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Volatile organic compounds from leaf litter decomposition alter soil microbial communities and carbon dynamics

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Abstract. Investigations into the transfer of carbon from plant litter to underlying soil horizons have primarily focused on the leaching of soluble carbon from litter belowground or the mixing of litter directly into soil. However, previous work has largely ignored the role of volatile organic compounds (VOCs) released during litter decomposition. Unlike most leaf carbon, these litter-derived VOCs are able to diffuse directly into the soil matrix. Here, we used a 99-d microcosm experiment to track VOCs produced during microbial decomposition of ^{13}C-labeled leaf litter into soil carbon fractions where the decomposing litters were only sharing headspace with the soil samples, thus preventing direct contact and aqueous movement of litter carbon. We also determined the effects of these litter-derived VOCs on soil microbial community structure. We demonstrated that the litter VOCs contributed to all measured soil carbon pools. Specifically, VOC-derived carbon accounted for 2.0, 0.61, 0.18, and 0.08% of carbon in the microbial biomass, dissolved organic matter, mineral-associated organic matter, and particulate organic matter pools, respectively. We also show that litter-derived VOCs can affect soil bacterial and fungal community diversity and composition. These findings highlight the importance of an underappreciated pathway where VOCs alter soil microbial communities and carbon dynamics.

Key words: *ammonium; carbon cycle; carbon sequestration; microbial biomass; microbial diversity; mineral associated organic matter; nitrate; particulate organic matter; stable isotope probing; target gene sequencing; VOC.*

Introduction

Much of the research on leaf litter decomposition focuses on factors that determine mass loss of the litter itself, for example, soil biota, climate, and litter quality (Aerts 1997, Bradford et al. 2016). From this focus, we know that the leaf litter type that accumulates on the soil surface can affect biotic and abiotic characteristics of the underlying mineral soils (Hobbie 1992, Binkley and Giardina 1998); that is, changes in the types of leaf litter inputs can alter soil microbial communities, nutrient dynamics, and soil organic C dynamics (Aerts 1997, Cotrufo et al. 2013). Furthermore, this focus leads to the understanding that leaf litter decomposition contributes to soil organic matter (SOM) formation primarily through two pathways: (1) high-quality, usually water-soluble C (e.g., leaf litter leachates) is rapidly decomposed, then assimilated into microbial biomass and other soil organisms (Soong et al. 2016, Joly et al. 2018) before stabilizing in the mineral-associated organic matter (MAOM); and (2) plant structural materials are mechanically pulled apart, and physically incorporated directly into the particulate organic matter (POM) of the underlying mineral soil horizons (Cotrufo et al. 2015, Kalbitz and Kaiser 2003, Bradford et al. 2013, Sokol and Bradford 2019). However, this research largely overlooks the potential of litter-derived volatile organic compounds (VOC) to shape soil microbial communities and soil biogeochemical processes. Given that VOCs are produced in high quantities during leaf litter decomposition (Ramirez et al. 2010), and can readily diffuse from decomposing litter into underlying soil horizons through air-filled pore spaces, these VOCs could represent an important mechanism by which plant-derived C can enter soil and contribute to SOM formation.

Volatile organic compounds are usually small C-containing compounds with high vapor pressure and low boiling points, which allows these compounds to transition between liquid and vapor phase readily. Biogenic

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VOC production during litter decomposition is 5–10 times higher than abiotic VOC production, and produces dozens of different volatiles, for example, alcohols, carbonyls, and monoterpenes (Gray et al. 2010). Microbially produced volatiles mediate many microbe–microbe, microbe–plant, and microbe–animal interactions (Bitas et al. 2013, Schmidt et al. 2015, Schulz-Bohm et al. 2017). Although variable, the total quantities of VOCs released during leaf litter decomposition can be surprisingly high, occasionally exceeding 100 μmol VOC-C·g-litter^{-1}·h^{-1} (Ramirez et al. 2010), with some litter types emitting VOCs at rates that approach those of CO_2-C from litter decomposition (Gray et al. 2010). Because of their abundance, litter-derived VOCs could represent an important, rarely considered, source of organic C to underlying soils; for example, we conservatively estimate that VOC emissions from *Pinus* litters (3–11 g of VOC-C·m^{-2}·yr^{-1} (Gray et al. 2010) are similar to reported rates of root-exudate C inputs from *Pinus taeda* (9 g·m^{-2}·yr^{-1} of root-exudate C; Phillips et al. 2008). Consumption of VOCs by microbes found in mineral soil can be significant (Owen et al. 2007, Gray et al. 2014). Indeed, soils exposed to litter VOCs absorbed 80% of the VOCs emitted from decomposing litter (Ramirez et al. 2010), and respiration in soils exposed to VOCs increases significantly (Asensio et al. 2012). Beyond C dynamics, methanol and acetone—common litter-derived VOCs—have been shown to affect nitrogen (N) transformations (McBride et al. 2019), as have monoterpenes (Paavolainen et al. 1998, Smolander et al. 2006). Likewise, monoterpenes may also inhibit soil enzyme activity (Adamczyk et al. 2015). The mechanisms of these VOC effects are not yet clear. However, it could be driven by VOC-induced changes to the microbial community through limiting or promoting the growth of specific microbial taxa, for example, methylotrophic bacteria (Wheatley 2002, Gray et al. 2015), increasing microbial activity (McBride et al. 2019), or inhibiting soil microbes (Asensio et al. 2012).

We designed a microcosm study using three litter types to test our expectation that VOCs emitted from decomposing litter influence soil C dynamics and soil microbial communities, even when the decomposing litters are not in direct contact with the soil surface. We chose three litter types for two primary reasons: (1) the litter types were expected to vary in the type and quantity of VOCs produced during decomposition and (2) the litter types differed in chemical recalcitrance of the litter material, which commonly affects the rate of decomposition and VOC production (Gray and Fierer 2012). We expected that if VOCs emitted during decomposition represent a significant C source to soils, then we would detect litter-derived C in multiple soil pools, and this may be dependent on litter type. Additionally, we expected that VOCs emitted during decomposition would lead to change in soil microbial communities, because VOCs may act as a resource for some microbes while inhibiting others (Ramirez et al. 2010, McBride et al. 2019). By using ^{13}C-labeled leaf litter, we tracked litter-derived VOC-C into several soil C pools throughout a 99-d incubation period to determine if and to what extent VOCs contribute to soil C pools and the composition of soil microbial communities.

Methods

Experimental design

To determine the influence of litter-derived VOCs on soil processes and soil microbial community composition, we employed a microcosm approach paired with ^{13}C tracking using chambers that physically separated leaf litter decomposition from the soil (Appendix S1: Fig. S1). To construct these microcosms, we added 25 g of dry weight equivalent soil to a 473-mL glass jar (~0.5 cm deep). The soil was sourced from a single site near Blacksburg, Virginia, USA (37.20, −80.58): the soil was identified using the U.S. Department of Agriculture soil classification system as a fine, mixed, semiactive, mesic Typic Hapludults in the Unison series (loam texture), similar to the World Reference Base classification Xanthic Acrisols (Paul McDaniel, *personal communication*); dominant plant cover are grasses (primarily *Festuca arundinacea*, as well as some herbaceous cover including members of the Lamiaceae and Plantaginaceae families). Six cores, 8 cm wide and 10 cm deep, were collected, sieved to 4 mm, and homogenized before being stored at 4°C. Within each of the large jars, we placed a second smaller jar (20 mL volume) (Appendix S1: Fig. S1). To each of the smaller jars, we added 2 g of air-dried ^{13}C-labeled leaf litter from one of three litter species (sourced from IsoLife, Wageningen, The Netherlands): eucalyptus (*Eucalyptus grandis*; 97 atom% enriched), tulip poplar (*Liriodendron tulipifera*; 95 atom% enriched), or switchgrass (*Panicum virgatum*; 97 atom% enriched). The leaf litter was then inoculated with the soil described above to establish an active microbial decomposer community by adding the inoculant (1 g dry wt soil:99 mL deionized water) at 700 μL/g^{-1} dry wt litter and covering with a 15-μm mesh to allow for VOC permeability but reduce the chance of solid matter escaping. Note that the microbial community inoculum likely does not share a common history with the litter species used in this experiment, which may lead to variation in decomposition dynamics (Strickland et al. 2009). Soil in the large jar and litter in the small jar were maintained at 65 and 50% water holding capacity, respectively, at 20°C throughout the 99-d experiment. Jars were loosely capped in order to minimize evaporative moisture loss; however, this may lead to an overestimation of VOC contribution to the measured soil C pools. In addition to each litter–soil treatment, we also included sets of "soil-only" and "litter-only" control microcosms. Both sets were constructed as described above, except the small 20-mL jar was left empty in the soil-only controls, and no soil was placed in the large

jars for the litter-only microcosms. The experiment consisted of 28 microcosms in total: 12 litter–soil treatment microcosms (4 replicates × 3 litter types), 12 litter-only microcosms (4 replicates × 3 litter types), and 4 soil-only microcosms (4 soil replicates).

Litter CO_2 production and soil C and N pools

To estimate rates of leaf litter decomposition, we tracked litter CO_2 production for all experimental units across the 99-d experiment (days: 2, 6, 9, 14, 21, 28, 37, 43, 50, 64, 71, 85, 99) using a static chamber technique. At the conclusion of the 99-d experiment, we destructively harvested each microcosm containing soil and determined microbial biomass C (MBC), extractable dissolved organic C (DOC), MAOM C and N, POM C and N, NH_4-N, NO_3-N, and the species composition of both the soil prokaryotic (bacteria plus archaea) and fungal communities (Appendix S1; Supporting methods). For MBC and extractable DOC, we conducted a modified chloroform fumigation extraction (Fierer and Schimel 2003). Hereon we will refer to extractable DOC simply as DOC, which we operationally define as the fraction of organic carbon that passes through a 0.45-μm filter after extraction by agitation in 0.5 mol/L K_2SO_4. We determined soil NO_3-N and NH_4-N concentrations of the unfumigated extracts using a Lachat QuikChem flow injection analyzer (Hach Company, Loveland, Colorado, USA). To determine MAOM and POM C and N pools, we used the fractionation method described in Paul et al. (2001). Additional details are in Appendix S1, and data are archived at figshare.[7]

Determining the contribution of litter-derived VOCs to soil C pools

To establish the amount of leaf-litter-derived VOC-C, we determined the $\delta^{13}C$ signatures of the following soil C pools: MBC, DOC, POM-C, and MAOM-C. For microbial biomass and DOC, $\delta^{13}C$ values of liquid extracts were determined using an isotope ratio mass spectrometer (IRMS; Thermo Finnigan, San Jose, California, USA, Model: Delta Plus XP) following the method described by Lang et al. (2012). For POM and MAOM C, $\delta^{13}C$ values were determined using an elemental analyzer paired with the IRMS. Resulting delta values were converted to atom% using the following equation:

$$\text{atom\%} = 100 \times \frac{\left(\delta^{13}C_{\text{sample}} + 1{,}000\right)}{\left(\delta^{13}C_{\text{sample}} + 1{,}000 + \left(\frac{1{,}000}{R_{\text{std}}}\right)\right)}$$

where R_{std} is the $^{13}C/^{12}C$ ratio of the Vienna Pee Dee Belemnite (VPDB) standard, and $\delta^{13}C_{\text{sample}}$ is the delta value for a given sample.

[7] https://doi.org/10.6084/m9.figshare.12323825.v1

The contribution of litter-derived VOCs to the soil C pools was estimated using stable isotope mixing models via the following equation (sensu Ineson et al. 1996):

$$C_{\text{VOC derived}} = C_{\text{pool}} \times (\text{atom\%}^{13}C_{\text{VOC exposed}} - \text{atom\%}^{13}C_{\text{Soil}}) / (\text{atom\%}^{13}C_{\text{Litter}} - \text{atom\%}^{13}C_{\text{Soil}})$$

where C_{pool} is the total amount of C in a given pool, $\text{atom\%}^{13}C_{\text{VOC exposed}}$ is the atom%^{13}C value of a given pool after exposure to litter-derived VOCs, $\text{atom\%}^{13}C_{\text{soil}}$ is the atom%^{13}C value of a given pool not exposed to litter-derived VOCs (i.e., the soil-only controls), and atom %^{13}C litter is the atom%^{13}C value of the actual litter. Data are archived at figshare.[8]

Determination of litter-derived VOC effects on soil microbial community composition

We assessed the diversity and composition of the microbial communities in the soils exposed to the litter-derived VOCs (the litter–soil microcosms) as well as in the soils incubated in the absence of any litter-derived VOCs (the soil-only microcosms) to determine how exposures to litter VOCs alone may alter soil microbial communities. To do so, we extracted total genomic DNA from the soil samples at the end of the 99-d experiment and sequenced the V4 hypervariable region of the 16S rRNA gene for bacterial and archaeal communities and the internal transcribed spacer (ITS1) region for fungal communities using amplicon sequencing methods described previously (Fierer et al. 2012, McGuire et al. 2013; additional details in Appendix S1). In total, 4,422 bacterial and archaeal exact sequence variants (ESVs) and 1,964 fungal ESVs across the 16 samples were used for all downstream analyses. ESV tables and sequence data from this project are available on figshare.[9]

Statistical analyses

Statistical analyses of cumulative litter CO_2 production, soil C and N pools, the contribution of litter-derived VOCs to soil C pools, and microbial communities were conducted in R (R Development Core Team 2017). Differences between litter species and the soil-only control were determined via analysis of variance (ANOVA). Pairwise treatment comparisons were assessed via Tukey's honestly significant difference. When reported, data were $\log_{10}$-transformed to meet model assumptions (verified using model checking) or if necessary generalized linear models (GLM) were employed. In cases where GLM was used, we first determined an appropriate distribution to fit the data; in all of those cases we used a gamma distribution with the log link function. Differences between microbial community richness across litter treatments were determined with

[8] https://doi.org/10.6084/m9.figshare.12323825.v1
[9] https://doi.org/10.6084/m9.figshare.6882899.v1

ANOVA. Differences in microbial community composition between treatments were visualized using principal coordinate analysis (PCoA) of Bray–Curtis dissimilarities after square-root transformation, and permutational ANOVA was used to assess statistical differences following 999 permutations using the R package 'vegan' (Oksanen et al. 2019). Finally, we used the nonparametric Kruskal–Wallis (KW) test to determine taxonomic groups (i.e., classification at phylum, class, order, and family) whose relative abundances differed between treatments, $\alpha = 0.05$ (uncorrected P value) using the R package 'mctoolsr' (https://github.com/leffj/mctoolsr/)—omitting rare taxa with relative abundances <0.025.

Results

Contribution of litter-derived VOCs to soil C pools

Litter decomposition was highest in the switchgrass litter, followed by tulip poplar, and eucalyptus (Fig. 1; $F_{3,12} = 343.6$; $P < 0.001$). The soil-only microcosms had CO_2 production rates that were approximately ninefold lower than those observed for any of the litter-only microcosms. Litter-derived VOCs contributed appreciably to all measured soil C pools (Fig. 2A). Across all leaf litter species, litter-derived VOCs accounted for between 0.44 and 4.06% of the C in the MBC pool (Fig. 2A). The greatest percentage of litter-derived VOC-Cs in the MBC pool was associated with decomposing eucalyptus litter, switchgrass had the lowest percentage, and tulip poplar was intermediate between the two (Fig. 2B; $F_{2,9} = 10.4$; $P < 0.01$). For the DOC pool, litter-derived VOCs accounted for between 0.32 and 1.41% of (Fig. 2 A). Although litter-derived VOCs contributed to the DOC pool, no significant differences between litter types were observed (Fig. 2C; $F_{2,9} = 0.59$; $P = 0.32$). For POM C, litter-derived VOCs accounted for between 0.04 and 0.31% of the C in this pool (Fig. 2A). As with the DOC pool, although litter-derived VOCs contributed to the POM C pool, no differences between litter species were observed (Fig. 2D; $F_{2,9} = 1.3$; $P = 0.32$). For MAOM C, litter-derived VOCs accounted for between 0.11 and 0.29% of the C in this pool (Fig. 2A). The greatest percentage of litter-derived VOC-C in the MAOM C pool was associated with decomposing eucalyptus litter as compared to the decomposing switchgrass and tulip poplar (Fig. 2E; $F_{2,9} = 5.95$; $P < 0.05$). Finally, enrichment of soil C pools coincided with differences in N pool sizes; NO_3^- concentrations were highest in switchgrass and tulip poplar, and NH_4^+ concentrations were highest in switchgrass (Appendix S1; Table S1). Additional results pertaining to soil C and N pools, and atom% and mass of ^{13}C associated with these pools are reported in Appendix S1.

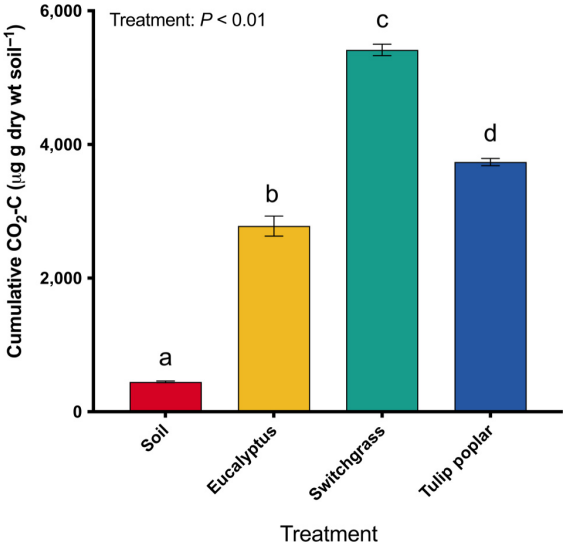

Fig. 1. Cumulative C mineralization determined via integration for the entire time course of the experiment associated with the three litters (i.e., eucalyptus, switchgrass, and tulip poplar) and the soil-only control. Different letters indicate significant pairwise treatment differences ($n = 4$). Error bars represent the mean ± 1 standard error. [Color figure can be viewed at wileyonlinelibrary.com]

Effect of litter-derived VOCs on microbial community composition

Exposure to litter-derived VOCs resulted in notable variation in soil microbial diversity and community composition of soil-litter microcosms compared to those communities found in the soil-only microcosms. Based on Fig. 3, the soil communities exposed to VOCs from switchgrass and tulip poplar litter were more similar to each other than they were to the communities exposed to eucalyptus litter VOCs. For instance, bacterial and archaeal diversity differed across litter treatments (Fig. 3; $F_{3,12} = 20.7$, $P < 0.0001$), with switchgrass and tulip poplar-exposed soil communities having lower diversity compared to the soils incubated in the absence of decomposing litters. There was no significant difference across treatments for fungal community diversity (Fig. 3; $F_{3,12} = 0.26$, $P = 0.85$). We observed variation in microbial community composition across litter treatments for bacteria and archaea (PERMANOVA; $R^2 = 0.486$, $P = 0.001$) as well as for fungi (PERMANOVA; $R^2 = 0.283$, $P = 0.001$), again with exposure to switchgrass and tulip poplar VOCs leading to the most distinct soil microbial communities as compared to the soils incubated alone (Fig. 3). Finally, the relative abundances of certain microbial taxa increased or decreased depending on exposure to VOCs from the different litters (Appendix S1: Table S2). For example, exposure to switchgrass and tulip poplar VOCs resulted in an increase in relative abundances of candidate phyla WPS-2 and the family Acidobacteriaceae, and these taxa are essentially absent in eucalyptus and soil-only treatments (Appendix S1: Fig. S3). Conversely, we observed a decrease in relative abundances of the phyla

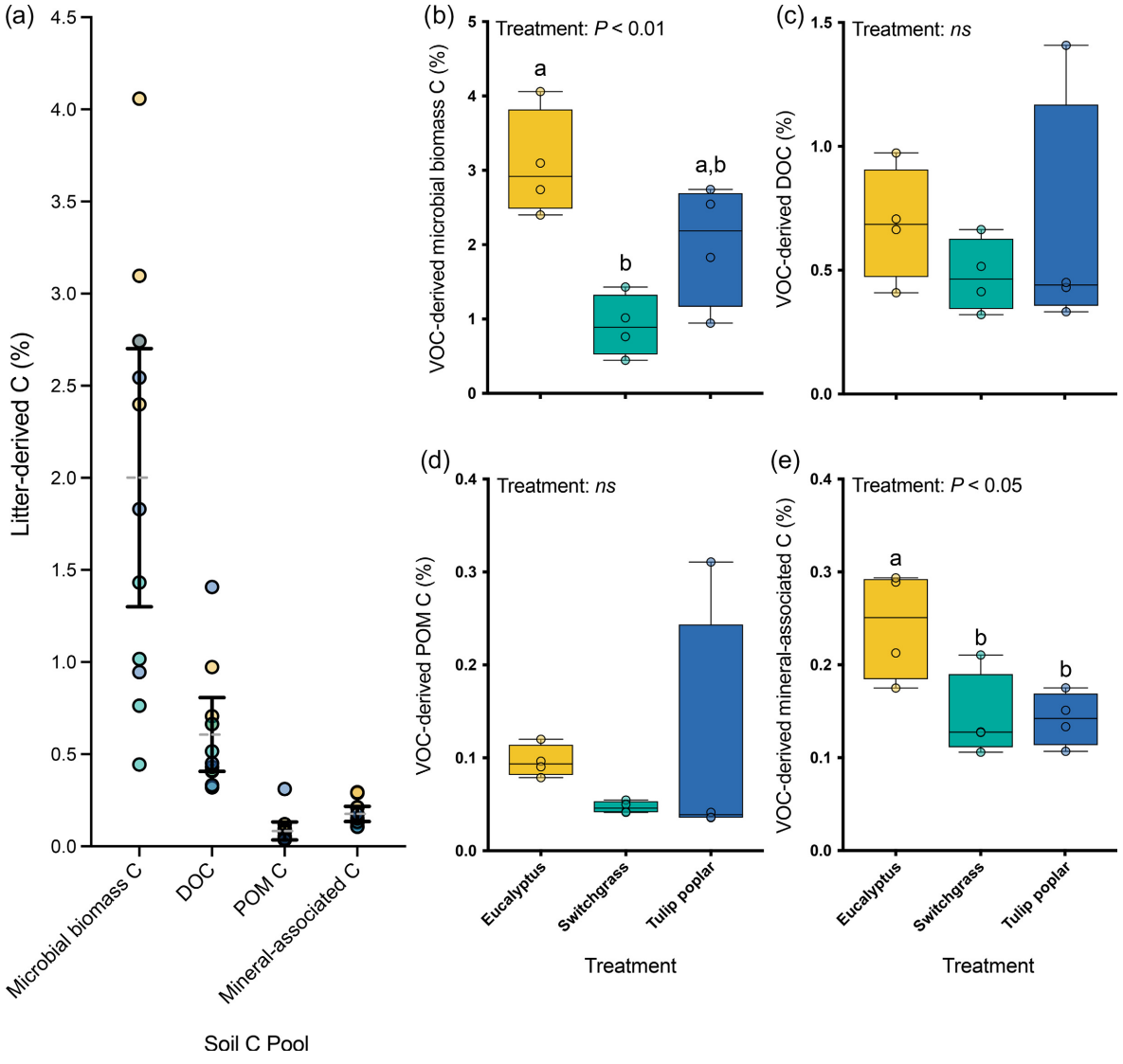

FIG. 2. The contribution of litter-derived volatile organic compounds (VOCs) to measured soil C pools assessed after a 99-d microcosm experiment. (a) Across all species, litter-derived VOCs contributed significant C to the measured pools. Shown is the mean and 95% confidence interval (CI) for each soil C pool. If the CI does not overlap zero, then it can be assumed that litter-derived VOCs contributed significantly to that pool. All data points are shown and colors correspond to the litter treatments as shown in panels (b)–(e). Box and whisker plots show the percentage of VOC-C per litter treatment (i.e., eucalyptus, switchgrass, and tulip poplar) associated with (b) microbial biomass C (MBC), (c) dissolved organic C (DOC), (d) particulate organic matter (POM) C, and (e) mineral-associated soil C. For both MBC and mineral-associated soil C the contribution of VOC-C was dependent on the litter type in question. Different letters indicate significant pairwise treatment differences between litter treatments. [Color figure can be viewed at wileyonlinelibrary.com]

Planctomycetes and the class Blastocatellia in soil communities exposed to switchgrass and tulip poplar VOCs compared to those soils exposed to eucalyptus VOCs and the soil-only treatments (Appendix S1: Fig S3).

Discussion

We investigated the possibility that those VOCs released during leaf litter decomposition can alter soil C dynamics, even without any direct contact between the litters and the soil. Across three leaf litter species of varying chemical recalcitrance, we observed litter-derived VOC-C in all of the measured soil C pools, with VOC-C contributing the most to MBC followed by DOC, MAOM soil C, and POM C. These results highlight the potential for VOC-C emitted from decomposing litters to contribute significantly to soil C pools. In fact, when comparing the contribution of VOC-C vs. soluble low molecular weight C (i.e., glucose) to soil C pools we note several examples where the contribution

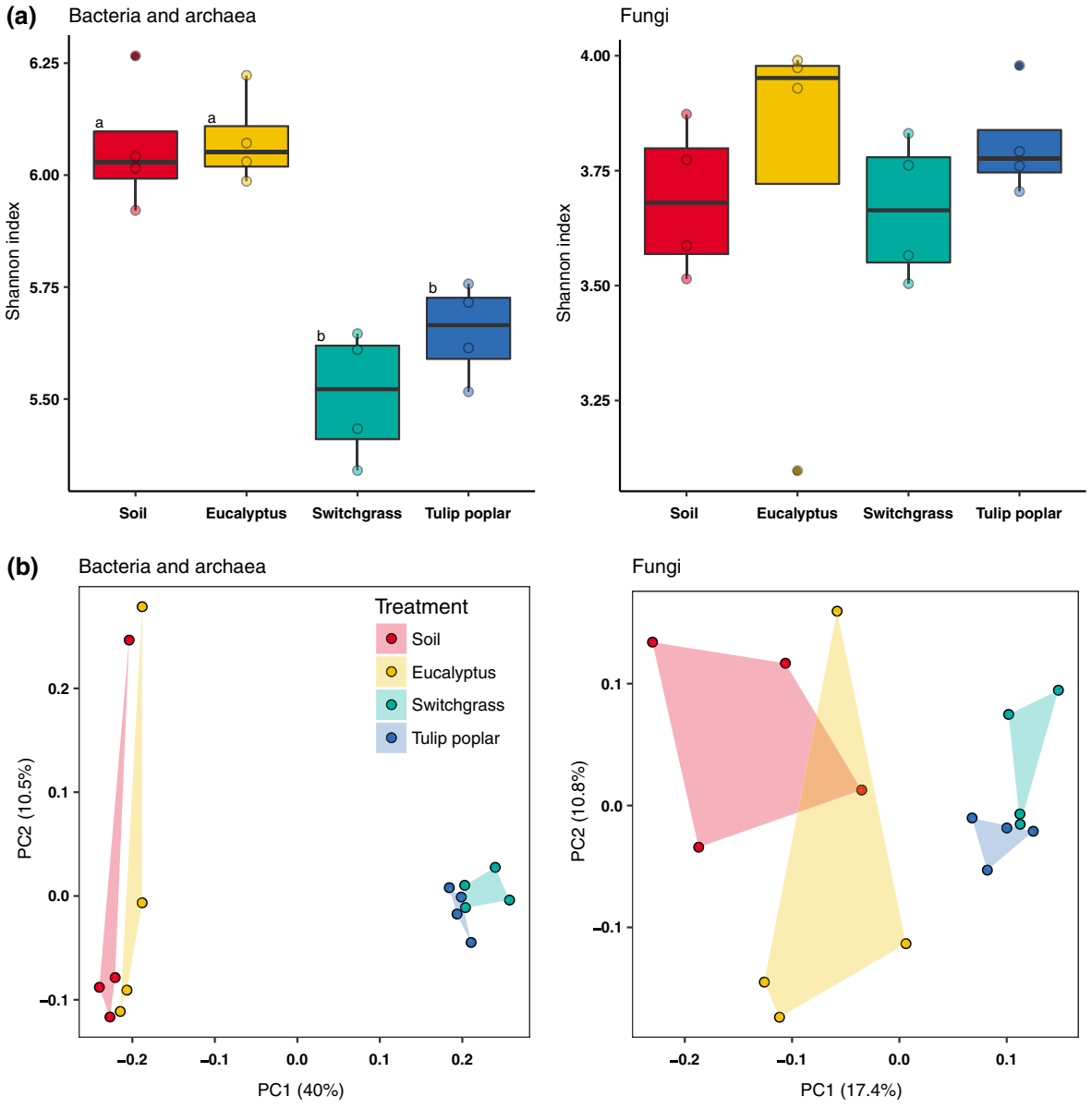

FIG. 3. Richness and species composition of soil microbial communities exposed to litter-derived volatile organic compounds. (a) Box and whisker plots show microbial richness estimates for each litter treatment. Bacterial and archaeal diversity differ across treatments (Shannon index; ANOVA; $F_{3,12} = 20.7$, $P < 0.0001$). Fungal diversity does not differ across treatments (Shannon index; ANOVA; $F_{3,12} = 0.26$, $P = 0.85$). (b) We used principal coordinate analysis (PCoA) to visualize how community composition differed between soil treatments. Each point represents the composition of the microbial soil community (Bray–Curtis dissimilarity with square-root transformation) for each litter treatment. Bacterial and archaeal community composition differs across treatments (PERMANOVA; $R^2 = 0.486$, $P = 0.001$). Fungal community composition also differs across treatments (PERMANOVA; $R^2 = 0.283$, $P = 0.001$). [Color figure can be viewed at wileyonlinelibrary.com]

of VOC-C determined in our experiment is similar to that observed for soils amended directly with glucose. For example, Sokol and Bradford (2019) found that between 0.7 and 7.59% of MBC was derived from ^{13}C glucose under laboratory conditions, and Strickland et al. (2012) observed that ~1% of MBC was derived from ^{13}C-glucose under field conditions. Here we observed on average that 2.0% of MBC was derived from VOC-C, and this ranged from a low of 0.44% to a high of 4.06% depending on the litter type (Fig. 2). We recognize that the levels of VOC-C enrichment may have been artificially inflated in our study because the jars were temporarily capped, trapping VOCs within the vessel, and that the thin layer of soil in our jars (~0.5 cm) may limit how our results would apply to deeper soil horizons, which should be explored in future studies.

Although our experiment was laboratory-based and verification under field conditions is needed, our results suggest that the contribution of VOC-C from decomposing litter to soil microbial biomass may be on par with that observed for glucose and potentially other labile C inputs to soil, including root exudates.

For the other soil C pools, the contribution of litter-derived VOC-C to DOC pools ranged between 0.32 and 1.4%. This is considerably less than is attributed to root-exudate C (Giesler et al. 2007); however, this is likely because low-molecular-weight C from root exudation and litter leachates immediately enters the DOC pool. Additionally, VOC-C contributed to both the POM and MAOM C pools. These results suggest that VOCs emitted from decomposing litter have the potential to contribute to stable MAOM formation. Although we cannot rule out direct abiotic sorption of VOCs to soil minerals, our results suggest that VOC-C may follow the same pathway proposed for soluble low-molecular-weight C, that is, the microbial efficiency-matrix stabilization (MEMS) model—C compounds are first assimilated by microbes before ultimately being incorporated into SOM (Cotrufo et al. 2013). However, future experiments will need to be designed to confirm that VOCs are indeed metabolized by soil microbes before being stabilized in the mineral soil.

The MEMS model also suggests that the efficiency by which litter-derived C is incorporated into SOM is a function of the initial organic matter recalcitrance, with more labile substrates being assimilated to a greater extent than more recalcitrant substrates (Cotrufo et al. 2013). Furthermore, litter chemistry is a major control of litter decomposition (Melillo et al. 1982, Bradford et al. 2016), and drives soil chemistry dynamics (Aber et al. 1990). Although we only used three litter species for this study, our results suggest that initial litter quality may not be a good predictor of VOC effects on soil chemistry or C stabilization, likely because litter chemistry is not predictive of VOC emission profiles (Gray et al. 2010). Future studies should aim to determine what characteristics of leaf litter and its decomposers are predictive of VOC profiles. Here we observe that the leaf litter with the lowest mineralization rate (Fig. 1), eucalyptus, was associated with a greater contribution of VOC-C to both MBC, and MAOM C. This is likely because of differences in the types and amounts of VOCs produced between the litter species in our study (Gray et al. 2010). For instance, eucalyptus litter has been associated with some of the highest emissions of total VOCs compared to other litter species. Although we did not measure VOCs in this study, we would expect that the decomposition of eucalyptus litter produces a different VOC profile than the other litters. Eucalyptus includes a greater proportion of monoterpenes and propanal/acetone than most other litters, which primarily release methanol during decomposition (Gray et al. 2010, Gray and Fierer 2012). Monoterpenes are chemically diverse and have an array of antimicrobial and inhibitory properties (Amaral et al. 1998, Trombetta et al. 2005, Adamczyk et al. 2015). Propanal and acetone are structural isomers that can be produced through a variety of pathways that include nonenzymatic Maillard reactions (Warneke et al. 1999), as well as fermentation of sugars and oxidation of lipids (Beesch 1952, Marco et al. 2006). Furthermore, although more research is needed to quantify the relationship between litter recalcitrance and VOC production, our results suggest that litter quality alone cannot predict the contribution of VOCs to microbial assimilation of C in soil.

The diversity of archaeal/bacterial communities, but not fungal communities, were affected by exposure to VOCs (Fig. 3). This effect on archaeal/bacterial diversity was due to lower diversity associated with the switchgrass and tulip poplar treatments. The composition of the archaeal/bacterial and fungal communities shifted in response to exposure to VOCs emitted from the decomposing litters; similar to archaeal/bacterial diversity, community shifts were most pronounced in soils exposed to the switchgrass and tulip poplar litters (Fig. 3). These results are in line with previous studies indicating that exposure to particular VOCs can alter the abundances of soil microbial taxa (Wheatley 2002, Yuan et al. 2017). It is possible that the VOC-C induced changes to N pool sizes also contributed to changes in community composition, as changes in N pools have been linked to changes in diversity and composition (Zeng et al. 2016). The lack of effect on fungal diversity is not surprising, as fungal diversity can remain unchanged even when there are significant differences in bacterial diversity (Osburn et al. 2019). However, these results may be indicative that litter-derived VOCs are not as readily metabolized by soil fungi. We were also able to identify major bacterial and fungal taxa whose relative abundances changed appreciably upon exposure to the litter-derived VOCs (Appendix S1: Fig. S3, Table S2). Many of these taxa are from poorly characterized groups, including candidate phyla for which no cultivated representatives currently exist, thus making it difficult to identify the specific physiological mechanisms underlying these responses. Given our evidence that litter-derived VOC-C can be incorporated into the MBC pool (Fig. 2), we hypothesize that these VOCs are serving as growth-promoting labile C substrates to support the growth of particular taxa and potentially driving differences in diversity and composition. For instance, we observed increases in the relative abundances of particular taxa, including Verrucomicrobia and Burkholderiales (Appendix S1: Table S2), that include known methylotrophs (Chistoserdova et al. 2009). Alternatively, it is possible that particular litter VOCs may also be antagonistic, inhibiting the growth of some microbial taxa (Wheatley 2002). As decomposing litters emit a wide range of VOCs (including many uncharacterized VOCs; Leff and Fierer 2008) and soil microbial communities are also highly diverse, unraveling the specific mechanisms by which exposure to litter VOCs affects the growth and activity of soil

microbes is clearly an important direction for future research, such as determining how individual VOCs affect soil microbial community composition, and identifying the mechanism of VOC-C stabilization in the mineral soil.

Conclusion

Generally, it is thought that the movement of DOC or POM C directly from litter into soil requires water movement or mixing of the litter layer; however, these processes are not necessary for litters to influence C dynamics and SOM formation in underlying soil horizons. With this study, we show that in general litter VOCs alter soil bacterial and fungal communities, and VOC-C enters all measured SOM pools, without physical contact between the soil and the decomposing litters. It is not clear whether VOC effects on microbial communities are direct or indirect. However, we find that soil microorganisms are consuming litter VOCs, and that this is affected by the specific leaf litter species (e.g., eucalyptus-derived VOC-C contributed the most to microbial biomass). VOC-C enrichment decreased from microbial biomass to DOC, and DOC to MAOM-C, suggesting that VOCs cycle through soil C pools in a manner similar to that of organic-matter leachates. Because VOCs are not constrained by diffusion in water or mass flow paths, VOCs can clearly serve as an important C source in bulk soils—especially near the soil surface—similar to the role of root-exudate C inputs to rhizosphere soils.

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Supporting Information

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Data Availability

Data are available from Figshare. https://doi.org/10.6084/m9.figshare.12323825.v1 and https://doi.org/10.6084/m9.figshare.6882899.v1