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Mycorrhiza - saprotroph interactions and carbon cycling in the rhizosphere

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Keywords:	Microbial traits, Microbial biomass, Mycorrhiza, Stable isotope probing, Rhizosphere bacteria, Root exudate, Soil carbon, Temperate forest
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Abstract

Labile carbon (C) inputs in soils are expected to increase in the future due to global change drivers such as elevated atmospheric CO_2 concentrations or warming and potential increases in plant primary productivity. However, the role of mycorrhizal association in modulating microbial activity and soil organic matter (SOM) biogeochemistry responses to increasing below-ground C inputs remains unclear. We employed $^{18}O–H_2O$ quantitative stable isotope probing to investigate the effects of synthetic root exudate addition (0, 250, 500, and 1000 μg C g soil^{-1}) on microbial growth traits and SOM biogeochemistry in rhizosphere soils of trees associated with arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) fungi. Soil respiration increased proportionally to the amount of exudate addition in both AM and ECM soils. However, microbial biomass C (MBC) responses differed, increasing in AM and decreasing in ECM soils. In AM soils, exudate addition increased taxon-specific and community-wide relative growth rates leading to enhanced production of MBC. Conversely, in ECM soils, relative growth rates were less responsive to exudate addition and estimates of MBC mortality increased with increasing exudate addition. In the AM soils, aggregated microbial growth traits were predictive of soil respiration but this relationship was not observed in ECM soils, perhaps due to substantial MBC mortality. These findings highlight the distinct responses of rhizosphere microbial communities to exudates in AM and ECM-associated trees. Considering that microbial products contribute to the formation of stable soil organic carbon (SOC) pools, future increases in labile exudate release in response to global change may consequently lead to greater SOC gains in AM soils compared ECM soils.

Keywords: Microbial traits; Microbial biomass; Mycorrhiza; Stable isotope probing; Rhizosphere bacteria; Root exudate; Soil carbon; Temperate forest

1 INTRODUCTION

Interactions between plants, mycorrhiza, and soil microbes in the rhizosphere are a dominant control on the response of soil organic carbon (SOC) and net primary production to global change in temperate forest ecosystems (Terrer et al., 2021). Global change drivers, including elevated CO_2 or warming, often increase the exudation of labile C from fine roots (Phillips et al., 2011; Yin et al., 2013). However, there is uncertainty in the extent to which shifts in rates of root exudation either prime (Cheng et al., 2014) or stabilize soil organic C and nitrogen (N) (Ridgeway et al., 2024). At the center of this uncertainty is a lack of understanding regarding how increases in root exudation in response to global change alter taxon-specific and community-level microbial function.

In the temperate forest ecosystem, mycorrhiza colonize the interface between plant roots and soil, effectively regulating the crucial entry point for plant C into the rhizosphere. Arbuscular mycorrhizal (AM) and ectomycorrhizal (ECM) are the two most dominant mycorrhizal types associated with the majority of terrestrial plant species (Brundrett, 2009; Bonfante and Genre, 2010). Root exudation rates, and the ability of roots to ramp up exudation rates in response to global change, varies between AM and ECM tree species (Phillips and Fahey, 2006; Phillips et al., 2013; Brzostek et al., 2015). Further, AM and ECM fungi have distinct life strategies with respect to resource acquisition and allocation (Aerts, 2003; Phillips et al., 2013) that alter their interactions with other soil microbes. AM fungi rely on saprotrophs for decomposition and nutrient release (Herman et al., 2012), and as such AM tree species may foster cooperative rhizospheres. Indeed, AM hyphae have been shown to act as conduits for photosynthate C that can feed saprotrophic microbial communities (Kaiser et al., 2015; Paterson et al., 2016; Frey, 2019). In contrast, ECM

rhizospheres may foster competitive rhizospheres because ECM fungi possess the capability to produce extracellular enzymes to mine organic N (Martin et al., 2016; Chen et al., 2018) and may suppressing the growth of saprotrophs by enhancing N limitation (Gadgil and Gadgil, 1975; Averill and Hawkes, 2016). These prior studies suggest a dichotomy wherein AM fungi cooperate, while ECM compete, with soil saprotrophs like rhizosphere bacteria. These differing interactions between mycorrhiza and soil saprotrophs (i.e. cooperation vs. competition) may shape microbial function and carbon cycling in rhizosphere soils of AM- and ECM-associated plants as root exudation increases with rising atmospheric CO_2 or warming. Understanding these plant-microbe-soil interactions is crucial for accurately modeling and predicting ecosystem responses to global change (Johnson et al., 2013).

Recent advances in quantitative stable isotope probing (qSIP) may enhance our ability to detect cooperative and competitive interactions and connect these microbial activities with carbon cycling process rates in rhizosphere soils. By tracking ^{18}O labeled water into microbial DNA, taxon-specific growth and biomass production in response to root exudation addition can be quantitatively estimated (Hungate et al., 2015). These taxon-specific traits may then be aggregated for comparison with community-level processes, such as soil respiration rates (Walkup et al. *In Review*). Similar approaches have been widely applied in macro-organism ecology, where community-weighted mean (CWM) traits have been used to predict various ecosystem processes (Díaz et al., 2007; Suding et al., 2008; De Bello et al., 2010). However, the application of quantitative trait-based approaches remains limited in microbial ecology (Stone et al., 2021; Wang et al., 2021; Piñeiro et al., 2024), making it unclear how changes in community-level trait measurements correspond to rates of ecosystem processes. As microbes consume and respire carbon during growth, community-level microbial growth and biomass production should

correlated with soil respiration rates. However, microbial interactions such as competition, that stimulate microbial turnover, may lead to a disconnect between aggregated microbial growth traits and ecosystem process rates.

The aim of this study was to determine how rhizosphere carbon inputs regulate microbial growth, biomass production, and soil carbon cycling in rhizosphere soils associated with AM and ECM tree species. To do this, we sampled rhizosphere soils from canopy-dominant AM and ECM trees in a ~120 year old forested watershed at the Fernow Experimental Forest in West Virginia, USA. To mimic changes in root exudation, we added synthetic root exudates across a range of concentrations (0, 250, 500, and 1000 µg C g soil^{-1}) and quantified taxon-specific and community-level bacterial growth and biomass production using ^{18}O qSIP in a lab microcosm study. In AM rhizosphere soils, cooperative interactions between AM fungi and saprotrophs may allow saprotrophs to enhance their growth and biomass production in response to labile C inputs (Frey, 2019). However, this might not be the case in ECM systems due to the competitive relationship between ECM fungi and saprotrophs (Averill and Hawkes, 2016). As such we hypothesized that (H_1): increasing labile C inputs will increase microbial growth and biomass production to a greater extent in AM rhizosphere soils than in ECM rhizosphere soil. Furthermore, we hypothesized (H_2) that aggregated microbial growth and biomass production traits would be positively correlated with soil respiration rates. In simpler terms, we expected higher C inputs to stimulate microbial growth, leading to increased soil respiration.

2 MATERIALS AND METHODS

2.1 Site description and soil sampling

For this experiment, we collected soil samples at the Fernow long-term experimental forest (hereafter "Fernow") (reference watershed 4). The watershed comprises an unmanaged, mature (<100 years old) warm temperate forest stand located in the central Appalachian Mountains in West Virginia, United States (39.03°N, 79.67°W). The dominant tree species include *Acer saccharum*, *Liriodendron tulipifera* L., and *Acer rubrum* (AM association, near 40% of the basal area), and *Betula lenta*, *Quercus rubra*, and *Fagus grandifolia* (ECM association, near 30% of the basal area) among other tree species. Soils are coarse-textured Inceptisols of the Berks and Calvin Series (Gilliam et al., 1994).

In June 2020 (peak growing season), we sampled five 25 x 25 m plots containing approximately 50% AM and ECM tree species. Mineral soil samples were collected from beneath the canopy of three AM and three ECM trees (dbh >70 cm) within each plot. Around each tree, three soil cores (5 cm diameter, 10 cm depth) were collected and pooled into a composite sample per plot and mycorrhizal type. Rhizosphere soil was collected by gently separating the soil attached to live fine roots following the method outlined by Wollum (1994). Samples were then transported on ice and stored at 4°C in the laboratory. Further, samples were sieved (2 mm), and soil moisture and field capacity were measured. Soil moisture was measured gravimetrically from a soil subsample after drying for 48 h at 100°C. Field capacity was calculated according to Veihmeyer and Hendrickson (1931). We air-dried soil samples for approximately 48 hours, which reduced the gravimetric moisture content from an average of 19.9% to 4.5%. This drying step was necessary to facilitate the addition of ^{18}O water and exudate solution, achieving optimal soil moisture levels for measuring microbial activities (Morrissey et al., 2017; Foley et al., 2023). Subsamples of dried soil were stored at −80 °C for subsequent molecular analysis (unlabeled t_0 samples).

130 *2.2 Laboratory incubations: quantification of C mineralization and MBC*

131 Microcosm incubations were carried out to quantify the amount of CO_2–C respired across different
132 levels of labile C input in AM and ECM rhizosphere soils. The experiment consisted of two soil
133 types (AM and ECM rhizosphere soils) with three synthetic root exudate addition levels (250, 500,
134 and 1000 μg C g soil^{-1}, exudate addition treatment) and a no substrate addition control (water only),
135 each with five replicates (from each plot-level samples), for a total of 40 microcosms. The
136 synthetic root exudate mixture was composed of carbohydrates (23% each of glucose, fructose,
137 and sucrose) and organic acids (11% each of succinic and malic acid, and 9% of aspartic acid). A
138 total of 12 g (dry weight equivalent) soil samples were used to establish microcosms, which were
139 then sealed in 950 mL mason jars, adjusted to 60% water holding capacity with water or exudate
140 solution, and incubated for 5 days in the dark at room temperature (~23 °C). Total CO_2
141 concentration in the headspace of each microcosm was measured on days 1, 3, and 5 using a LI-
142 6400XT (LI-COR Biosciences, Lincoln, NE, USA) similar to Walkup et al. (2020). Following the
143 end of the 5-day incubation, MBC was determined from a subsample of 10 g soil and assessed via
144 the chloroform fumigation extraction method (Witt et al., 2000) followed by persulfate digestion
145 (Doyle et al., 2004) as described in Dang and Morrissey (Dang and Morrissey, 2024). The MBC
146 values were corrected for an extraction efficiency factor of 2.64 (Vance et al., 1987).
147
148 *2.3 Laboratory incubations: quantitative stable isotope probing (qSIP)*
149 A parallel incubation for qSIP used 2 g (dry weight equivalent) of soil per replicate. These samples
150 were placed into 15 ml Falcon tubes and adjusted to 60% water holding capacity (38 ± 5 %
151 gravimetric moisture) with either ^{18}O water (97 atom %) alone or ^{18}O water containing synthetic
152 root exudate. The incubation conditions and treatments mirrored those of the C mineralization,

except for soil volume and the use of ^{18}O water. The addition of 0.67 ± 0.11 mL of ^{18}O water achieved 75 ± 15% ^{18}O water enrichment. After the 5-day incubation, soil samples were immediately frozen at −80 °C (^{18}O-labelled t_5 samples).

Soil DNA was extracted from 0.25 g of each sample using the PowerLyzer PowerSoil DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, USA) in accordance with the manufacturer's instructions. The DNA was quantified using a Qubit Fluorometer (Life Technologies, Carlsbad, California, USA). Approximately 1.5 µg of DNA from each sample was separated by isopycnic centrifugation using 3.5 ml of saturated CsCl and gradient buffer (200 mM Tris-HCL, 200 mM KCl, 1 mM Ethylenediaminetetraacetic acid at pH 8) solution in a 4.6 ml OptiSeal ultracentrifuge tube (Beckman Coulter, Fullerton, CA). The samples were centrifuged in an Optima Max ultracentrifuge (Beckman Coulter, Indianapolis, IN) using a Beckman TLA 110 rotor at 127,000 x g for 72 h at 18°C. After centrifugation, the density gradient was fractionated by collecting ~28-30 fractions (each with 145-155 µl) per sample. The density of each fraction was measured using a Laxco RHD-B Series digital refractometer (Laxco Inc., Mill Creek, WA, USA). The DNA was then precipitated using isopropanol, cleaned with ethanol, and resuspended in 50 µl of nuclease-free water. The 16S rRNA gene abundance of all the fractions was measured using quantitative PCR with primer pair 515F/806R targeting the hypervariable V4 region, as described previously in Walkup et al. (2020). For all samples, fractions within the density range 1.644 to 1.738 g mL^{-1} were sequenced for 16S rRNA gene using the Illumina MiSeq sequencing platform (2 × 250 bp) at the Michigan State University Research Technology Support Facility Genomics Core. All sequence data is available under the NCBI SRA BioProject ID PRJNA1017906.

2.4 Data processing and statistical analysis

The resulting 16S rRNA gene sequence data were analyzed using QIIME2 (version.2021.2) (Bolyen et al., 2019). First, the raw forward and reverse sequence reads were imported into the QIIME2 format, and paired-end reads were assembled using the q2-vsearch plugin. To denoise sequences and resolve amplicon sequence variants (ASVs), all reads with low-quality scores or ambiguous base calls were then quality-filtered using the q2-deblur plugin with a trim length of 250 bp. The ASVs were taxonomically classified using the q2-feature-classifier (sklearn) trained on the SILVA v138.1 database. The resulting ASV feature table of each qSIP fraction was collapsed at the genus level and imported into R for further analysis. The 16S rRNA gene abundance (qPCR) and sequence read counts from non-fractionated samples were used to calculate the relative abundance of each taxon. The ^{18}O excess atom fraction (EAF) of each taxon was calculated as previously described (Hungate et al., 2015), using a custom R script (https://bitbucket.org/qsip/). Briefly, a weighted average density (WAD) was first calculated for each taxon. The difference in WAD (^{18}O-labelled t_5 - unlabeled t_0 samples) was used to quantify the amount of ^{18}O assimilation (^{18}O EAF) by individual taxa. A previously described method (Morrissey et al., 2017) was employed to account for technical error arising from slight variations in CsCl density gradients among ultracentrifuge tubes during WAD calculation. Finally, to determine the labile exudate addition response ($\Delta^{18}O$ EAF), the ^{18}O EAF values of bacterial taxa in each exudate-amended treatment were subtracted from the values in the no substrate addition control (water only).

The relative growth rate (RGR) of each taxon was estimated as a function of the rate of ^{18}O assimilation into DNA, assuming 40% of oxygen atoms derived from ^{18}O-water (based on the maximum observed ^{18}O EAF value of approximately 0.4 in this study). This approach aligns with

Purcell et al. (2022) and assumes steady-state microbial populations (Hungate et al., 2015; Li et al., 2019).

$$RGR_i = EAF_i/(average\ soil\ water\ ^{18}O\ enrichment \text{ x } 0.4) \quad (1)$$

The microbial community-weighted mean (CWM) of relative growth rate was calculated as follows:

$$CWMj = \sum_i^n\ p_i \text{ x } RGR_i \quad (2)$$

where p_i is the relative abundance and RGR_i is the relative growth rate of the taxon i in community j, and n is the total number of taxa in the community.

The production of new microbial biomass (μg C g^{-1} soil) was estimated as the product of the CWM and the community's MBC, as described previously (Wang et al., 2021):

$$New\ MBC_j = CWM_j \text{ x } MBC_j \quad (3)$$

To estimate the MBC mortality in each community, we used the following mass balance equation:

$$(Initial\ MBC_j + New\ MBC_j) = (Final\ MBC_j + MBC_j\ Mortality) \quad (4)$$

Here, $Initial\ MBC_j$ represents the MBC in community j under water-only treatment (assuming no change in MBC without exudate addition), while $Final\ MBC_j$ refers to the MBC of community j with exudate addition. Rearranging the equation yields:

$$MBC_j\ Mortality = (Initial\ MBC_j + New\ MBC_j) - \ Final\ MBC_j \quad (5)$$

The contribution of each taxon to new biomass production (NB_i) was estimated as the product of its relative abundance (p_i) and its relative growth rate (RGR_i), divided by the sum of the product of relative abundance and growth for all taxa, and then multiplied with new MBC, following Wang et al. (2021).

$$NB_i = \frac{p_i \text{ x } RGR_i}{\sum_i^n p_i \text{ x } RGR_i} \text{x } New\ MBC \quad (6)$$

New MBC production is a crucial factor in understanding both the overall productivity of the microbial community and the growth dynamics of individual taxa within it.

All statistical analyses were performed in R version 4.2.1 (Team, 2020). All figures were created using ggplot2. The pairwise Bray-Curtis dissimilarities ('vegdist' function) of compositional profile of microbial communities (non-fractionated DNA samples) were calculated using the "vegan" package (Oksanen et al., 2016) and visualized using principal coordinates analysis (PCoA). Permutational multivariate analysis of variance (PERMANOVA, 'adonis' function, with 999 permutations) was used to assess the effect of labile exudate addition and mycorrhizal type on microbial community composition. Mixed-effects models in "nlme" package (Pinheiro et al., 2017) were employed to test the effects of exudate addition, mycorrhizal association, and their interaction on soil respiration, MBC, CWM, new MBC, and MBC mortality. Sampling plot was included as a random factor to account for variation among plots. Post hoc pairwise comparisons were computed using the 'emmeans' package (Lenth et al., 2019). Correlation analyses between CWM and soil respiration, MBC and soil respiration, and new MBC and soil respiration were conducted in R.

3 RESULTS

The PERMANOVA results showed that both exudate addition and mycorrhizal association shaped microbial community composition (Figure S1). However, exudate addition had a stronger impact, explaining roughly 20% of the variation in community structure (PERMANOVA: $R^2 = 0.20$, $p < 0.001$) compared to mycorrhizal associations (AM and ECM), which explained only about 8% of the variation (PERMANOVA: $R^2 = 0.08$, $p < 0.01$). Microbial community composition in the highest exudate addition treatment (1000 µg C g soil^{-1}) was distinct from the other treatments (Figure S1). This shift in community composition likely resulted from changes in the relative abundance of microbial taxa responsive to exudate addition (Figure S2). Specifically, the relative abundance of *Burkholderia-Caballeronia-Paraburkholderia*, *Dyella*, and *Granulicella* increased while unassigned Xanthobacteraceae, Elsterales, Caulobacteraceae, and *Roseiarcus* decreased sharply in the 1000 µg treatment (Figure S2). While changes in the relative abundance of the aforementioned taxa occurred in both AM and ECM soils, the changes were more pronounced in AM systems. For example, *Burkholderia-Caballeronia-Paraburkholderia* increased 2.2 fold in the AM but only 1.8 fold in the ECM in the 1000 µg C exudate addition treatment. Similarly, 1000 µg C exudate addition treatment decreased *Roseiarcus* relative abundance by 59% in the AM but only 49% in the ECM soils.

Soil respiration increased proportionally with the amount of exudate addition in both AM and ECM soils (Figure 1A; Table S1). However, the response of MBC to exudate addition depended on the mycorrhizal association (Figure 1B; Table S1), as evidenced by a significant interaction between exudate addition and mycorrhizal association (Figure 1B; Table S1). Overall, MBC in ECM soils decreased as exudate addition increased, while in AM soils showed the opposite trend (Figure 1B).

Exudate addition altered the relative growth rates of individual taxa in both AM and ECM soils, but these effects were generally more pronounced in AM soils (Figure 2). Moreover, the differences in taxon-specific RGRs between AM and ECM soils increased with higher levels of exudate addition. For example, exudate addition tended to increase the RGRs of taxa within Proteobacteria, with these increases being larger in the AM soils (Figure 2). Notably, as the level of exudate addition increased, the differences in RGRs between AM and ECM soils became more pronounced, highlighting the contrasting responses of these ecosystems to exudate availability (Figure 2). Similarly, exudate addition significantly increased the CWM relative growth rates in both soils (Figure 3A; Table S1), with AM soils displaying relatively higher CWM values compared to ECM soils.

The production of new MBC increased with exudate addition only in AM soils, remaining relatively constant in the ECM soils (Figure 3B; Table S1). Conversely, estimates of MBC mortality increased with exudate addition in ECM soils (Figure 3C; Table S1), but showed minimal change across all exudate addition levels in AM soils. A few key taxa, including *Dyella*, *Burkholderia-Caballeronia-Paraburkholderia*, and Streptomycetaceae, were responsible for most of the new biomass production within the microbial community (Figure 4). Notably, these taxonomic groups displayed higher new biomass production in the AM soils compared to the ECM soils (Table S2). Interestingly, some taxa that responded positively to exudate addition in the AM soils responded negatively in the ECM soils. For instance, *Dyella* and Streptomycetaceae had reduced new biomass production following exudate addition in the ECM rhizosphere, except for *Dyella* at 1000 µg treatment (Figure 4). Bacterial CWM relative growth rates, MBC, and new MBC production were strongly correlated with soil respiration in the AM soils but not in the ECM

287 soils (Figure 5). Notably, across the exudate addition levels, new microbial biomass production
288 could explain 70% of the variation in soil respiration rates in the AM rhizosphere soil.
289
290 **4 DISCUSSION**
291 As global change continues to alter ecosystem function, understanding how plant-microbe
292 interactions will influence ecosystem responses is of paramount importance. Here, we aimed to
293 determine how mycorrhizal association influences the composition, traits, and carbon processing
294 of rhizosphere bacteria. Plants can respond to global change drivers by modifying the rates of
295 rhizosphere exudation, thereby influencing belowground C-cycling processes and microbial
296 community dynamics (Phillips et al., 2011; Yin et al., 2013). Using a range of exudate additions,
297 we determined that mycorrhizal association influences the ability of rhizosphere bacterial taxa to
298 grow and produce biomass. Specifically, in the rhizosphere of AM trees, microbes were able to
299 enhance their growth in proportion to exudate availability, while in ECM rhizosphere soils, growth
300 and biomass production were comparatively suppressed. These results highlight that the effects of
301 rhizosphere exudation on soil carbon storage depend on the interactions between plants,
302 mycorrhizae, and free-living saprotrophs.
303
304 *4.1 Plant-mycorrhizae-saprotroph interactions following exudate addition*
305 Our findings support the hypothesis (H1) that differences in mycorrhizal association can dictate
306 the responses of rhizosphere microbes to exudate addition. While soil respiration increased
307 proportionally to the amount of exudate addition in both AM and ECM soils (Figure 1A), estimates
308 of MBC exhibited contrasting trends, increasing in AM soils but decreasing in ECM soils (Figure
309 1B). These contrasting responses may reflect fundamentally different microbial dynamics

occurring in the rhizospheres of AM and ECM trees. AM fungal hyphae serve as conduits for plant-derived photosynthate C to microbial saprotrophs (Drigo et al., 2010; Kaiser et al., 2015; Frey, 2019), stimulating their growth and activity (Toljander et al., 2007; Chowdhury et al., 2022; Zhang et al., 2022). Our results agree with this past work, as exudate addition led to increases in MBC (Figure 1B), relative growth rates (Figure 2), and new biomass production (Figure 4) in AM soils. Moreover, the aggregated taxon-specific relative growth rates, represented as CWM relative growth rates, increased with exudate addition, particularly in AM soils. This increase in CWM traits in response to exudate addition is a consequence of increases in the relative growth rates of bacterial taxa (Figure 2), many of which increased in relative abundance (Figure S2) following exudate addition. AM fungi can secrete low-molecular-weight organic C exudates (Frey, 2019; Chowdhury et al., 2022; Zhang et al., 2022) that may prime saprotrophs to liberate nutrients in the rhizosphere. Exudates are well known to prime rhizobacteria to enhance soil organic matter decomposition and liberate nutrients (Cheng et al., 2014). However, rhizobacteria can also provide nutrients to their host plants and mycorrhizae via nitrogen fixation and phosphorus solubilization (Zeng et al., 2022). As such, AM fungi may engage in mutualistic interactions with rhizobacteria, delivering exudates in exchange for assistance with nutrient acquisition. Moreover, it has been hypothesized that AM fungal-bacterial relationships may have coevolved (Garbaye, 1994; Olsson et al., 2017), leading to a consistent priming of the bacterial community with labile C exudates secreted through AM fungal hyphae (Paterson et al., 2016; Frey, 2019; Kakouridis et al., 2024). This selection process may have favored members of certain bacterial phyla, such as Proteobacteria and Actinobacteria, which are known to dominate in the AM hyphosphere (Zhang et al., 2022), making them well suited to grow rapidly when exudates are abundant. Our results support this, as many taxa in Proteobacteria and Actinobacteria had larger increases in relative

growth rates following exudate addition in the AM rhizosphere than the ECM rhizosphere (Figure 2).

Conversely, our results suggest ECM fungi limit microbial growth and biomass production when exudates are present. While some taxa increased their relative growth rates in response to exudate addition (Figure 2), these increased growth rates were countered by accelerated microbial biomass mortality (Figure 3C) leading to decreases in new biomass production (Figure 3B and Figure 4). Unlike AM fungi, ECM fungi may rely less on saprotrophs for nutrient acquisition, as they can produce extracellular enzymes to acquire small organic N-containing compounds (Talbot et al., 2008; Tedersoo et al., 2012). This organic N uptake may create nutrient limitation and limit saprotrophic activity, a phenomenon known as the Gadgil effect (Gadgil and Gadgil, 1975). Therefore, N limitation could potentially explain the limited increases in microbial relative growth rates observed at both the individual (Figure 2) and community level (Figure 3A) in ECM soils. Under nutrient-limited conditions, the addition of labile C substrates with a high C:N ratio can cause a decline in the microbial population, potentially due to their inability to adapt to stoichiometric imbalances, thereby promoting microbial turnover (Kaiser et al., 2014). Our results are in agreement with this past work, as we observed substantial MBC mortality following exudate addition in the ECM rhizosphere soils. However, it is possible that other mechanisms, beyond nutrient limitation, could cause the observed responses. ECM fungi can produce a range of secondary metabolites, many of which may have antibiotic properties (Rasanayagam and Jeffries, 1992; Olsson et al., 1996). Competition for the added exudates could fuel chemical warfare and other antagonistic interactions in the ECM soils, leading to MBC mortality (Figure 3C) and reducing new biomass production (Figure 4). These antagonistic interactions and the rapid MBC

355 mortality could benefit ECM trees and fungi because MBC mortality could enhance the availability
356 of dissolved organic nitrogen and phosphorus.

357 Our findings demonstrate that AM and ECM-associated rhizospheres exert contrasting
358 influences on bacterial growth and biomass production following exudate addition. Specifically,
359 the AM rhizosphere fostered microbial growth and biomass production following exudate
360 addition, possibly due to positive interactions between AM fungi and bacteria. In contrast,
361 microbial growth was more constrained and mortality was high in the ECM rhizosphere exposed
362 to increasing exudation rates, potentially due to nutrient limitation or antagonistic competition.
363 These results shed light on the intricate responses of microbial communities to labile exudate
364 addition in contrasting soil ecosystems, emphasizing the need for further research to unravel the
365 underlying mechanisms driving these dynamics and their implications for ecosystem functioning.
366

367 *4.2 Taxon-specific responses to exudate addition*

368 The addition of labile exudates in soil selectively favors certain bacterial taxa that exhibit rapid
369 growth in nutrient-rich environments, while many others either grow slowly or remain
370 unresponsive (Fierer et al., 2007; Papp et al., 2020; Stone et al., 2023). Our results support this
371 explanation, as we observed an increase in the relative abundance (Figure S2), growth rate (Figure
372 2), and new biomass production (Figure 4) of some bacterial taxa following exudate addition.
373 Notably, *Dyella* (7.2%) and *Burkholderia-Caballeronia-Paraburkholderia* (8.9%) were among
374 the most abundant genera in both AM and ECM soils. These taxa increased their relative growth
375 rates in response to exudate addition. Both of these genera are well-known rhizobacteria that can
376 produce phytohormones, solubilize phosphate, and fix nitrogen (Palaniappan et al., 2010;
377 Domínguez-Castillo et al., 2021). As such, these bacteria have been reported to colonize the

surface of both AM and ECM hyphae (Bonfante and Anca, 2009; Taktek et al., 2015; Marupakula et al., 2017) and likely engage in forming tripartite symbiotic associations with mycorrhizae and their host plants (Zhang et al., 2024). However, the responses of these bacterial taxa to exudate addition were much stronger in AM soils compared to ECM soils. AM fungal hyphae release compounds that enhance SOM priming by stimulating the activity of specific rhizosphere bacteria referred to as 'hyper symbionts' (Jansa et al., 2013). *Burkholderia* and *Dyella* are commonly reported from the hyphosphere or mycorrhizosphere of AM fungi (Taktek et al., 2015). Interestingly, despite the positive effect of exudate addition on the relative growth rates of *Dyella* in both AM and ECM rhizospheres, *Dyella* had reduced biomass production in the ECM rhizosphere under certain exudate addition treatments (250 and 500 μg C g soil^{-1}), suggesting exudate addition may stimulate rapid turnover in some rhizobacteria populations, where mortality exceeds growth.

4.3 Connecting microbial growth, biomass production, and ecosystem function

The functional trait values within an ecosystem are shaped by the species composition of ecological communities and the prevailing environmental conditions, making trait-based approaches a robust framework for linking organismal responses to environmental changes with shifts in ecosystem functioning (Hicks et al., 2022). The results showed a significant correlation between CWM relative growth rates and soil respiration with exudate addition in AM soils (Figure 5A). Moreover, there was an even stronger relationship between newly synthesized MBC and soil respiration in AM soils (Figure 5C). The new MBC represents the proportion of microbial biomass synthesized over the course of the experimental incubation and approximates the growth of metabolically active bacteria, therefore offering better predictability for soil processes. The

connection between CWM relative growth rate and new biomass production suggests soil respiration in AM soil was driven by the need for energy production to fuel growth in response to exudate addition. Further, our findings agree with past qSIP-based studies that identified CWM traits (Piñeiro et al., 2024, Walkup et al. In Review) and new biomass production (Wang et al. 2021) as predictive of soil respiration. For instance, reductions in the C and N assimilation traits of bacterial communities under chronic N deposition mirrored decreases in soil respiration and N mineralization in AM forest soils (Piñeiro et al., 2024). Our approach to calculating CWM relative growth rates focused only on bacteria, and thus may have been more successful in the AM rhizosphere where bacteria are posited to play a larger role. Relative to ECM systems, AM ecosystems are characterized by a higher bacteria to fungi ratio (Cheeke et al., 2017) and a greater proportion of bacterial genes linked to nutrient and C cycling (Bahram et al., 2020), highlighting the importance of bacterial saprotrophs in AM soil function (Carrara et al., 2021; Piñeiro et al., 2024). Linking microbial community composition to function is a long-standing challenge in microbial ecology, and our results suggest that the trait-based framework provides an appropriate measure to connect microbial diversity with ecosystem functions (Morrissey et al., 2023).

In contrast, none of the community-level microbial traits (CWM, MBC, and new MBC) showed a significant correlation with soil respiration in ECM soils, indicating a decoupling between community-level traits and soil processes in these environments, as previously reported (Piñeiro et al., 2024). This disconnect highlights that other microbial dynamics or populations may be needed to accurately understand soil respiration in the ECM rhizosphere. Perhaps most notably, our results suggest that high MBC turnover (Figure 3C) may have fueled CO_2 production in the ECM rhizosphere. This turnover could be driven by nutrient limitation or antibiosis as described above. For instance, the ECM rhizosphere could be particularly deficient in N due to N mining by

ECM fungi (Phillips et al., 2013). To overcome N deficiency, microorganisms in these soils would likely switch to necromass-N recycling to cope with acute stoichiometric imbalances under high C input (Kaiser et al., 2014; Cui et al., 2020), resulting in C overflow as increased microbial respiration (Manzoni et al., 2008; Spohn, 2015). In this scenario, microbes relying on labile C would increase their growth rate (Reischke et al., 2014; Wei et al., 2019), but with minimal changes in their biomass likely due to their shortened lifespan (Behera and Wagner, 1974), facilitating necromass generation and rapid microbial turnover. Such dynamics could explain how exudate addition increased soil respiration (Figure 1) but resulted in limited increases in taxon-specific and CWM relative growth rates (Figure 2, Figure 3a) and no increases in biomass production (Figure 3B). Alternatively, the disconnect could relate to the absence of fungal communities in our calculation of community-level traits. Given the dominance of fungi in ECM soils (Cheeke et al., 2017), their inclusion may be necessary to establish connections between microbial traits and soil processes (Averill et al., 2014; Carrara et al., 2021).

Taken together our results suggest that AM ecosystems will respond very differently from ECM ecosystems to increases in root exudation, likely associated with global change drivers such as elevated atmospheric CO_2 or warming. Increased root exudation in AM ecosystems would stimulate microbial growth in the rhizosphere, leading to greater biomass production. As this microbial biomass gradually turns over, the resulting microbial residues could contribute to the formation of mineral-associated organic matter (MAOM) (Sokol et al., 2019), thereby increasing the SOC stock in AM ecosystems (Keller et al., 2021). MAOM cycles slowly and shields C from microbial decomposition (Schlesinger and Lichter, 2001), making it a significant process for enhancing the long-term carbon sequestration potential of AM ecosystems. In contrast, the enhanced exudation in ECM ecosystems may not fuel microbial growth and biomass production

as significantly due to increased microbial mortality and rapid microbial necromass recycling. This dynamic could result in smaller changes in the stable SOC pool in ECM ecosystems under global change drivers such as elevated CO_2 levels and warming.

5 CONCLUSIONS

In summary, labile exudates elicited contrasting responses in rhizosphere bacteria between AM and ECM ecosystems. Taxon-specific relative growth rates and new biomass production increased more in AM soils after exudate addition. Similarly, CWM traits, reflecting aggregated taxon-specific relative growth rates, further increased in AM soils. These findings suggest that while new biomass production thrived in AM soils with exudate addition, it declined in ECM soils, potentially due to accelerated microbial mortality. Notably, community-level aggregated traits predicted soil respiration, MBC, and new MBC in AM soils, but not in ECM soils. This discrepancy might be linked to mycorrhizal-driven interactions with saprotrophic bacteria within these contrasting ecosystems. The strong correlation between bacterial community composition traits and soil processes in the AM system underscores the crucial role of bacterial saprotrophs in AM soil functioning. Overall, our results demonstrate that the trait-based framework offers a valuable tool to connect microbial diversity with ecosystem functions, and plant-mycorrhizae-saprotroph interactions determine the fate of root exudation and shape ecosystem feedbacks to global change.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

The sequence data and related metadata are accessible under the NCBI SRA BioProject ID PRJNA1017906.

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706

FIGURE LEGENDS

FIGURE 1 Soil respiration rate (**A**) and microbial biomass carbon (**B**) in AM and ECM soils with different levels of C input (250 - 1000 μg C g soil^{-1}). Significant *p* values are denoted by asterisks (***$p < 0.001$).

FIGURE 2 The change in relative growth rate (Δ^{18}O RGR) following exudate addition for select microbial taxa (which showed Δ^{18}O RGR > 0.1 in any of the treatments) that responded to exudate addition (250, 500, and 1000 μg C g soil^{-1}) in AM and ECM rhizosphere soils. Different colors indicate the phylum-level microbial taxonomy.

FIGURE 3 Community weighted mean (CWM) relative growth rates (A), new MBC production (B), and MBC mortality (C) in AM and ECM soils along the C input gradient. The asterisks indicate *p* values of the test statistic (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

FIGURE 4 Change in new biomass (ΔNB_i) between exudate amended soils (250, 500, and 1000 μg C g soil^{-1}) and unamended soils for selected microbial taxa that demonstrated changes in their biomass production in both AM and ECM rhizosphere soils. Data are mean ± SE and colored by phylum, note square root scale.

FIGURE 5 Relationship between soil respiration rate and CWM relative growth rates, MBC, and new MBC production in AM (A, B, and C) and ECM (D, E, and F) soils along the exudate addition gradient. Significant *p*-values are represented by asterisks (***$p < 0.001$).

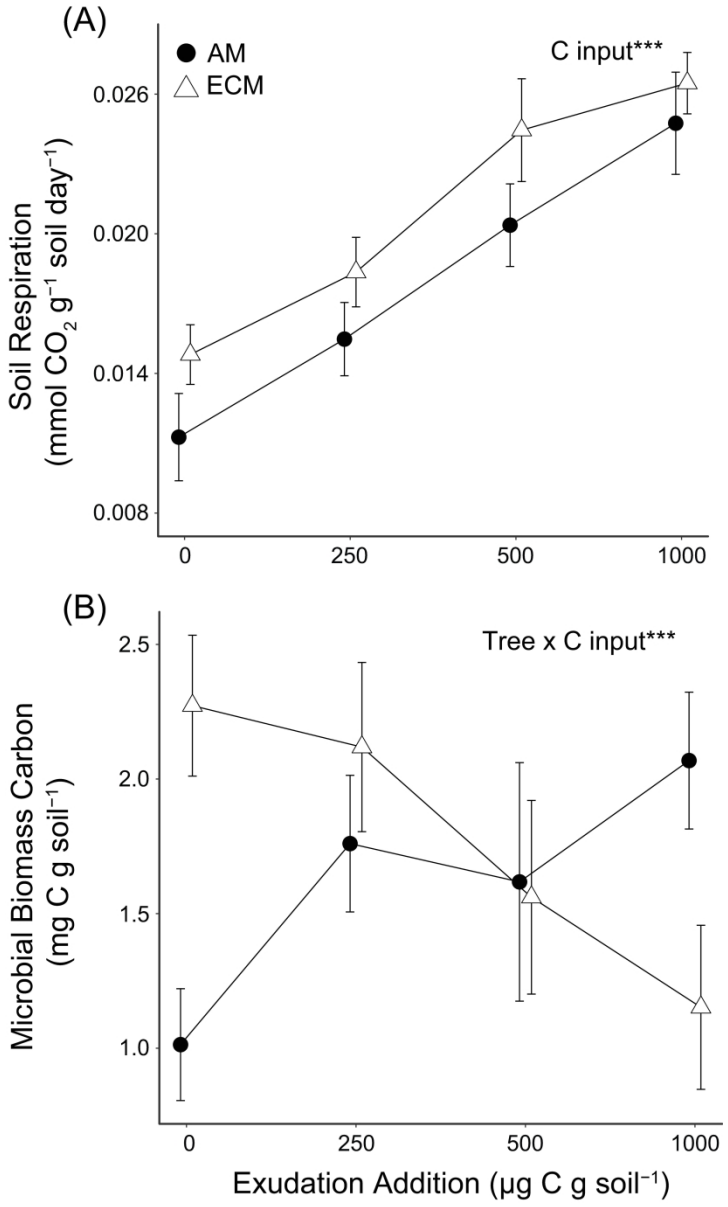

FIGURE 1 Soil respiration rate (A) and microbial biomass carbon (B) in AM and ECM soils with different levels of C input (250 - 1000 µg C g soil-1). Significant p values are denoted by asterisks (***$p < 0.001$).

272x453mm (300 x 300 DPI)

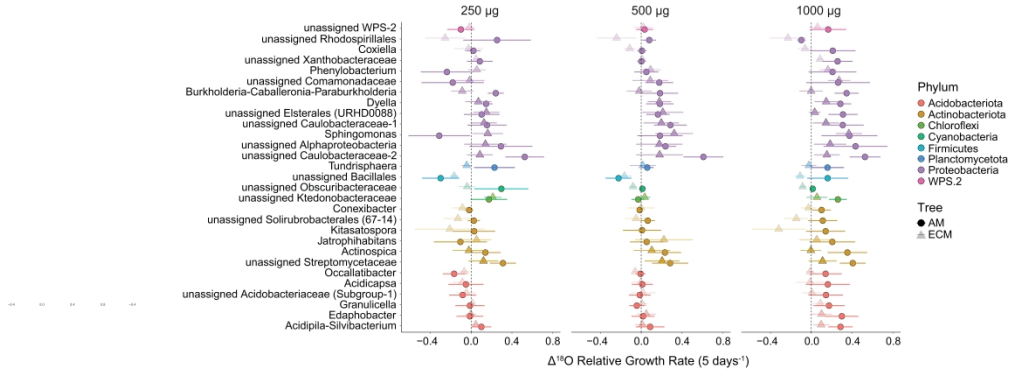

FIGURE 2 The change in relative growth rate (Δ18O RGR) following exudate addition for select microbial taxa (which showed Δ18O RGR > 0.1 in any of the treatments) that responded to exudate addition (250, 500, and 1000 µg C g soil-1) in AM and ECM rhizosphere soils. Different colors indicate the phylum-level microbial taxonomy.

913x332mm (600 x 600 DPI)

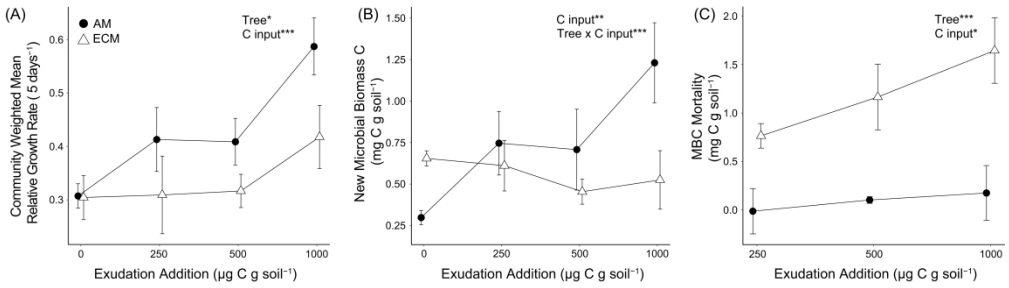

FIGURE 3 Community weighted mean (CWM) relative growth rates (A), new MBC production (B), and MBC mortality (C) in AM and ECM soils along the C input gradient. The asterisks indicate p values of the test statistic (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

817x225mm (300 x 300 DPI)

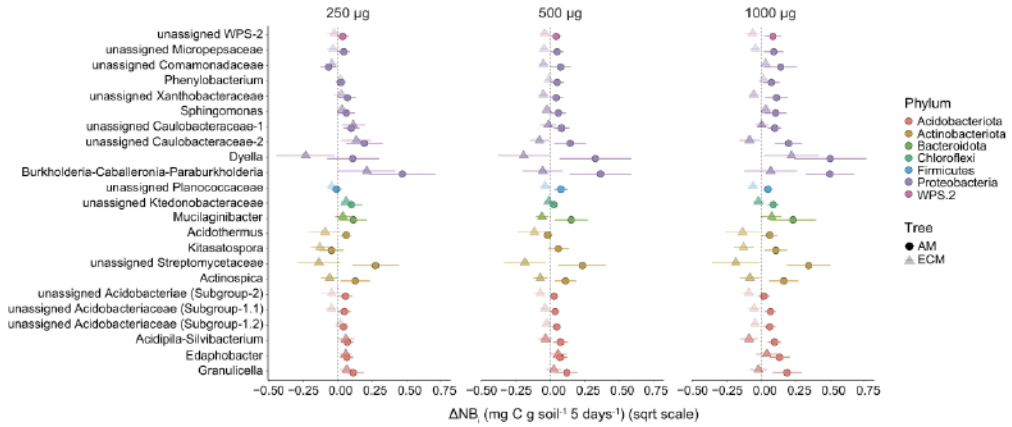

FIGURE 4 Change in new biomass (ΔNBi) between exudate amended soils (250, 500, and 1000 µg C g soil-1) and unamended soils for selected microbial taxa that demonstrated changes in their biomass production in both AM and ECM rhizosphere soils. Data are mean □ SE and colored by phylum, note square root scale.

795x337mm (600 x 600 DPI)

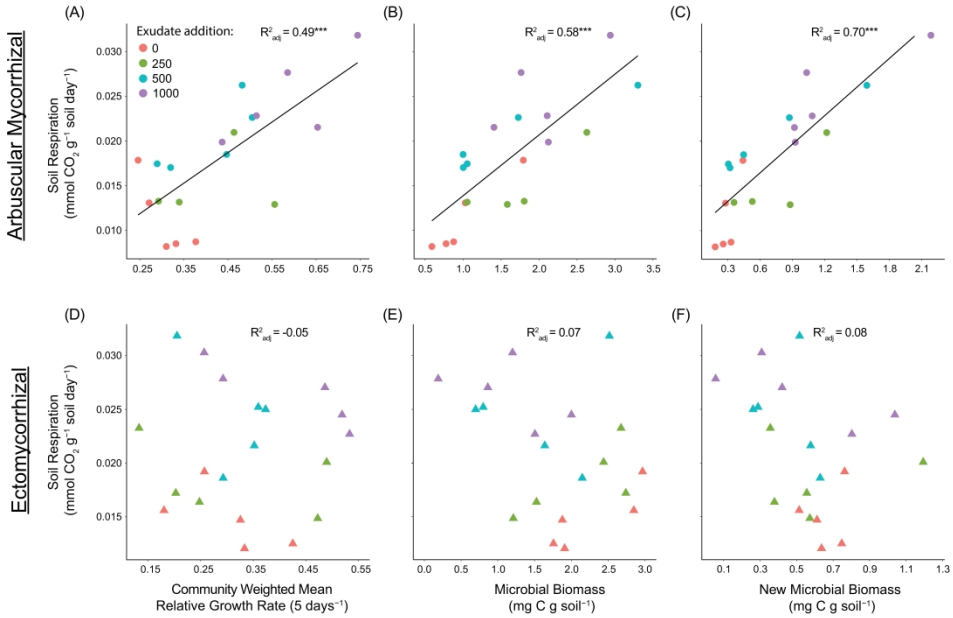

FIGURE 5 Relationship between soil respiration rate and CWM relative growth rates, MBC, and new MBC production in AM (A, B, and C) and ECM (D, E, and F) soils along the exudate addition gradient. Significant p-values are represented by asterisks (***p < 0.001).

824x503mm (600 x 600 DPI)