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Volatile and Dissolved Organic Carbon Sources Have Distinct Effects on Microbial Activity, Nitrogen Content, and Bacterial Communities in Soil

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Abstract

Variation in microbial use of soil carbon compounds is a major driver of biogeochemical processes and microbial community composition. Available carbon substrates in soil include both low molecular weight-dissolved organic carbon (LMW-DOC) and volatile organic compounds (VOCs). To compare the effects of LMW-DOC and VOCs on soil chemistry and microbial communities under different moisture regimes, we performed a microcosm experiment with five levels of soil water content (ranging from 25 to 70% water-holding capacity) and five levels of carbon amendment: a no carbon control, two dissolved compounds (glucose and oxalate), and two volatile compounds (methanol and α-pinene). Microbial activity was measured throughout as soil respiration; at the end of the experiment, we measured extractable soil organic carbon and total extractable nitrogen and characterized prokaryotic communities using amplicon sequencing. All C amendments increased microbial activity, and all except oxalate decreased total extractable nitrogen. Likewise, individual phyla responded to specific C amendments—e.g., Proteobacteria increased under addition of glucose, and both VOCs. Further, we observed an interaction between moisture and C amendment, where both VOC treatments had higher microbial activity than LMW-DOC treatments and controls at low moisture. Across moisture and C treatments, we identified that Chloroflexi, Nitrospirae, Proteobacteria, and Verrucomicrobia were strong predictors of microbial activity, while Actinobacteria, Bacteroidetes, and Thaumarcheota strongly predicted soil extractable nitrogen. These results indicate that the type of labile C source available to soil prokaryotes can influence both microbial diversity and ecosystem function and that VOCs may drive microbial functions and composition under low moisture conditions.

Keywords Dissolved organic carbon · Microbial community composition · Amplicon sequencing

Introduction

The response of soil ecosystems to climate change depends on microorganisms' access to a variety of soil carbon (C) substrates that shape their growth and activity. Soil moisture content is an important regulator of these C substrates [1, 2], as many C compounds are limited by slow diffusion through soil water [3]. These compounds include low molecular weight-dissolved organic carbon (LMW-DOC), e.g., carbohydrates, amino acids, and organic acids. However, not all microbial C substrates require water to move through the soil matrix. Volatile organic compounds (VOCs), which are also abundant in soils [4], can diffuse through soil air and are therefore not limited by diffusivity through water, e.g., alcohols, aldehydes, and terpenes. This study examines

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soil microbial responses to LMW-DOCs and VOCs, and the extent to which they are dependent on soil water content.

LMW-DOC is heterogeneously distributed throughout the soil [5]. The majority of LMW-DOC is concentrated in the rhizosphere where it enters through rhizodeposition before it is rapidly metabolized, and entry into the bulk soil is largely regulated by mass flow of water [6]. Indeed, microbial availability of LMW-DOC is positively related to soil moisture content [7]. Because higher water content correlates with increased LMW-DOC, wet microsites in the soil matrix are nutrient-rich and likely to favor copiotrophic microbial taxa [8]—e.g., Bacteriodetes and Proteobacteria [9]. In contrast, low moisture environments likely favor oligotrophic taxa that are specialized to persist when nutrients are depleted—e.g. Acidobacteria and Verrucomicrobia [9, 10]. Furthermore, increased availability of LMW-DOC increases microbial activity and alters N processing [11–13], suggesting that high moisture environments will be hotspots of microbial activity and nutrient cycling.

Like LMW-DOC, VOCs, including methanol and monoterpenes, function as microbial C substrates [14, 15], drive microbial activity [16, 17], alter N transformations [17, 18], and alter microbial community composition [19]. VOCs can enter the soil through a variety of mechanisms including root emission, organic matter decomposition, and soil-atmosphere exchange [4, 20–22]. VOC production during decomposition can be considerable [23]. The VOCs produced during decomposition are dominated by methanol in many leaf species—e.g., *Rhododendron maximum*, and *Populus deltoides*—though some species such as *Pinus* spp. and *Eucalyptus* spp. release a large proportion of monoterpenes, and other VOCs [24]. VOCs differ from LMW-DOC in their ability to diffuse through soil air—the high vapor pressure of VOCs leads to evaporation at ambient temperature and diffusion through air-filled pore spaces [20]. In addition, VOC diffusion is dependent on soil moisture content—at low moisture, these compounds can diffuse freely through soil pore spaces, but at high moisture they either dissolve in soil water (e.g., methanol), or, when hydrophobic, are prevented from moving past water barriers (e.g., monoterpenes). Therefore, the relative importance of VOCs for microbial activity and soil function is likely to change depending on local variation in soil water content.

Here, we used a microcosm approach to determine the effect of LMW-DOC and VOCs on soil C and N cycling, microbial activity, and soil microbial communities under different soil moisture regimes. We designed a full factorial experiment with five levels of moisture (25%, 35%, 45%, 60%, and 70% of water holding capacity), and five levels of C amendment: a no C control, two dissolved compounds (glucose, and oxalate), and two volatile compounds (methanol, and α-pinene—a monoterpene). We predicted that the two LMW-DOC compounds (glucose and oxalate) would have the largest effect on soil and microbial variables under high moisture conditions because these compounds require water connectivity to diffuse through the soil matrix. In contrast, we predict VOC effects on soil communities and nutrient availability to be driven by their ability to diffuse through soil water. Because methanol is both miscible in water and capable of vaporization, we predicted it would affect microbial communities at all moisture levels. However, since the α-pinene is hydrophobic, we predicted it only affects soil microbial processes under low moisture content. While we do not know which taxa will respond to C addition, we expect all of these carbon compounds to increase microbial activity as the soil microorganisms consume them. Further, we expect the microbial consumption of C to lead to a subsequent decrease in soil nitrogen as N demand increases with increased microbial activity.

Methods

Experimental Design

We collected six individual A-horizon soil cores (8-cm diameter, 0–10-cm depth) from Kentland Farm in Montgomery County, VA (37.1987, − 80.5833): Guernsey silt loam; *Pinus strobus* plant cover. At this site, soil pH was measured as ~ 6.77, and annual rain in 2017 (when soil was collected) was 81.9 cm. Soil temperature at 15 cm ranged from a high temperature of 27.2 °C to a low temperature of 2.2 °C; the average monthly soil moisture at 15 cm ranged from the wettest month in January 14.05 kPa, to the driest in September at 147.46 kPa (https://montgomery.weatherstem.com/data?refer=/kentlandfarm). Soil cores were composited, sieved (4.75 mm), homogenized, and stored at 4 °C. We constructed microcosms by first adding 10 g dry weight equivalent soil to a 50-mL conical tube. Soil was allowed to dry down to 25% of water holding capacity (WHC). We then adjusted WHC to five levels (25%, 35%, 45%, 60%, and 70% WHC), and all tubes were incubated for 2 weeks at 20 °C and 100% humidity, with weekly moisture adjustments to maintain assigned water contents.

Microbial activity, dissolved organic C, and total extractable nitrogen

At the end of the initial 2-week incubation, we began weekly amendments of four C sources—2 volatile organic compounds (methanol, and α-pinene) and 2 dissolved organic compounds (glucose, and oxalate)—at a rate of 120 µg C g^{-1} dry soil. This rate is similar to that of previous studies (e.g., [16, 17]. Additionally, we had a no C control at each moisture level that was only water-adjusted. This resulted in 25 treatments with five levels of moisture, five levels of C addition, and five replicates for a total of 125 experimental

units. Weekly VOC-C amendments were made by adding liquid phase methanol or α-pinene into a 0.2-mL conical tube placed into the center of the soil. This was to allow for only vapor phase transport of VOC compounds into the soil. Weekly DOC amendments were added directly to the soil. Immediately after C addition, the 50 ml conical tubes were sealed, and we used a static incubation procedure to measure CO_2 production (sensu [25]. Briefly, after adding C, an initial 5-mL headspace sub-sample was measured to determine CO_2 using an infrared gas analyzer (Li-7000; Li-Cor Biosciences, Lincoln, Nebraska, USA). A second 5-mL subsample was measured after a ~ 24-h incubation period. Microbial activity was calculated by subtracting the initial concentration of headspace CO_2 from the CO_2 produced after incubation and divided by hours incubated to calculate a respiration rate (μg CO_2–C g dry soil^{-1} h^{-1}). Additional measurements in between C additions were made after flushing microcosms with CO_2-free air for 3 min, then measuring headspace CO_2 after ~ 24 h. At the end of the 28-day experiment, we subsampled each microcosm to assess extractable soil organic C (SOC) and total extractable N concentrations. Briefly, 0.5 M K_2SO_4 was added to soil (1:5 soil:solution ratio) and shaken for 1 h before extracting supernatant by filtering through Whatman no. 42 filters and measuring C and N content with an Elementar Variocube TOC/TN (Elementar Americas Inc., Mt. Laurel, NJ, USA).

Prokaryotic community assessment

We assessed prokaryotic community composition via amplicon sequencing of the 16S rRNA gene. DNA was extracted from each soil sample using the Qiagen© PowerSoil kit (Qiagen, Hilden, Germany), according to the manufacturer's protocols. We amplified the gene using the 515F/806R primer pair in a 2-step PCR [26, 27]. After completing the first round of PCR, amplicons were purified using ExoSAP-IT™ PCR clean-up reagent (Affymetrix Inc., Santa Clara, CA, USA) according to the manufacturer's protocol. During the second round of PCR, unique barcoded primers were added to each sample. At the completion of the second round of PCR, we used SequelPrep™ 96-well plates (Invitrogen, Carlsbad, CA, USA) to clean and normalize samples. We pooled amplicons in equimolar ratios, and the Genomics Resource Core (GRC) sequencing facility at the University of Idaho sequenced the amplicon pools using an Illumina MiSeq instrument and 2 × 300 bp sequencing kits. We used a no-DNA control throughout the laboratory process to identify potential contamination.

The University of Idaho's Genomic Resource Core demultiplexed sequences using the dbcAmplicons program [28]. We then trimmed, dereplicated, and denoised reads using the DADA2 pipeline, producing a total of 3,267,213 sequences, which comprised 6,417 unique amplicon sequence variants (ASVs). Taxonomy assignments were determined using the naïve Bayesian classifier [29] against the SILVA reference database (ver. 132, [30]. To account for differences in sequencing depths among samples, 17,085 sequences were randomly selected from each sample. Five samples were excluded from analysis due to insufficient sequence depth.

Statistical analyses

We determined effects of C amendment, moisture, and C amendment by moisture interactions on cumulative CO_2 evolution as an index of microbial activity, DOC content, total extractable N content, and prokaryotic phyla using two-way analysis of variance (ANOVA). The residuals of the ANOVA models were analyzed for normality using the Shapiro–Wilk test [31]. When the residuals did not meet normality, we used generalized linear models (GLMs), with a gamma distribution and log link function ('glm' function in lme4 package [32]. When no significant interaction was detected, we determined pairwise differences between C treatments averaged across all moisture levels, and between moisture treatments averaged across all C levels. When there were significant C × moisture interactions, we determined pairwise differences between C treatments within moisture levels, and between moisture levels within C treatments. All pairwise differences were determined using the Tukey post hoc test in the *emmeans* package [33]. Differences in prokaryotic α diversity were assessed using the Shannon and Simpson indices. We determined the effects of C amendment and its interaction with moisture on prokaryotic community composition using PERMANOVA ('adonis2' function in vegan package,[34], and pairwise differences between C sources were determined using the 'pairwise.perm.manova' function in RVAidemoire package (Hervé 2021). Patterns in community composition were visualized using NMDS ('metaMDS' function in vegan package). Both PERMANOVA and NMDS were performed with Bray–Curtis dissimilarities. Significant treatment effects were considered at $P < 0.05$, and marginal significance was considered at $P < 0.10$. To elucidate links between prokaryotic communities and their biogeochemical functions, we used best subsets GLM, which allowed us to identify prokaryotic phyla that best predicted microbial activity (i.e., CO_2 flux) and soil extractable N content. The regression model containing the best-supported subset of phyla for each biogeochemical function was identified using AIC (sensu Osburn et al., [35]).

Results

Microbial activity, dissolved organic C, and total extractable N

C addition—for all amendments—increased microbial activity 125–211% ($\chi^2_4 = 886.47$; $P < 0.001$; Fig. 1(A)).

There was a significant interaction between C amendment and soil moisture (χ^2_{16} = 67.98; $P < 0.001$; Fig. 1(B)) which was primarily due to α-pinene marginally increasing activity above both LMW-DOC compounds at 25% WHC, and oxalate at 35% WHC (pairwise $P < 0.1$). Further, at 25% WHC, the two VOC treatments elicited 26–40% higher cumulative microbial activity rates than the two LMW-DOC treatments (Fig. 1(B)). Additionally, methanol increased microbial activity above both LMW-DOC compounds at 25%, 35%, and 45% WHC, but only above glucose at 60% WHC, while all C amendments had statistically equal activities at the highest moisture level (70% WHC).

In general, C addition—averaged across moisture levels—decreased total extractable N (F_4 = 33.97; $P < 0.001$; Fig. 1(C)). Total extractable N concentrations were also affected by an interaction between C and moisture level (F_{16} = 1.91; $P = 0.029$; Fig. 1(D)). Methanol reduced total extractable N at all moisture levels except 45% WHC, while α-pinene decreased extractable N at 25%, 35%, and 60% WHC, and glucose only had an effect at 70% WHC. Oxalate did not significantly affect total extractable N concentrations at any moisture level.

C additions only marginally affected DOC (χ^2_4 = 8.85; $P = 0.06$), which was driven by DOC in methanol-amended soils being 11% lower than that in glucose-amended soils (pairwise $P = 0.09$; Supplemental Fig. 1). And there was no moisture effect or interaction between moisture and carbon source on DOC (χ^2_{16} = 19.39; $P = 0.249$).

Microbial community composition

There was a main effect of C amendment on Shannon Diversity (χ^2_4 = 18.40; $P = 0.001$; Fig. 2(A)), which was 2.9–3.5% lower in the methanol treatment than the control and the two LMW-DOC treatments. This decrease in diversity was driven by lower ASV evenness, i.e., Simpson index (χ^2_{16} =19.40; $P < 0.001$; Supplemental Fig. 2A), which was marginally reduced in the methanol treatment by 0.5–0.7% (all pairwise $P < 0.1$). There was also a significant interaction between C amendment and moisture on Shannon Diversity (χ^2_{16} = 33.01; $P = 0.007$; Fig. 2(B)). Specifically, at 25%

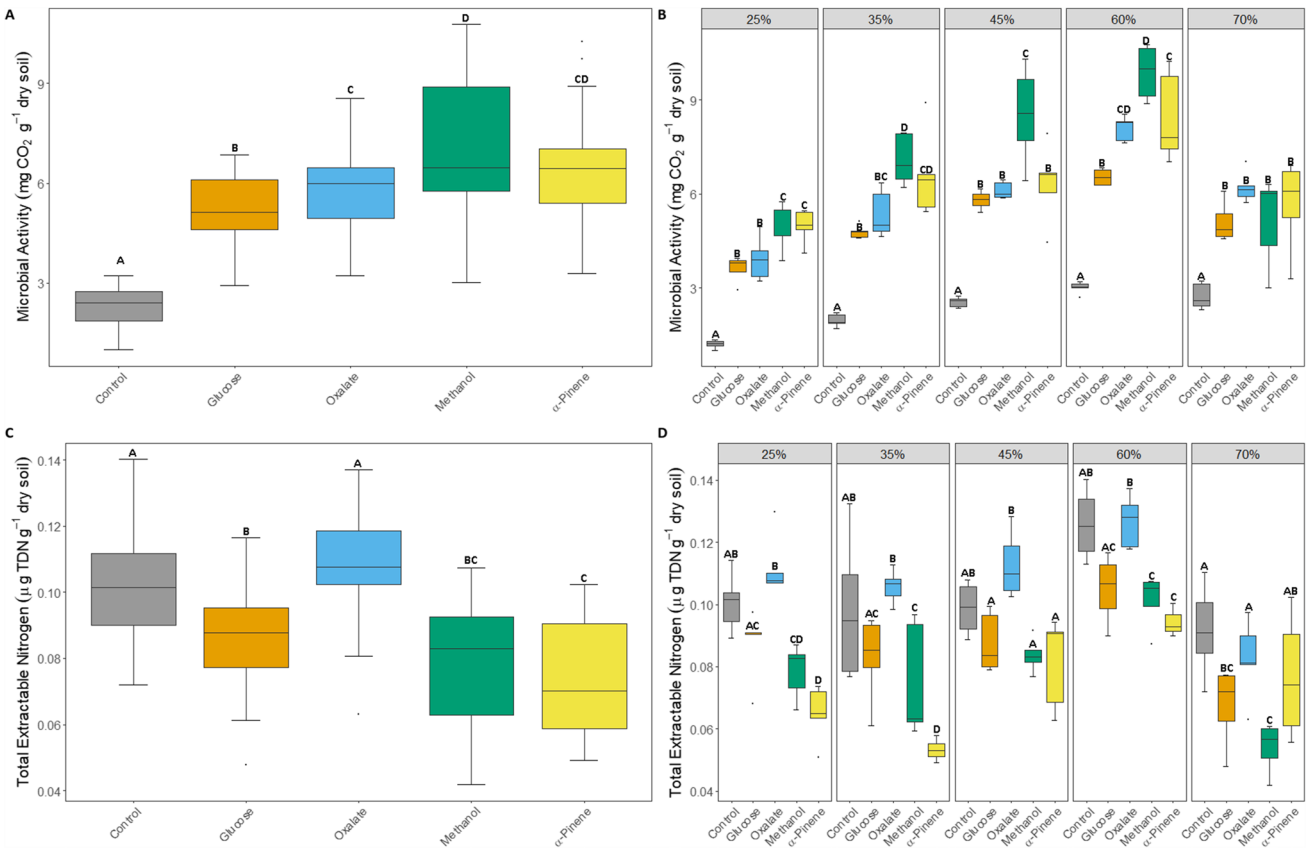

Fig. 1 Microbial activity as determined by integrating respiration over the entire 28-day experiment for both carbon source (A), and the carbon × soil moisture effects (B). Total extractable nitrogen assessed at the end of the experiment for both the carbon source effect (C), and the carbon × soil moisture effects (D). Different letters denote statistically significant treatment effects based on pairwise comparisons adjusted using Tukey's test, α = 0.1

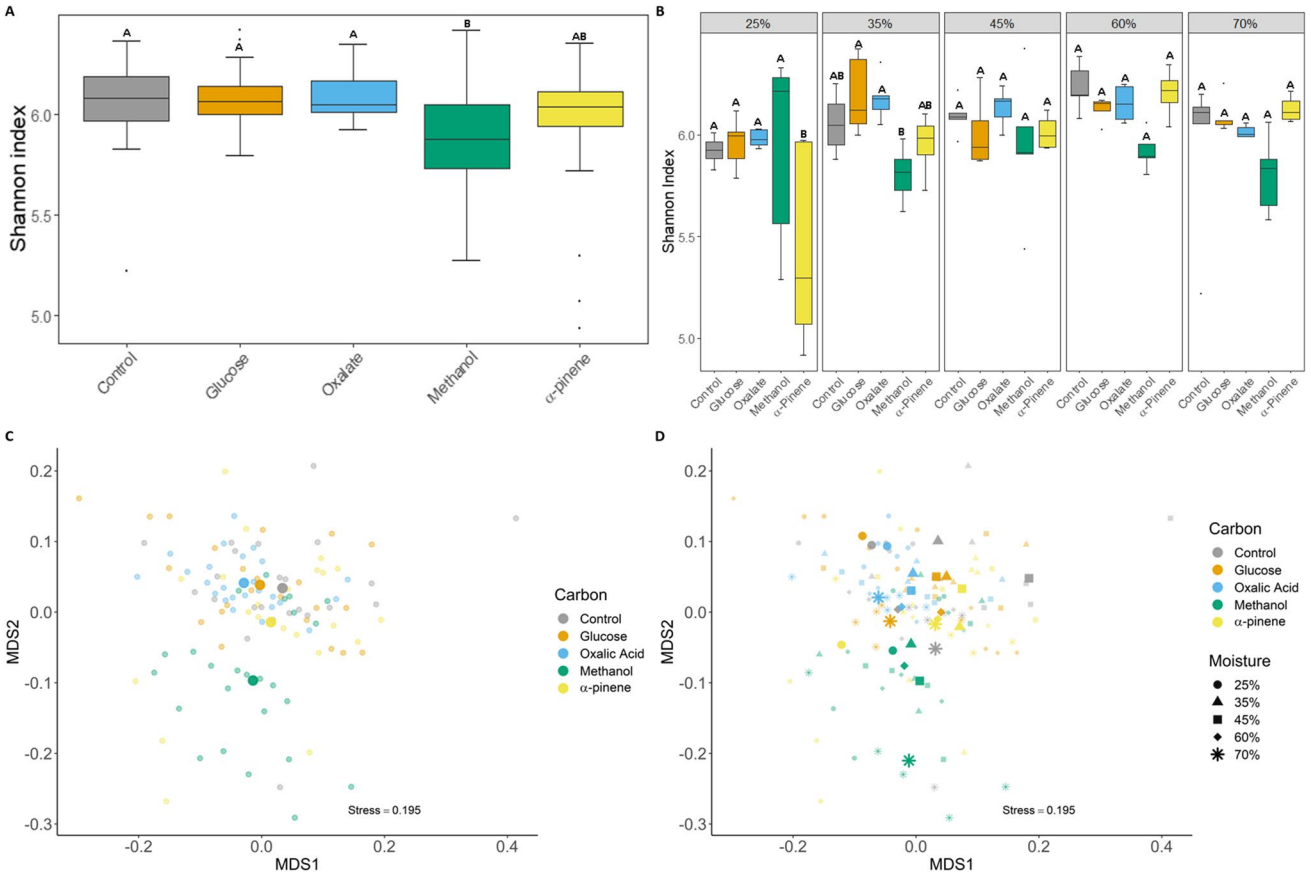

Fig. 2 Shannon diversity for the carbon source (A), and the carbon source × soil moisture effects (B). And NMDS visualization of prokaryotic communities using Bray–Curtis dissimilarity for the carbon source (C), and the carbon source × soil moisture effects (D). Different letters denote statistically significant treatment effects based on pairwise comparisons adjusted using Tukey's test, $\alpha = 0.1$. Displayed on the ordinations are centroids (large dark shapes), and individual samples (small light shapes)

WHC, α-pinene had 8.1–9.0% lower diversity than the control (pairwise $P < 0.1$), and all other C treatments (all pairwise $P < 0.05$). This reduction in diversity was also driven by lower ASV evenness, with α-pinene having 1.0–1.8% lower Simpson diversity than the other treatments at 25% WHC ($\chi^2_{16} = 28.48$; $P = 0.028$; Supplemental Fig. 2B).

PERMANOVA analysis of microbial ASVs using Bray–Curtis distances also identified both an overall C effect (pseudo $F_4 = 2.98$, $P = 0.001$), and an interactive effect of moisture and C amendment (pseudo $F_{16} = 1.41$, $P = 0.001$). Pairwise PERMANOVA of microbial community compositions indicated that all C treatments were significantly different from the control when averaged across all moisture levels (Fig. 2(C)). The most apparent differences in Bray–Curtis distances visualized with non-metric multidimensional scaling (NMDS) were clear separation of methanol from the other C treatments at all moisture levels, and α-pinene at low moisture only (Fig. 2(D)).

Across all treatments, the ten most abundant phyla were the Proteobacteria (41.3%), Acidobacteria (16.4%), Bacteroidetes (10.1%), Verrucomicrobia (7.7%), Actinobacteria (7.2%), Planctomycetes (3.4%), Chloroflexi (2.5%), Firmicutes (2.3%), Nitrospirae (2.0%), and Thaumarchaeota (1.9%) (Fig. 3A). To link specific prokaryotic taxa to microbial functional responses (i.e., microbial activity, and total extractable N), we performed best-subsets multiple regression. The best-subsets regression model for predicting microbial activity—using the most abundant 10 phyla as candidate predictor variables—contained Chloroflexi, Nitrospirae, Proteobacteria, and Verrucomicrobia. Higher Verrucomicrobia abundance was associated with lower microbial activity while the other three phyla were associated with higher microbial activity (Table 1). The best-subsets regression model for predicting total extractable N contained Actinobacteria, Bacteroidetes, and Thaumarchaeota, of which Actinobacteria was associated with lower total extractable N while the other two were associated with higher total extractable N (Table 1).

All four phyla in the best-supported microbial activity GLM model responded significantly to C amendment (Fig. 3B): Proteobacteria ($\chi^2_4 = 44.60$; $P < 0.001$),

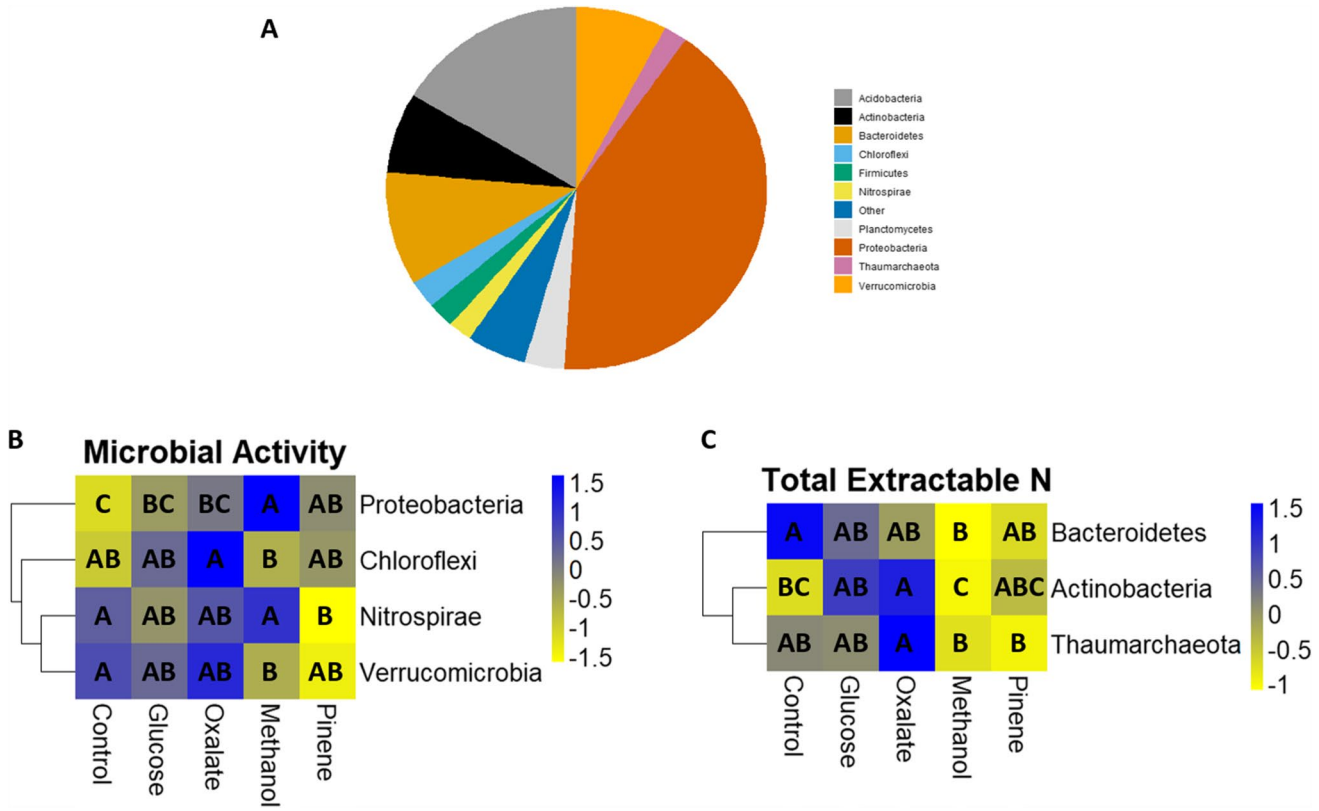

Fig. 3 The 10 most abundant phyla across all treatments (**A**), and the phyla retained in the best-subsets multiple regression for microbial activity (**B**), and total extractable N (**C**). Different letters denote statistically significant treatment effects based on pairwise comparisons adjusted using Tukey's test, α = 0.1. Dendrograms on heat maps reflect similarity of relative abundance patterns of phyla between carbon treatments (complete-linkage clustering)—they do not reflect phylogenetic relationships

Table 1 Best-subsets multiple regression models of predictor phyla for each functional response

Response	Predictors in Top model	Coefficients	Adj. R^2
Microbial activity	Chloroflexi	0.389	19.3
	Nitrospirae	0.515	
	Proteobacteria	0.486	
	Verrucomicrobia	−0.538	
	Intercept	5.47	
Total extractable N	Actinobacteria	−0.007	14.7
	Bacteroidetes	0.006	
	Thaumarchaeota	0.013	
	Intercept	0.09	

Nitrospirae (F_4 = 44.60; $P = 0.029$), Chloroflexi (F_4 = 2.48; $P = 0.049$), and Verrucomicrobia (F_4 = 2.03; $P = 0.096$). Methanol increased the relative abundance of Proteobacteria 6.4–16.4% relative to the control and all other C treatments, and α-pinene increased Proteobacteria 9.4% more than control. In contrast, methanol reduced the relative abundance of Verrucomicrobia by 12.4% relative to the control. Chloroflexi were significantly affected by C amendments, driven by oxalate-treated soils having relative abundance 13.2% higher than methanol-treated soils. Proteobacteria (X^2_{16} = 28.54; $P = 0.027$) and Nitrospirae (F_{16}= 3.36; $P < 0.001$) were affected by a significant interaction between C amendment and moisture level (Supplemental Fig. 3). Notably, at 25% WHC, α-pinene increased Proteobacteria relative abundance by 27–28% above the control and the two LMW-DOC treatments, while at 60% WHC, methanol addition increased the relative abundance of Nitrospirae by 44.9% above the control and 32–34% above the other C treatments. However, at the highest moisture, both glucose (pairwise $P = 0.054$) and methanol (pairwise $P < 0.001$) reduced Nitrospirae relative abundance relative to the control (Fig. 3B), by 21% and 34% respectively.

All of the phyla retained in the best-subsets regression model for predicting total extractable N exhibited a significant C amendment response: Thaumarchaeota (F_4 = 4.25; $P = 0.003$), Actinobacteria ($X^2_4 = 25.57$; $P < 0.001$), and Bacteroidetes (F_4 = 2.25; $P < 0.069$). However, none of those phyla had a significant interaction with moisture. Methanol

marginally reduced Thaumarcheota relative abundance by 24.6% below the control (pairwise $P = 0.075$). Likewise, there was higher relative abundance of Thaumarcheota in the oxalate treatment than in both methanol and α-pinene (pairwise $P < 0.05$). Oxalate marginally increased relative abundance of Actinobacteria relative to the control (pairwise $P = 0.091$), and both glucose and oxalate treatments had ~ 31% greater relative abundance of Actinobacteria than the methanol treatment (pairwise $P < 0.05$; Fig. 3C). Methanol marginally reduced the relative abundance of Bacteroidetes by ~ 15.9% below the control (pairwise $P = 0.058$; Fig. 3C).

Discussion

Volatile organic compounds have distinct effects on microbial activity, soil N cycling, and prokaryotic community composition compared to LMW-DOC. While α-pinene—and to some extent methanol—consistently interacts with soil moisture to affect soil functional and compositional variables, our other predictions related to the moisture dependence of C source effects were largely not supported. Of particular interest, effects of C amendments on microbial activity and extractable N were associated with changes in specific prokaryotic taxa, thereby demonstrating clear links between community composition and community processes that could scale to the ecosystem level links between community.

Though interactions between C and soil moisture were mostly insignificant, we did observe notable effects of the hydrophobic VOC α-pinene at low moisture levels. Although both VOC treatments (α-pinene, and methanol) increased microbial activity more than both LMW-DOC treatments (glucose and oxalate) at the lowest moisture (Fig. 1(B)), α-pinene did not differ from either DOC treatment at any other moisture (Fig. 1(A)). This supports our hypothesis that VOCs may play an important role as a C source in drier soils. However, the hypothesis that LMW-DOC would only have effects at higher moisture levels was not supported. It is possible that our study did not reach a low-enough soil moisture level to cause significant inhibition of LMW-DOC diffusion or that the microbial availability of these compounds is so high that the community within the zone of application was able to use all of the amended C.

The general effect of C addition was to increase microbial activity and decrease total extractable N (Fig. 1). Since the microcosms had no other C inputs, all C amendments alleviated C limitation and provided soil microorganisms with readily available substrates [36, 37]. The increase in microbial activity likely drove a concomitant increase in microbial N demand [38]. Indeed, C amendments decreased total extractable N in all C treatments except oxalate (Fig. 1(C)). Although oxalate increased microbial activity, its effect on total extractable N may have been mediated by the ability of organic acids like oxalate to act as a chelating agent—extracting organic N from soil [39]. These results support the importance of both VOCs and LMW-DOC as C substrates for soil microbial communities and suggest that VOCs can have equivalent if not greater effects on microbial activity—including N demand.

Some of the variability in microbial activity and N demand can be explained by specific characteristics of the C source. VOCs have a large diversity of chemical properties such as an ability to dissolve in water. For example, α-pinene does not dissolve in water so its ability to diffuse through soil air spaces decreases with increasing water content. This inability to diffuse through soil water is reflected in a reduced effect of α-pinene on microbial activity and total extractable N with increasing moisture level. Alternatively, methanol is miscible with water so it is able to diffuse freely through both air and water. Methanol enters several metabolic pathways primarily as an energy source instead of building biomass [40]—therefore, it is likely that most of the methanol C was mineralized. However, future studies would benefit from using stable isotope labeled compounds since our study is unable to determine the exact fate of the added C. Interestingly, both VOCs increased the relative abundance of Methylophilaceae (Supplemental Table 1; Supplemental Fig. 4A), a diverse group of known methylotrophs [41]. This enrichment of methylotrophs could explain the decrease in Shannon and Simpson diversity observed (Fig. 2, Supplemental Fig. 2), as the Methylophilaceae became a dominant taxa in all methanol treatments, and with α-pinene addition at the two lowest moisture levels (Supplemental Fig. 4A), while being rare in other samples. Likewise, Opitutaceae, which contain facultative methylotrophs [42], were enriched by methanol addition at the highest moisture level (Supplemental Table 1; Supplemental Fig. 4A). Methylotrophs are known to metabolize methylated compounds like α-pinene in addition to methanol and methane (Lindstrom 2006). Since Methylophilaceae are Proteobacteria, which were positively associated with microbial activity in the best-supported GLM model and also had strong positive responses to both VOCs, it is likely that VOC additions to soils increased abundance of Methylotrophs in the VOC treatments.

Several taxa decreased in relative abundance due to VOC addition (Supplemental Fig. 4). It is possible that these decreases were due to other taxa increasing in abundance, which is supported by the fact that VOCs reduced microbial community evenness (Supplemental Fig. 2). However, it is also possible that some of these taxa experienced reductions in actual abundance since both α-pinene and methanol have previously been shown to have inhibitory effects, especially on nitrification and nitrifying taxa—e.g., *Nitrosomonas* spp., *Nitrospira* spp.,

and Thaumarcheaota [16, 17, 43]. We saw lower relative abundance of Thaumarchaeota in the two VOC treatments (Fig. 3B), which contains all known ammonia-oxidizing archaea. Further, the Thaumarchaeota were positively associated with extractable N in the best-supported model for predicting soil total extractable N content (Table 1). This suggests that their abundance is linked to N availability, which has previously been suggested as a key covariate of ammonia oxidizer abundance [44]. Interestingly, α-pinene also inhibited the Nitrospirae, a group of chemolithotrophs that contain nitrite-oxidizing bacteria and the complete ammonia oxidizers (commamox). Commamox have previously been shown to be the most abundant ammonia oxidizers in some forest soils [45], and Nitrospirae overall were positively associated with microbial activity in the best-supported GLM model (Table 1). The inclusion of Nitrospirae in the microbial activity model is potentially linked to their interactions with heterotrophic microorganisms, or simply reflects environmental conditions that promote both nitrification and overall microbial activity, rather than a direct link to CO_2 flux.

LMW-DOC compounds such as glucose have high C-use efficiencies [46] which means more C is incorporated into biomass instead of being mineralized [47]. These LMW-DOC compounds are thought to be favored by copiotrophic taxa such as the Proteobacteria, while oligotrophic taxa such as the Verrucomicrobia typically utilize more complex C sources [9, 48]. Indeed, the Proteobacteria were positively associated with microbial activity, and the Verrucomicrobia were negatively associated with microbial activity in our study. Likewise, Actinobacteria, which also contains many copiotrophs [49], trended toward higher relative abundance under both LMW-DOC treatments (Fig. 3). However, Actinobacteria were only included in the best-supported total extractable N GLM model and were negatively associated with soil N content (Table 1). This is possibly because several Actinobacteria families including Micrococcaceae and Nocardiaceae have the ability to fix atmospheric N [50, 51]. Some taxa responded specifically to oxalate addition such as Roseiflexaceae (Supplemental Fig. 4), which increased in relative abundance, and are thought to have the ability to directly assimilate low molecular weight organic acids like oxalate [52]. Likewise, total extractable N increased under oxalate addition, which coincided with an increase in Thaumarcheota, possibly because oxalate lowered soil pH and ammonia-oxidizing archaea are known to thrive in acidic environments [53, 54].

In our study, we highlight the importance of considering different C types—LMW-DOC vs VOC—when investigating soil communities and biogeochemical processes. Different C types appear to have distinct effects on microbial community composition. In particular, VOCs like methanol and α-pinene appear to be available to a taxonomically narrow group of organisms such as the methylotrophs, while LMW-DOC is metabolized by a broad range of microorganisms. Though both C types tend to affect microbial functions similarly, the magnitude of responses depends on C types and sometimes interaction between C type and moisture. While our findings are limited to only a single soil, these results highlight the importance of considering different C source effects on soil processes under intensifying drought, and the potential importance that VOCs may play in drier soils.

Data

Data are archived at figshare, doi:10.6084/m9.figshare.16918900.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00248-022-01967-0.

Author Contribution Steven G. McBride, Michael S. Strickland, and John E. Barrett contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Steven G. McBride, Ernest D. Osburn, Jane M. Lucas Taylor Brown, and Julia S. Simpson. The first draft of the manuscript was written by Steven G. McBride and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

Competing Interests The authors declare no competing interests.

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