2	AICc	ΔAICc
A	Alphaproteobacteria + Bacteroidetes	Fungal richness	TC + C:N	0.780	-23.47	
B	Excluded	Fungal richness	TC + C:N	0.649	-17.21	6.26
C	Excluded	Fungal richness	pH + TC + C:N	0.714	-19.75	3.72

Table 6 Correlation coefficients (Spearman's ρ) between main microbial predictors and soil properties. *P* values below 0.05 are in bold.

	SWC	pH	TC	C:N	SOC	Inorganic N	LC
Fungal richness	**-0.508**	**0.694**	0.16	-0.21	0.322	**0.436**	-0.18
	.007	.000	0.425	0.304	0.102	0.023	0.376
Alphaproteobacteria	**0.483**	**-0.499**	.327	**0.681**	-0.18	-0.27	0.189
	.011	.008	0.096	0	0.377	0.174	0.346
Bacteroidetes	-0.35	**0.459**	-.030	-0.32	**0.681**	0.269	**0.458**
	0.077	0.016	0.882	0.101	0	0.174	0.016

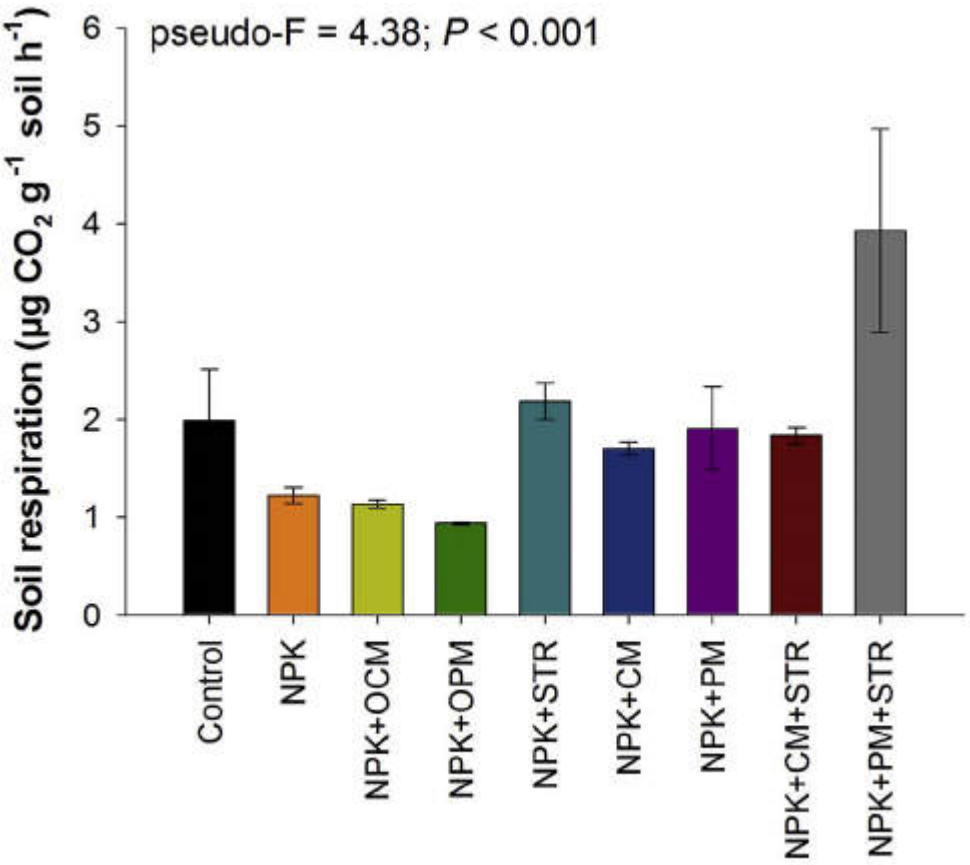

Fig. 1 Soil respiration rates under different treatment regimes. Control, no fertilization; NPK, chemical NPK fertilizers; NPK+PM, NPK fertilizers plus fresh pig manure; NPK+CM, NPK fertilizers plus fresh cow manure; NPK+ST, NPK fertilizers plus wheat straw; NPK+PM+ST, NPK fertilizers plus fresh pig manure and wheat straw; NPK+CM+ST, NPK fertilizers plus fresh cow manure and wheat straw; NPK+OPM, NPK fertilizers plus commercial organic fertilizer that is made of pig manure; NPK+OCM, NPK fertilizers plus commercial organic fertilizer that is made of cow manure.

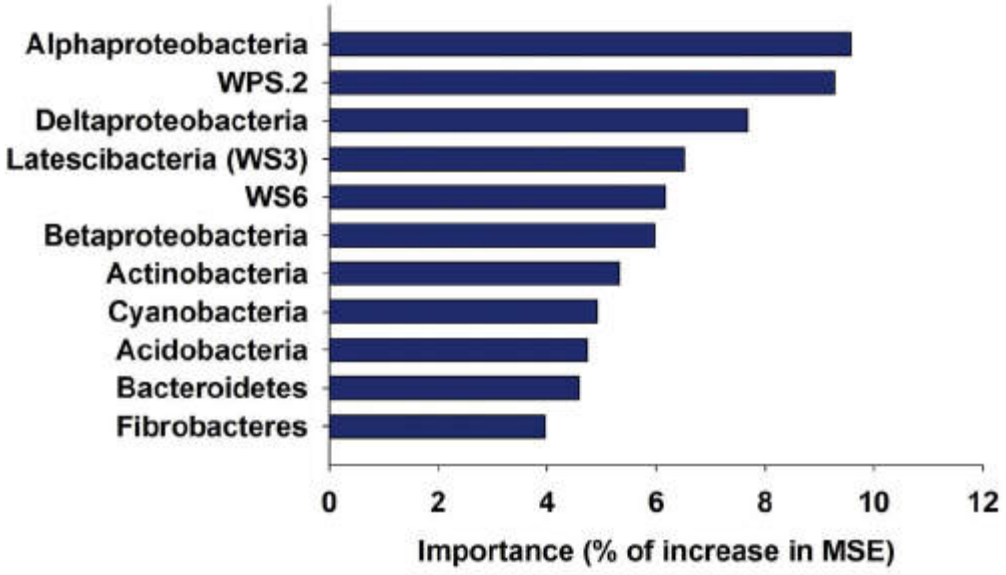

Fig. 2 Predictor importance (percentage of increase of mean square error, MSE) of major bacterial and fungal phyla/classes as drivers of soil respiration based on random forest model.

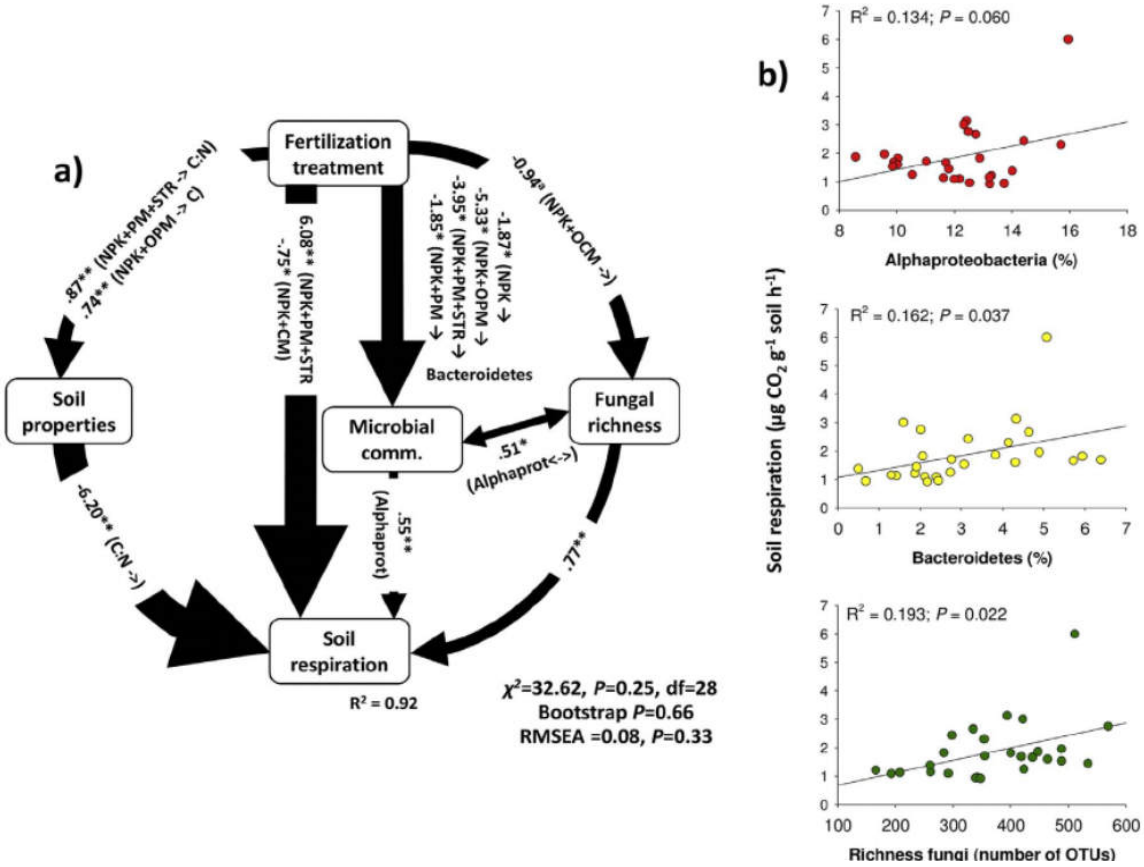

Fig. 3 (a) Structural equation modeling (SEM) showing effects of soil abiotic and biotic properties on soil respiration. Black lines and arrows indicate significant positive effect and negative effect, respectively. Numbers adjacent to arrows are path directions and coefficients, and width of the arrows is proportional to the strength of path coefficients. For simplicity, only the largest direct effects of fertilization on soil properties are shown. Minus represents negative effect of factors on soil respiration. Significance levels are as follows: $^{a}P = 0.06$, $*P < 0.05$, $**P < 0.01$; **(b)** Relationships between the major microbial parameters and soil respiration rates

Supplementary Materials

New insights into the role of microbial community composition in driving soil respiration rates

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Table S1. The quantities of nutrients yearly added to the plots (10 × 5 m in size) with different treatments. Control, no fertilization; NPK, chemical NPK fertilizers; NPK+PM, NPK fertilizers plus fresh pig manure; NPK+CM, NPK fertilizers plus fresh cow manure; NPK+ST, NPK fertilizers plus wheat straw; NPK+PM+ST, NPK fertilizers plus fresh pig manure and wheat straw; NPK+CM+ST, NPK fertilizers plus fresh cow manure and wheat straw; NPK+OPM, NPK fertilizers plus commercial organic fertilizer that is made of pig manure; NPK+OCM, NPK fertilizers plus commercial organic fertilizer that is made of cow manure.

	Control	NPK	NPK+PM	NPK+CM	NPK+STR	NPK+PM+STR	NPK+CM+STR	NPK+OPM	NPK+OCM
Total N (kg)	0.00	2.75	1.90	1.61	2.90	2.05	1.76	1.65	1.65
Total P (kg)	0.00	0.48	0.35	0.29	0.48	0.35	0.29	0.36	0.29
Total K (kg)	0.00	0.46	0.30	0.26	0.90	0.74	0.70	0.29	0.44
Total C (kg)	0.00	0.00	10.95	11.19	16.11	27.06	27.31	7.87	8.81

Figure S1. *a priori* model showing effects of soil abiotic and biotic on soil respiration