

Substrate stoichiometric regulation of microbial respiration and community dynamics across four different ecosystems

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ABSTRACT

Microbes decompose soil organic matter (SOM), yet it is unclear how substrate inputs (i.e., stoichiometry) directly mediate microbial activities and community dynamics. We hypothesized that C+N input has the largest effect on microbial respiration and community structure, followed by C input and N input. Soils were collected from four ecosystems (grassland, piñon-juniper, ponderosa pine, mixed conifer) and amended with NH_4NO_3 (N only; $100 \mu\text{g g}^{-1} \text{wk}^{-1}$), ^{13}C -glucose (C only; $1000 \mu\text{g g}^{-1} \text{wk}^{-1}$), or C+N in a five-week laboratory incubation. We found that C+N input induced the greatest total respiration while C input induced the greatest SOM-derived respiration (i.e., priming effect) across ecosystems. Shifts in community composition were the largest with C+N input, followed by C input, and showed little response to N input. C only and C+N inputs increased both of the relative and absolute abundances of Actinobacteria and Proteobacteria (α , β , γ), but reduced the relative abundances of Verrucomicrobia and δ -Proteobacteria. C+N input increased the relative abundances of Bacillales, Rhizobiales, Burkholderiales and of 9 families, and reduced the relative abundances of Myxococcales and of 12 families, but showed little effect on the absolute abundances of these bacterial taxa. N input reduced the absolute abundances of Actinobacteria, Proteobacteria, and Verrucomicrobia but did not affect their relative abundances in the mixed conifer soil; by contrast, N input reduced relative abundances of δ -Proteobacteria and increased the relative abundances of γ -Proteobacteria but did not affect their absolute abundances in the ponderosa pine soil. We also found that substrate inputs were the main driver of SOM decomposition, microbial respiration and diversity, while soil ecosystem was the main driver of community composition and abundances of most bacterial phyla. Our work suggests that substrate stoichiometry has predictable effects on soil C cycling, microbial diversity and community composition, but has variable effects on microbial abundances, and that incorporating bacterial gene copies in abundance calculations can help more accurately estimate microbial responses across taxonomic levels and ecosystems.

1. Introduction

Fresh organic matter (FOM) inputs affect the rate of microbial decomposition of soil organic matter (SOM) (Li et al., 2019; Morrissey et al., 2019; Razanamalala et al., 2018a, 2018b). These organic matter inputs can increase substrate availability that influences resource stoichiometry (i.e., carbon:nitrogen (C:N) ratios) (Kaiser et al., 2014; Manzoni et al., 2017; Waring et al., 2020), which also mediates microbial nutrient and energy demands, resulting in changes in microbial diversity and community structure (Mooshammer et al., 2014; Delgado-Baquerizo et al., 2017; Hartman et al., 2017). Substrate C:N ratios

can mediate SOM decomposition by different mechanisms. High substrate C:N ratio can induce the priming effect via microbial N mining, where microbes increase enzyme activity and SOM decomposition to alleviate N limitation (Blagodatskaya et al., 2007; Liu et al., 2017a, 2020; Pascault et al., 2013; Finley et al., 2018). Low substrate C:N ratio can also induce the priming effect by stoichiometric decomposition due to balanced microbial growth (Chen et al., 2014), or may not trigger priming owing to microbial energy limitation, where microbes cannot synthesize enzymes to decompose SOM (Blagodatskaya et al., 2007; Liu et al., 2017b, 2020; Soong et al., 2020; Zheng et al., 2019). For instance, compared to high rates of glucose addition, glucose plus N induced

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greater total respiration but lower SOM-derived respiration (Blagodatskaya et al., 2007), indicating the critical role of substrate stoichiometry in mediating microbial activities (Wild et al., 2019). Thus, microbes need to overcome imbalances between substrate inputs and microbial biomass stoichiometry (Kaiser et al., 2014; Manzoni et al., 2017; Liu et al., 2020; Soong et al., 2020).

Microbes drive soil C cycling processes, but their community responses to substrate inputs are still uncertain. Actinobacteria, Firmicutes, and β - and γ - Proteobacteria have been suggested to be phyla comprising a high proportion of fast-growing bacterial taxa that are able to decompose lower molecular weight (i.e., labile) substrates, while Acidobacteria, Chloroflexi, Gemmatimonadetes, Verrucomicrobia, and α - and δ -Proteobacteria have been suggested to be phyla comprising many SOM decomposers (Fierer et al., 2007; Eilers et al., 2010; Pascault et al., 2013; Razanamalala et al., 2018a). However, many bacteria may share co-metabolic processes (Fontaine et al., 2003). For instance, several families of β -Proteobacteria were FOM (wheat straw) decomposers, while families of α -Proteobacteria were both FOM and SOM decomposers (Bernard et al., 2012). Increased labile substrate (e.g., earthworm mucus) boosted the relative abundances of fast-growing bacterial families in Bacteroidetes and in Firmicutes that also had the capacity to degrade complex SOM (Bernard et al., 2012). These findings suggest variable microbial responses to resource availability, but it remains unclear how substrate stoichiometry directly affects microbial diversity, community composition, and gene abundances, especially across different ecosystems.

Here, to investigate the effects of substrate stoichiometry on microbial respiration, community structure, and abundances, we sampled soils from four ecosystems (grassland, piñon-juniper, ponderosa pine, and mixed conifer) that have different soil, microbial, vegetation and other climatic properties (Table 1; Liu et al., 2017a), providing a platform for comparing microbial responses across a wide range of soil physiochemical properties in a 35-day laboratory incubation. We hypothesized that: 1) C+N input stimulates the greatest respiration, followed by C input, while N input shows little effect; 2) C+N input has the greatest effect on bacteria diversity, community structure, and abundances, followed by C input and N input, and 3) substrate inputs are the main driver of microbial respiration and community dynamics compared to the effect of soil ecosystems.

2. Materials and methods

2.1. Experimental design

Soil samples were collected from four ecosystems along an elevation gradient in Arizona, USA in October 2014: cool desert grassland (1760 m), piñon-juniper woodland (2020 m), ponderosa pine forest (2340 m), and mixed conifer forest (2620 m). These soils vary in mean annual precipitation (229, 381, 660, and 787 mm), temperature (13.6, 11.4, 10.8, and 6.7 °C) (Liu et al., 2017a), and soil chemical and microbiological properties (Table 1).

Four soil cores (dia. 5.0 cm) were randomly collected (0–10 cm) from each ecosystem, independently passed through a 2-mm sieve. Subsamples were stored at 4 °C and –80 °C for microbial biomass, respiration, and subsequent molecular analyses, respectively. Additional soil samples were measured with an elemental analyzer (NC2100; Thermo

Fisher Scientific, West Palm Beach, FL, USA) for soil C and N content.

2.2. Microbial respiration

40 g soil (dry weight equivalent) was added to 125 ml specimen cups, placed in 473 ml mason jars at room temperature (22 ± 1 °C) and incubated for one week with moisture maintained at 70% water holding capacity (WHC; measured at the 233 kPa matric potential (Haubensak et al., 2002) to equilibrate the microbial communities (Liu et al., 2017a). After preincubation, we added ^{13}C -glucose solution as labile C and NH_4NO_3 as labile N to soils.

In total, four substrate treatments were implemented: non-amended control, N only ($100 \mu\text{g g}^{-1} \text{ week}^{-1}$), C only (C only; $1000 \mu\text{g C g}^{-1} \text{ week}^{-1}$), and C+N input (combination of C and N treatments), with each treatment being replicated four times. Substrates were added in a 200 μL solution volume ($\delta^{13}\text{C} = 1357\text{‰}$) weekly to 2.0 g soil for five weeks. Gas samples were collected from the headspace of the mason jars three times per week, and measured for CO_2 concentration (Li-Cor 6262, Li-Cor Inc., Lincoln, NE, USA) and for $\delta^{13}\text{C}\text{CO}_2$ (Picarro G2201, Picarro Inc., Sunnyvale, CA, USA). CO_2 was partitioned into the amount of C from native SOM and that from glucose in the glucose amended soils (C_{SOM}), and was calculated as described in prior studies (Liu et al., 2020):

$$\text{C}_{\text{SOM}} = \frac{\text{C}_{\text{total}} * (\delta_{\text{total}} - \delta_{\text{glucose}})}{\delta_{\text{control}} - \delta_{\text{glucose}}}$$

where C_{total} is the total $\text{CO}_2\text{--C}$ (mg g^{-1}) derived from glucose amended samples; δ_{total} , δ_{glucose} and δ_{control} are $\delta^{13}\text{C}$ of CO_2 from glucose-amended samples, glucose (1357‰), and from non-amended control samples (i.e., native SOM). A portion of weekly CO_2 fluxes from C and C+N inputs was used to calculate the priming effect before (Liu et al., 2020). However, weekly CO_2 fluxes from N input and CO_2 fluxes for all treatments and bacterial community data are unique to this article.

2.3. Microbial community soil incubation

Soil samples (2.0 g) were added to 15 ml falcon tubes, and a 200 μL solution of each substrate treatment (as described above) was added weekly to the tubes for five weeks ($n = 4$). Soil moisture was adjusted weekly and maintained at 70% WHC at room temperature (Liu et al., 2017a). After each week, soil samples were harvested and frozen at –80 °C. Soil DNA was extracted from 0.25 g soil using the PowerSoil DNA isolation kit (Qiagen, Germantown, MD, USA) following the manufacturer's instructions (Li et al., 2019; Morrissey et al., 2019). The extracted DNA was stored at –80 °C.

2.4. Real-time polymerase chain reaction and DNA sequencing

The 16S rRNA gene copies were determined using a BioRad CFX384 Touch Real-time detection system. Standards (10^1 to 10^9) were prepared by amplifying template DNA from the soil using 16S rRNA gene primer sets with P5 and P7 oligonucleotide tails. We used the forward primer EUB338F (5'-ACTCCTACGGGAGGCAGCAG-3') and the reverse primer EUB518R (5'-ATTACCGCGCTGG-3') (Fierer et al., 2005). qPCR was conducted in triplicate in 10 μL reactions that contained 1X Forget-Me-Not Master Mix (Biotium), 0.2 μM of each primer, and 1 μL of DNA template. Thermal cycler conditions were: initial denaturation for

Table 1
Soil chemical and microbiological properties of four ecosystems along an elevation gradient in Flagstaff, AZ, USA[§].

Ecosystems	SOC (%)	Soil N (%)	Soil C:N ratio	MBC ($\mu\text{g g}^{-1}$)	MBN ($\mu\text{g g}^{-1}$)	MBC:MBN ratio	pH	Soil type
Grassland	1.6	0.13	12.2	152	10.5	14.5	6.9	Typic Haplustoll
Pinon-juniper	1.6	0.13	11.9	142	10.8	6.9	6.2	Calcic Haplustand
Ponderosa pine	1.7	0.10	17.2	129	9.0	14.2	5.8	Typic Argiboroll
Mixed conifer	4.4	0.33	13.2	254	38.8	6.5	6.3	Pachic Udic Argiboroll

[§]: MBC:MBN = microbial biomass C:N ratio; Soil type and pH data are from Dijkstra et al. (2006) and Liu et al. (2017a).

2 min at 95 °C, 40 cycles at 95 °C for 15 s, 59 °C for 30 s, and 72 °C for 30 s, followed by a melting curve of 0.5 °C steps between 55 and 95 °C (30 s each step).

Soil DNA was prepared for amplicon sequencing with a two-step PCR protocol (Hayer et al., 2016). First, sample amplification was performed in triplicate, in 10 µl reactions with the Earth Microbiome Primer set (515F/806R) targeting the V4 region of the 16S rRNA gene: 1.0 µL of DNA template, 1X Phusion Green Hot Start High-Fidelity PCR Master Mix (Thermo Scientific), and 0.2 µM of each primer, with the following PCR conditions: initial denaturation at 95 °C for 2 min followed by 15 cycles at 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 1 min. Triplicate PCR products were pooled, checked for quality on a 1% agarose gel, 10-fold diluted, and used as the template for subsequent tailing reactions. PCR conditions for the tailing reactions were the same as described above, though the cycles were repeated 20 times, and the 515F and 806R primers were included the Illumina adapter P5/P7 sequences and 12-nucleotide barcodes. Products of the tailing reaction were purified with SeraMag Speed Beads (Sigma-Aldrich, St. Louis, MO, USA) at 1:1 ratio (v/v), and quantified by PicoGreen fluorescence assay (Invitrogen, Carlsbad, CA, USA). Equal concentrations of the reaction products were then pooled, bead-purified, and quantified by qPCR using the Library Quantification Kit from Illumina. Libraries were sequenced using an Illumina MiSeq and a V2 300-cycle kit (2 × 150 paired-end reads).

2.5. Bacterial community data processing

Raw DNA sequences were analyzed using QIIME (v1.8) (Caporaso et al., 2010). Open reference picking was used to cluster OTUs with a cutoff value of 97% identity with the UCLUST algorithm, and aligned with PyNAST against the Greengenes v13.8 database to assign bacterial taxonomy. Any OTUs that accounted for less than 0.05% of the total sequences were removed, and all data were rarefied (5000 sequences per sample) so that sequencing biases did not affect diversity comparisons (Hayer et al., 2016; Li et al., 2019). The raw sequencing data were deposited in the MG-RAST database (<https://www.mg-rast.org/linkin.cgi?project=mgp81835>). To more accurately compare the effects of substrate inputs on relative and absolute abundances, raw OTUs were filtered to keep only those with assigned bacterial families (Morrissey et al., 2019). Relative bacterial abundances were calculated from OTUs across taxa within the same sample, and absolute abundances were calculated by multiplying taxa relative abundances by the total 16S rRNA gene copies of individual samples.

2.6. Data analysis

Statistical analyses were conducted in R (R Core Team, 2020) using the “vegan” package (Oksanen et al., 2019). Bray-Curtis dissimilarities were calculated with square-root transformed relative abundances of OTUs. We used Shannon’s diversity index to measure community evenness and richness. Three-way ANOVA (substrates, soils, incubation time) was conducted to determine treatment effects on relative and absolute abundances and diversity of bacterial communities, and a repeated measures three-way ANOVA was used for respiration, followed by a posthoc test for multiple comparisons (glht) to adjust resulting p-values (Benjamini-Hochberg method) (Liu et al., 2021a). The variance of respiration explained by bacterial taxa was calculated from the output of the three-way repeated ANOVA for respiration and three-way ANOVA for bacterial community data in R, where the sum of squares of individual phyla were divided by the total sum of squares, and converted to percentages (Liu et al., 2021b). Permutational multivariate analysis of variance (Permanova) and homogeneity of variances for bacterial communities were tested in R with the functions of *adonis* and *betadis* with $P \leq 0.05$.

3. Results

3.1. Substrate inputs affected microbial respiration, diversity and community composition

Total microbial respiration increased with C and C+N inputs but the sources of respired C varied among the substrate treatments (Figs. 1 and S1A). Compared to the control, C+N input increased the total microbial respiration, of which 92.6% was derived from glucose and 7.4% from the native SOM (Fig. 1). The C input increased total microbial respiration, and respiration derived from SOM and glucose. In general, the amounts of total respiration were similar for C+N input and C input, except respiration was greater for C+N input in the first week for the grassland and piñon-juniper soils. Yet, C input induced greater SOM-derived respiration and less glucose-derived respiration compared to the C+N input (Fig. 1). Both of C and C+N inputs also increased bacterial gene copies, with the greatest gene copies being observed in the mixed conifer soil (Fig. S2); N input reduced gene copies in the mixed conifer soil for the first and last weeks of incubation.

Soil bacterial diversity showed limited response to the substrate inputs (Fig. S1B). Compared to the control, bacterial diversity decreased with C+N input in the second week of the grassland soil and in the last week of the piñon-juniper and ponderosa pine soils, driven by decreased bacterial richness and evenness (Fig. S3). C input and N input showed little effect on bacterial diversity, except that N input reduced bacterial diversity in the last week of the piñon-juniper soil.

Substrate inputs significantly altered bacterial community composition across the four soil ecosystems (Fig. 2). Compared to the control, C+N input caused the greatest shifts in bacterial community composition, followed by C input, while N input showed little effect. The degree of changes in bacterial community composition increased over time, with the greatest shifts of community composition being observed in the last two weeks of incubation.

3.2. Variable substrate effects on bacterial relative and absolute abundances

Substrate inputs induced different effects on bacterial abundances; more so on relative than on absolute abundances. At the phylum level, C and C+N inputs increased the relative abundances of Actinobacteria and Proteobacteria, but reduced the relative abundances of Chloroflexi and Verrucomicrobia (Fig. 3). C+N input also increased the absolute abundances of Actinobacteria and Proteobacteria, and reduced the absolute abundances of Verrucomicrobia in the mixed conifer soil. N input increased the relative abundances of Actinobacteria in the piñon-juniper soil but reduced the absolute abundances of Proteobacteria and Verrucomicrobia in the mixed conifer soil. In general, bacterial relative abundances were similar among soils, while bacterial absolute abundances were the greatest in the mixed conifer soil (Figs. 3 and S4). Relative abundances of Actinobacteria and Proteobacteria increased over time, while their absolute abundances showed little temporal change (Figs. 4 and S5).

At the class level, C and C+N inputs increased the relative abundances of α -Proteobacteria, β -Proteobacteria, γ -Proteobacteria, but reduced the relative abundances of δ -Proteobacteria and Planctomycetacia in most soils (Figs. 3 and S4). N input increased the relative abundances of γ -Proteobacteria in the ponderosa pine soil but reduced the relative abundances of δ -Proteobacteria in the piñon-juniper and ponderosa pine soils. C input increased the absolute abundances of α -Proteobacteria in the piñon-juniper soil, γ -Proteobacteria in the ponderosa pine soil, and Thermoleophilia in the grassland soil; C+N input increased the absolute abundances of α -Proteobacteria in the piñon-juniper and ponderosa pine soils, β -Proteobacteria and γ -Proteobacteria in the ponderosa pine and mixed conifer soils; while N input reduced the absolute abundances of α -Proteobacteria in the mixed conifer soil. Relative abundances of γ -Proteobacteria and Thermoleophilia increased

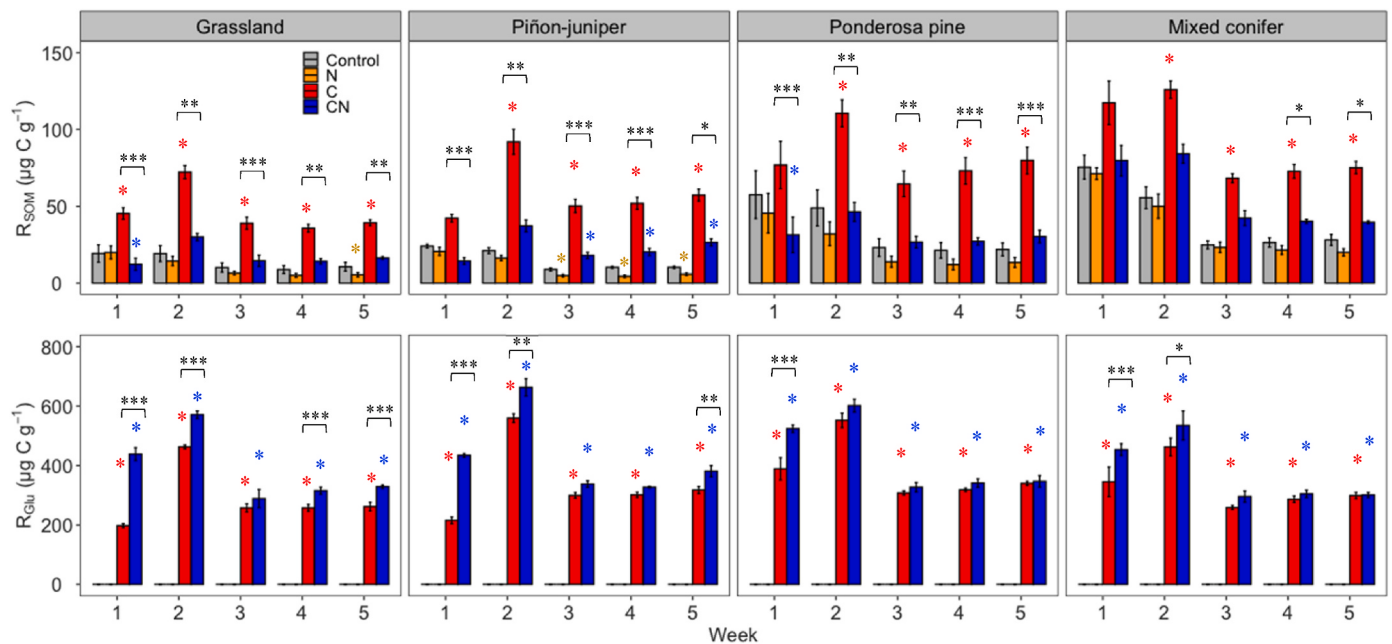


Fig. 1. Microbial respiration derived from soil organic matter (SOM) and from glucose (mean $\pm$ s. e.m.) in response to substrate inputs. Control = no amendment, N = $100 \mu\text{g N g}^{-1} \text{ week}^{-1}$, C = $1000 \mu\text{g C g}^{-1} \text{ week}^{-1}$, CN = both N and C inputs with C:N ratio at 10. P values are obtained from two-way repeated ANOVA. Orange, red and blue asterisks indicate significant differences compared to control, and black asterisks indicate differences between substrate C and C+N inputs. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

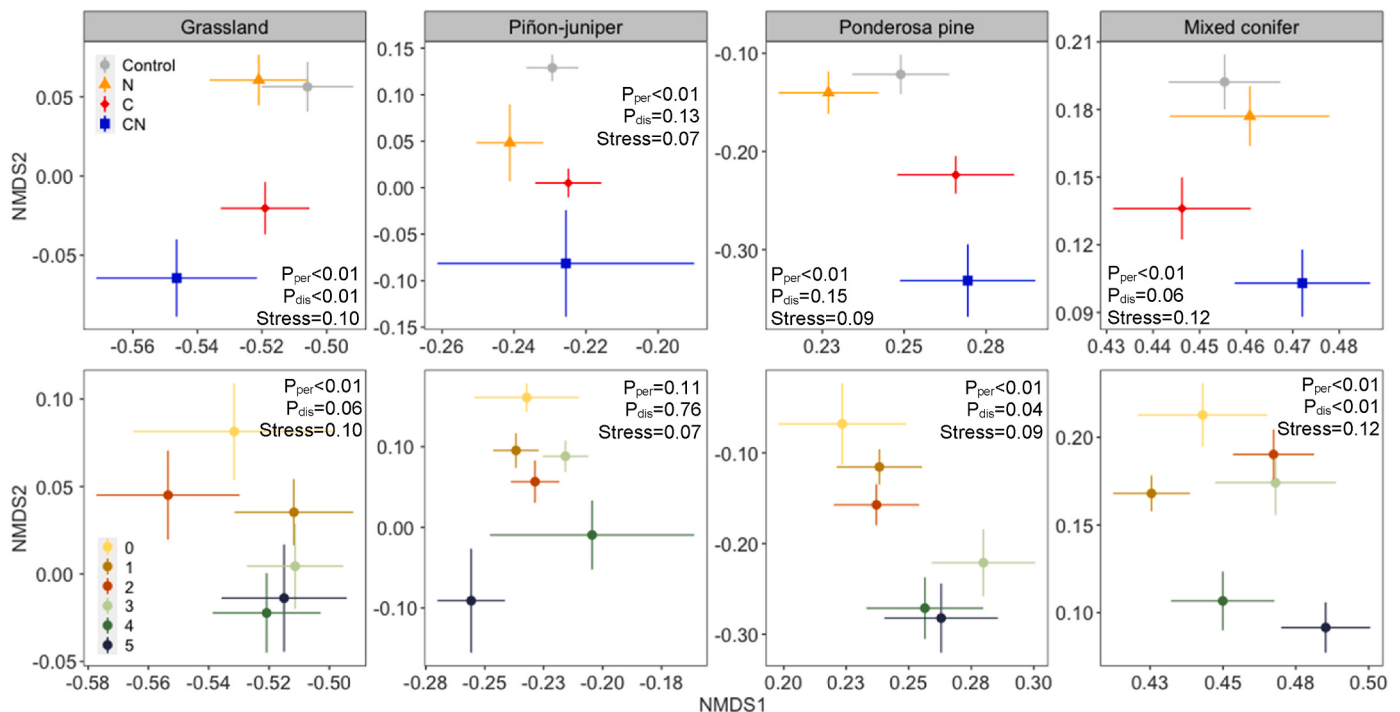


Fig. 2. Microbial community composition mediated by substrate stoichiometry in a 5-week incubation across four ecosystems (Bray-Curtis distance matrix; mean $\pm$ s. e.m.). Control = no amendment, N = $100 \mu\text{g N g}^{-1} \text{ week}^{-1}$, C = $1000 \mu\text{g C g}^{-1} \text{ week}^{-1}$, CN = both N and C inputs with C:N ratio at 10. P values were obtained from PERMANOVA test. P values (P_{per} , P_{dis}) indicate tests from Permanova (adonis) and Dispersion (permutest). Values are averaged across either weeks (upper panel) or substrate treatments (lower panel).

over time in most soils, while the absolute abundances of α -Proteobacteria, Planctomycetacia, and Thermoleophilia decreased over time in the mixed conifer soil (Figs. 4 and S5).

At the order level, C and C+N inputs increased the relative abundances of Bacillales, Burkholderiales, Rhizobiales, Rhodospirillales,

Sphingomonadales, and Xanthomonadales, but reduced the relative abundances of Gemmatiales, Myxococcales and Nitrospirales in most soils (Figs. 5 and S6). N input increased the relative abundances of Sphingomonadales and Xanthomonadales in the ponderosa pine soil, and reduced the relative abundances of Myxococcales in the piñon-

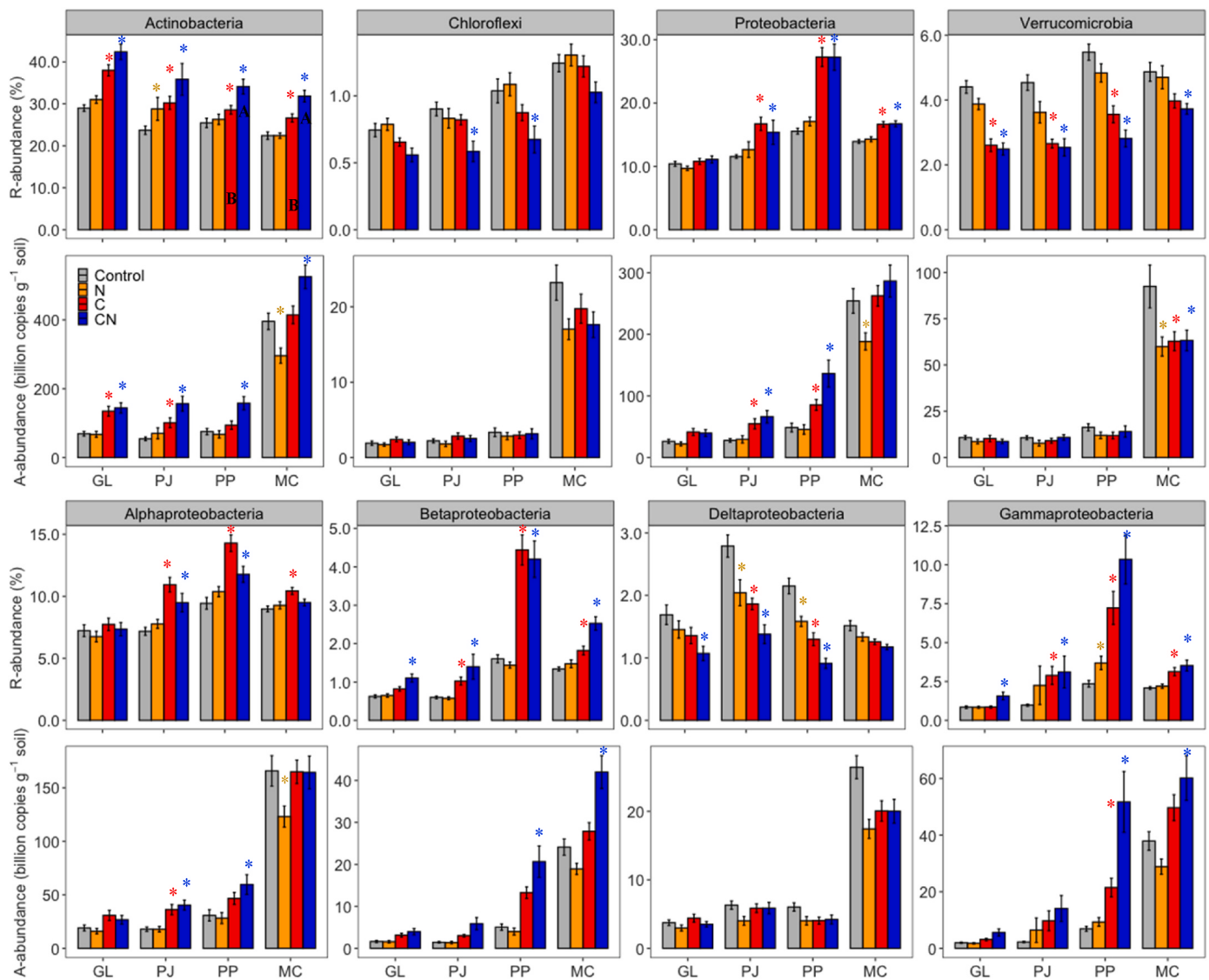


Fig. 3. Relative and absolute abundances at the phylum and class levels as affected by substrate stoichiometry in different soil ecosystems (mean $\pm$ s.e.m.). Control = no amendment, N = $100 \mu\text{g N g}^{-1} \text{ week}^{-1}$, C = $1000 \mu\text{g C g}^{-1} \text{ week}^{-1}$, CN = both N and C inputs with C:N ratio at 10. P values are obtained from two-way ANOVA. Asterisks indicate significant differences against the control, and different letters indicate significant differences between C and C+N inputs. R- and A- abundance indicate relative and absolute abundances. GL, PJ, PP, and MC represent grassland, piñon-juniper, ponderosa pine, and mixed conifer soils. Values are averaged across incubation weeks.

juniper and ponderosa pine soils. However, substrate inputs showed little effect on the absolute abundances of most bacterial orders. Relative abundances of Bacillaceae and Gemmatales decreased, while relative abundances of Rhizobiales and Burkholderiales increased over time; however, the absolute abundances of Sphingomonadales decreased over time (Figs. S7 and S8).

At the family level, C and C+N inputs caused more families to increase in abundances than to decrease, whether expressed in relative or absolute terms (Figs. 5 and S9-S13). With N input, the relative abundances of 8 families increased and that of Haliangiaceae decreased; the absolute abundances of Paenibacillaceae increased while that of 2 families decreased. With C input, the relative abundances of 22 families increased but that of 10 families decreased; the absolute abundances of 8 families increased but that of 2 families decreased. With C+N input, the relative abundances of 21 families increased and that of 12 families decreased; the absolute abundances of 9 families increased but that of 4 families decreased. Relative abundances of 13 families increased while that of 3 families decreased over time; however, absolute abundances of most bacterial families showed little temporal change (Figs. S14-S18).

3.3. Drivers of microbial respiration and communities

Substrate inputs were the main driver for soil microbial respiration and bacterial diversity while ecosystems were the main driver of community composition and bacterial gene copies. Across the four soil ecosystems, labile substrate inputs explained more than 80% of the variances in total microbial respiration and in the glucose-derived respiration, but explained only 40% of the variance in the SOM-derived respiration (Tables 2 and S1). Furthermore, substrate inputs explained more than 40% of variances for bacterial diversity and evenness in the grassland and ponderosa pine soils while they explained little variance in the mixed conifer soil (Tables S2 and S3). Lastly, soil ecosystems explained more than 70% of the variance in bacterial gene copies, except that in the mixed conifer soil where more than 60% of variance was explained by the incubation time.

Substrate inputs explained more variance, while soils explained less variance, in relative abundances than in absolute abundances for most of the bacterial taxa. For example, labile substrate inputs explained more than 20% of the variance in the relative abundances of Actinobacteria

and δ -Proteobacteria but explained less than 3% of the variance of their absolute abundances (Table 2). By contrast, soil ecosystems explained less than 20% of variance in the relative abundances of the Actinobacteria and δ -Proteobacteria, but explained nearly 70% of variance in their absolute abundances. Besides, variances of relative abundances and absolute abundances were mostly explained by soil ecosystems, except that the variances of relative abundances of Actinobacteria, δ -Proteobacteria and Verrucomicrobia were mostly explained by the substrate inputs (Tables 2 and S1). For individual soils, substrate inputs explained more variances in bacterial relative abundances, while incubation time explained more variances in the absolute abundances of most bacterial phyla (Tables S2 and S3).

4. Discussion

4.1. Substrate stoichiometry mediates microbial respiration and SOM decomposition

Labile C input accelerated SOM-derived respiration by 164% ($43.2 \mu\text{g g}^{-1} \text{ week}$) over the control, while C+N input increased SOM-derived respiration by 24% (Fig. 1), suggesting a strong microbial C priming effect as reported previously (Craine et al., 2007; Liu et al., 2017a, 2020; Wild et al., 2019). This priming effect was likely induced by the N mining strategy (Craine et al., 2007; Liu et al., 2017b), resulting from stoichiometric imbalances in C and N availability, when microbes harvest energy in the added labile C to produce enzymes to acquire N (Blagodatskaya et al., 2007; Finley et al., 2018; Liu et al., 2020). The C input induced the largest SOM decomposition in the first and second

weeks, supporting a priming saturation response to C input and that microbes might need a short period to adapt to high C availability and reduced N availability (Blagodatskaya et al., 2007; Chen et al., 2014; Liu et al., 2020).

Addition of C+N increased glucose-derived respiration but showed little effect on SOM-derived respiration (Fig. 1), suggesting that substrate stoichiometry mediates the microbial SOM decomposition and the priming effect (Blagodatskaya et al., 2007; Ramirez et al., 2012; Di Lonardo et al., 2017; Liu et al., 2020). Compared to the C input, the C+N input reduced SOM decomposition, possibly due to the alleviation of nutrient limitation (Chen et al., 2014; Liu et al., 2020). We also found that soil respiration derived from glucose was greater than respiration derived from SOM (Fig. 1), indicating flexible C use strategies of microbial taxa (Morrissey et al., 2017) where microbes either switched substrate utilization from SOM to glucose (Blagodatskaya et al., 2007; Guenet et al., 2010; Liu et al., 2017a, 2020) or continue to use both SOM and the added labile substrate (Fontaine et al., 2003; Chen et al., 2014). However, N only addition reduced respiration at the end of the incubation, possibly associated with inhibition of microbial enzyme activities and increasing recalcitrance of SOM over time (Fog, 1988; Chen et al., 2014), causing a negative feedback on microbial respiration (DeForest et al., 2004; Frey et al., 2004; Treseder, 2008; Sinsabaugh, 2010).

4.2. Substrate inputs alters microbial community composition but not diversity

Substrate inputs changed bacterial community composition but

Table 2

Variance in microbial respiration, bacterial community composition, diversity, and abundances (phylum level) explained by soil ecosystems, substrate inputs, and incubation time[§].

Parameter	Type	S	T	W	S:T	S:W	T:W	S:T:W	Total
Respiration	Total	0.4	83	6.8	0.6	0.6	6.0	0.5	98
Bacterial composition	NMDS1	97	0.0	0.0	0.1	0.1	0.1	0.4	98
	NMDS2	54	11	7.6	1.6	2.2	1.8	2.2	80
Bacterial diversity	Shannon	4.6	11	3.9	5.4	15.5	4.1	8.6	53
Phylum	Abun								
Acidobacteria	R	47	4.1	2.7	1.5	6.3	2.4	4.1	68
	A	68	0.3	1.0	0.3	0.7	0.0	0.0	70
Actinobacteria	R	14	24	23	1.6	4.1	3.6	3.1	73
	A	70	2.5	0.6	0.2	0.3	0.1	0.1	74
Bacteroidetes	R	5.9	0.9	5.1	3.6	9.2	7.8	21	53
	A	55	0.7	1.4	0.2	0.7	0.1	0.1	59
Firmicutes	R	18	2.7	20	5.3	14	4.4	8.1	72
	A	37	0.1	1.8	0.1	1.3	0.2	0.1	40
Planctomycetes	R	25	16	20	2.6	2.7	2.0	5.5	74
	A	71	0.2	1.5	0.1	0.7	0.0	0.0	74
α -Proteobacteria	R	30	15	1.1	6.0	7.0	4.6	5.6	69
	A	71	0.8	1.0	0.1	0.5	0.0	0.0	74
β -Proteobacteria	R	38	18	2.6	14	4.5	3.0	5.3	84
	A	59	3.6	0.4	0.6	0.4	0.1	0.1	64
δ -Proteobacteria	R	17	22	5.0	5.4	4.8	4.7	3.6	63
	A	73	0.8	0.7	0.3	0.3	0.0	0.0	75
γ -Proteobacteria	R	24	11	8.0	11	5.8	4.8	7.3	71
	A	40	3.7	0.6	0.7	0.5	0.3	0.2	46

§: Abun indicates relative abundances (R) or absolute abundances (A). S, soil; T, substrate treatment; W, week. Bold numbers indicate the most variances explained by treatment factors, while numbers highlighted by orange and blue indicate high and low variances between relative abundances and absolute abundances.

showed little effect on microbial diversity (Figs. 2 and S1B), suggesting variable responses of microbial communities to substrate stoichiometry. C+N input caused the greatest shifts in community composition across the four soil ecosystems, followed by the C only input, while N only input showed little effect, indicating the important role of substrate stoichiometry in regulating competitions and/or predations among different microbial communities (Razanamalala et al., 2018a; Li et al., 2019). These findings demonstrate predictable changes in microbial community composition in response to substrate inputs over time, regardless of variations among soil ecosystems.

4.3. Variable responses of relative and absolute abundances to substrate inputs

Bacterial relative and absolute abundances showed different responses to substrate inputs (Fig. 3), suggesting the important role of bacterial gene copies in estimating microbial abundances. Our findings show that the numbers of bacterial taxa with changes in relative abundances were greater than the numbers of taxa with changes in absolute abundances. Here we show that the absolute abundances of bacteria

were greater in the mixed conifer soil than all other soils, and that such differences were absent in the relative abundances (Figs. 3–5; S4, S6, S9–S13). This suggests that in studies only looking at relative abundances, severe biases in overestimating substrate treatment effects and underestimating ecosystem effects may occur. Therefore, it is critical for the field to more accurately estimate microbial abundances by incorporating bacterial gene copies into measurements of bacterial composition.

4.4. Variable bacterial life strategies among soil ecosystems

Our findings suggest that most bacterial taxa cannot be clearly defined as copiotrophs or oligotrophs, based on how their bacterial absolute abundances respond to substrate inputs. For instance, N input increased the absolute abundances of Paenibacillaceae (Firmicutes) in ponderosa pine and mixed conifer soils, but reduced the absolute abundances of Actinobacteria (Thermoleophilina), Proteobacteria (α -), and Verrucomicrobia in the mixed conifer soil (Figs. 3, 5, S4), suggesting that although N addition may alleviate the cost of nutrient stress and increase microbial growth (Hessen et al., 2004; Li et al., 2019), it can

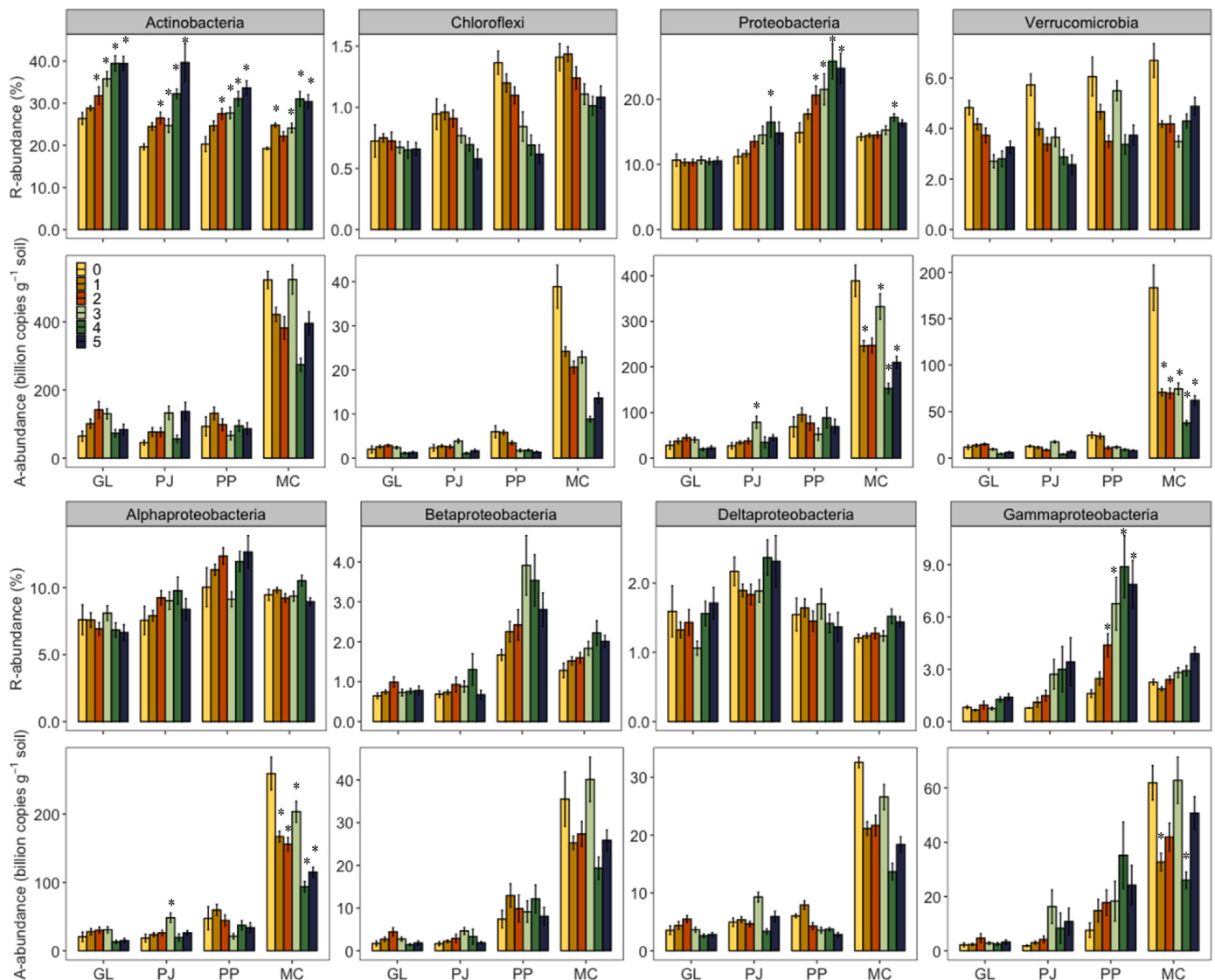


Fig. 4. Relative and absolute abundances at the phylum and class levels as affected by incubation time (weeks) in different soil ecosystems over time (mean $\pm$ s. e. m.). Asterisks indicate significant differences against the week 0 based on P values obtained from two-way ANOVA. R- and A- abundance indicate relative and absolute abundances. GL, PJ, PP, and MC represent grassland, piñon-juniper, ponderosa pine, and mixed conifer soils. Values are averaged across substrate treatments.

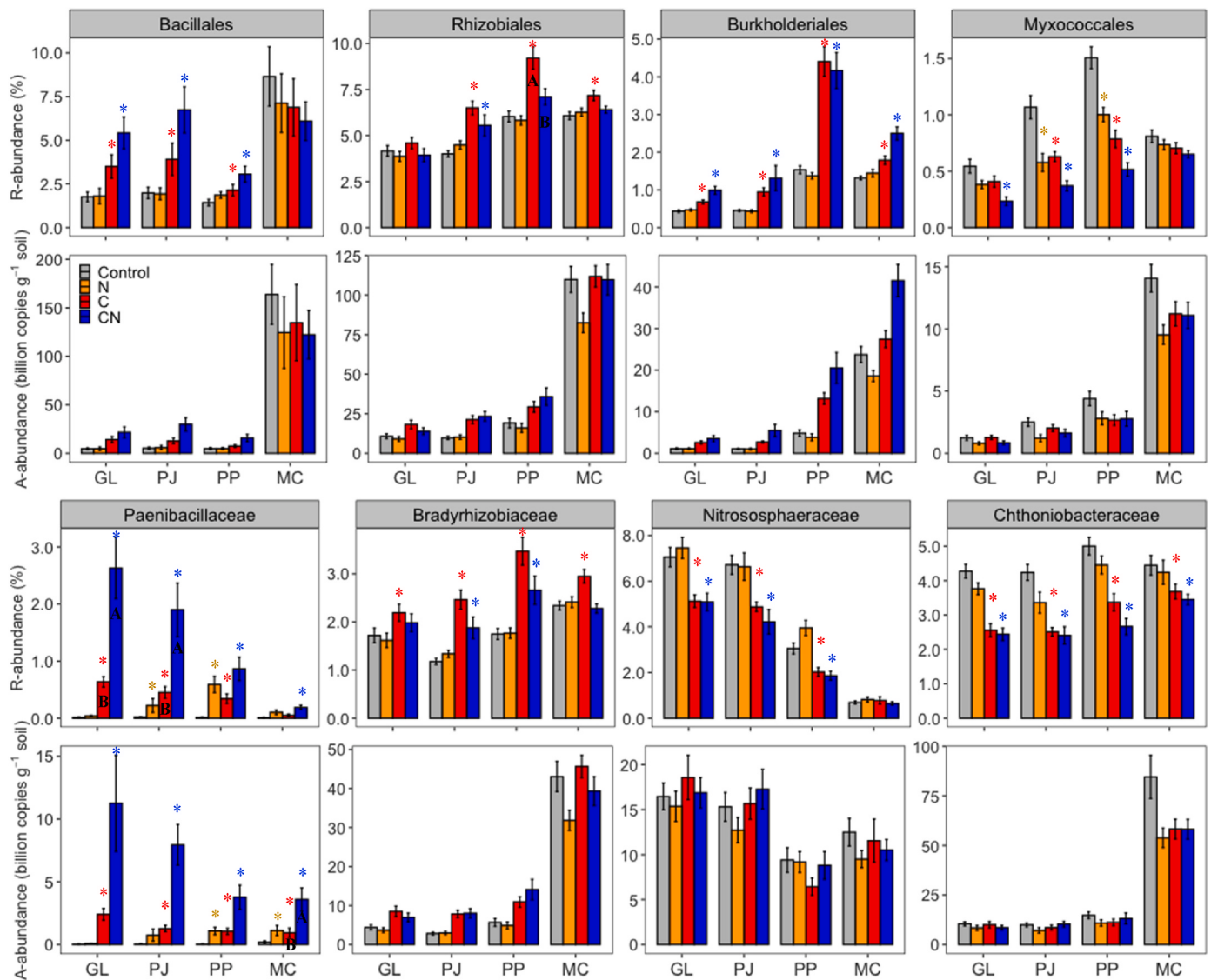


Fig. 5. Relative and absolute abundances at the order and family levels as affected by substrate stoichiometry in different soil ecosystems (mean $\pm$ s. e.m.). Control = no amendment, N = 100 $\mu\text{g N g}^{-1} \text{ week}^{-1}$, C = 1000 $\mu\text{g C g}^{-1} \text{ week}^{-1}$, CN = both N and C inputs with C:N ratio at 10. P values are obtained from two-way ANOVA. Asterisks indicate significant differences against the control, and different letters indicate significant differences between C and C+N inputs. R- and A- abundance indicate relative and absolute abundances. GL, PJ, PP, and MC represent grassland, piñon-juniper, ponderosa pine, and mixed conifer soils. Values are averaged across incubation weeks.

also reduce microbial responses in others, possibly mediated by soil nutrients status (Liu et al., 2017a; Morrissey et al., 2019).

Unlike the limited effect of N input, C and C+N inputs increased the absolute abundances of bacteria in two orders (Actinobacteria, Proteobacteria), three classes (α -, β -, γ - Proteobacteria), and 7 families (Acidobacteriaceae, Paenibacillaceae, Phyllobacteriaceae, Xanthobacteraceae, Xanthomonadaceae, Pseudomonadaceae) (Figs. 3, 5, S9, S11–12), suggesting that these bacterial taxa may have aspects of their life strategies that might be described as more copiotrophic. Partially consistent with our findings, prior studies showed that labile substrate additions increased relative abundances of Actinobacteria, α -Proteobacteria and β -Proteobacteria and γ -Proteobacteria, but did not affect the relative abundances of Acidobacteria (Eilers et al., 2010; Dungait et al., 2013). Actinobacteria and Proteobacteria are known to degrade both labile and recalcitrant compounds, such as lignin (Bernard et al., 2007; Davis et al., 2011; Di Lorenzo et al., 2017), consistent with the accelerated SOM decomposition (i.e., priming effect) in our study. We observed increasing abundances of Acidobacteriaceae following substrate inputs (Fig. S9), supporting the idea that Acidobacteria have the ability to utilize labile substrates, implying that this

phylum either can compete with other fast-growing bacteria for labile C (Di Lorenzo et al., 2017), or benefit through enzymes produced by fast-growing bacteria to break down labile C, resulting in efficient degradation of SOM (Fontaine et al., 2003). However, other studies showed that C input reduced relative abundances of Acidobacteria but did not affect relative abundances of α -Proteobacteria, Firmicutes, and Actinobacteria (Fierer et al., 2007), which might be due to inaccurate estimation of microbial abundances using the relative abundances that did not include responses of bacterial gene copies.

However, C and C+N inputs reduced the absolute abundances of Verrucomicrobia (Verrucomicrobiaceae) and SBR1301 (Chloroflexi), and Polyangiaceae (δ -Proteobacteria) (Figs. S6, S11, S13), consistent with the idea that most Verrucomicrobia are oligotrophic and thus less competitive with increased labile substrates compared to copiotrophs (Hibbing et al., 2010; Eilers et al., 2012; Li et al., 2019). Glucose addition was reported to increase relative abundances of Verrucomicrobia, Nocardioideae, and Streptomycetaceae and to reduce the relative abundances of Bacilli (Morrissey et al., 2017), but we found that only Streptomycetaceae increased in absolute abundances with substrate

inputs. These discrepancies in microbial abundances might reflect biases in relative abundance estimates (when missing bacterial gene responses), but could also reflect real differences in microbial strategies in different ecosystems.

4.5. Respiration and microbial communities are driven by different factors

Substrate inputs were the main driver for microbial respiration across ecosystems, and were the main driver of bacterial diversity in three of the four soils (Tables 2 and S2), suggesting a consistent and predictable role of substrate stoichiometry in mediating microbial activities in diverse communities. Substrate inputs explained nearly 90% of total respiration and glucose-derived respiration across ecosystems, suggesting microbial activities are mainly mediated by microbial preferential utilization of added labile C. However, respiration derived from SOM was explained less by substrate inputs but more by incubation time (Table S1), indicating the important roles of microbial community changes in substrate utilization strategy and nutrients and energy tradeoffs among microbes over time (Blagodatskaya et al., 2007; Liu et al., 2020). Substrate inputs explained nearly 50% of variance in bacterial diversity in grassland and ponderosa pine soils (Table S2), but explained little in the mixed conifer soil, suggesting that higher soil nutrients and microbial biomass N can increase the resilience of microbial communities to changes in diversity (Razanamalala et al., 2018a). In other words, microbial diversity might change more dramatically in soils with lower nutrients and microbial biomass, supporting the microbial N mining strategy (Liu et al., 2017a; Razanamalala et al., 2018b; Hicks et al., 2019).

Soil ecosystems, but not substrate inputs, explained most of the variances in bacterial community composition and gene abundances (Tables 2 and S1), suggesting a more important role of soil environment than substrate inputs in mediating microbial community composition (Fierer et al., 2012; Li et al., 2019; Morrissey et al., 2019). Substrate inputs explained 20–40% of variance in gene abundances of Actinobacteria, Firmicutes, α -Proteobacteria, β -Proteobacteria, and γ -Proteobacteria, depending on soil ecosystems (Table S2), indicating the importance of considering microbial responses defined by specific ecosystems. Incubation time also explained large variances of gene abundances of bacterial phyla in different soils, suggesting dynamic and complex temporal responses of microbial communities. For example, incubation time explained nearly 60% of variance in gene abundances of Acidobacteria, but only less than 40% of the variance in gene abundances of Actinobacteria, suggesting different life strategies between microbial communities. In general, our findings indicate that predicting bacterial gene abundances is dependent on substrate stoichiometry but can be hampered by variations among soil ecosystems.

4.6. Limitations of the study

Our main objective was to assess how substrate stoichiometry affects microbial respiration, community structure, and abundances, and whether these effects are consistent across the four soil ecosystems. Despite variable climatic and plant parameters, these ecosystems have limited differences in soil characteristics. For instance, soil pH can dramatically alter microbial richness, diversity, and community structure (Fierer and Jackson, 2006). Because the pH values in the four ecosystems have a relatively narrow range (5.8–6.9), microbial activities and community responses to substrate inputs could be different in soil ecosystems that have pH values outside of the pH range of our study. Hence, interpretation of the findings in this study should be cautious, given that soil properties play an important role in controlling microbial community structure and soil C cycling, though it seems that priming effect is an universal response to substrate inputs across five continents (Liu et al., 2017a; Perveen et al., 2019).

5. Conclusions

Our work shows that substrate inputs are the main driver of SOM decomposition, and that microbes shift to utilize more labile C when additional N is applied. However, soil ecosystems are the main driver of bacterial community composition, and substrate inputs are the main driver within individual ecosystems, with the greatest shifts of community composition being observed with the C+N input, followed by the C input, but showed little response to the N input. However, substrate inputs showed variable effects on relative abundances and absolute abundances of bacteria at different taxonomic levels among soil ecosystems. Our research suggests predictable responses of SOM decomposition and microbial community composition and abundances to substrate inputs, and suggests the urgent need to include gene copies to more accurately estimate microbial abundances (i.e., absolute abundances) across taxonomic levels and ecosystems.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix B. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2021.108458>.

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