The role of Volatile Organic Compounds on Soil Microbial Communities and Ecosystem Processes

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Academic Abstract

Soil microorganisms are primarily limited by carbon (C) availability. The majority of C entering belowground food webs comes directly from local flora. Plant derived labile C compounds affect microbial community structure and function, which in turn drive ecosystem function. Research has focused on dissolved organic C (DOC) from litter leachates and root exudates. These compounds are often readily assimilable by soil microorganisms and are precursors for stable soil organic matter formation. Due to diffusion limitation DOC rarely travels far beyond its origin, meaning most soil microorganisms are unable to access these compounds unless they are located near the C source. However, recent studies have illuminated the importance of volatile organic compounds (VOCs) in soil ecosystems. VOCs are produced in abundance and, as vapors, they are able to travel through soil more rapidly than DOC. This dissertation aims to investigate the importance of VOCs commonly produced during the decomposition of leaf litter. We used three separate microcosm experiments to answer the following questions. 1) How do abundant VOCs affect microbial activity in soil? 2) How do VOCs affect nitrogen (N) transformations and the microbes associated with N transformations? 3) How do VOCs affect microbial community composition? 4) Are VOCs from decomposing litter incorporated into soil C pools? In chapter 2, we show that methanol and acetone – common litter derived VOCs – increase microbial activity and labile soil C, while also decreasing available nitrate, and ammonia oxidizing archaea. Interestingly,

this decrease in nitrifiers did not affect nitrification rate after VOC addition was ceased. In chapter 3, we demonstrate that soil microbial taxa respond differently to DOC and VOCs at different soil moisture levels. Specifically, DOC primarily affected taxa abundance in wetter soils, while the insoluble VOC α-pinene had the largest impact at lower moisture levels, and methanol affected abundance at all moisture levels. Finally, in chapter 4, we demonstrate that VOCs from decomposing leaf litter altered soil bacterial and fungal communities, and VOC derived C entered all measured soil organic matter pools without direct contact between decomposing litters and the soil. This work demonstrates the importance of VOCs on soil microbial communities and ecosystem function. The VOC induced increase in microbial activity, and the effects of VOCs at low moisture levels suggest that VOCs may function in the bulk soil in a manner similar to DOC in rhizosphere soil. Additionally, the incorporation of VOC-C into soil organic matter pools identifies a hitherto unrecognized mechanism for soil organic matter formation.

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GENERAL AUDIENCE ABSTRACT

Soil microorganisms live in an environment where their access to carbon containing compounds limits their growth. In these belowground environments most of the carbon flows from aboveground plant matter through soil microbes into the organisms that consume those microbes. The carbon from plants not only feeds the soil microbes but also changes the type of microbes and how those microbes process important chemicals in the environment – e.g., carbon and nitrogen. Previously, research has focused on carbon compounds that are able to dissolve in water. Often, these compounds originate from liquids that plants release from their roots, or dissolve like tea when leaves are soaked in water. Soil microorganisms can often use these dissolved carbon compounds and directly incorporate them into their biomass. Additionally, these compounds can be stored in soil - sequestering that carbon in the soil, potentially long term. However, dissolved compounds are unable to move very quickly through soil, and the soil microorganisms that live far from the source of these compounds do not have access to them. However, recent studies have found that another form of carbon, volatile organic compounds, are also produced in abundance in the soil environment. These compounds can travel through the air in the soil, as well as in the soil water. When in the air, VOCs travel very quickly and can also travel farther than dissolved compounds. This dissertation aims to investigate the importance of volatile organic compounds that are produced during the decomposition of leaves. We carried out three experiments using

small volumes of soil under controlled conditions in the laboratory. We aimed to answer the following questions. 1) How do abundant volatile organic compounds affect microbial activity in soil? 2) How do volatile organic compounds affect microbial processing of nitrogen containing compounds, and the populations of microorganisms that process those compounds? 3) How do volatile organic compounds affect the composition of microorganism in the soil? 4) Are volatile organic compounds from decomposing leaves able to be stabilized in the soil. In chapter 1, we show that methanol and acetone – common volatile compounds produced during the decomposition of leaves– increase microbial activity, and microbial available carbon in soil. Methanol and acetone also decreased available nitrate (an important N containing compound) and a group of organisms that produce nitrate called ammonia oxidizing archaea. Interestingly, once we stopped adding methanol and acetone to the soil the production of nitrate did not differ, meaning that the nitrate producing community was able to recover from the reduction in ammonia oxidizing archaea. In chapter 2, we demonstrated that soil microbial taxa respond differently to dissolved carbon and volatile organic compounds across a gradient of soil moisture. Specifically, dissolved carbon primarily affected taxa abundance in wetter soils, while the insoluble volatile α-pinene had the largest impact at lower moisture levels, and the volatile compound methanol affected abundance of microbial taxa at all moisture levels. Finally, in chapter 3, we demonstrate that volatile organic compounds produced during the decomposition of leaves altered the composition of both bacterial and fungal communities in the soil. Also, and possibly most interestingly, carbon from those volatile organic compounds was stored in all of the pools of carbon that we measured. Together these chapters demonstrate the importance of

volatile organic compounds on soil microbial communities and ecosystem function. Since volatile organic compounds induced an increase in microbial activity we are able to infer that soil microorganisms are using these compounds; paired with our observation that volatile organic compounds affected microbial taxa at lower moisture levels than the dissolved compounds did, we can infer that volatile compounds may function as a carbon source in parts of the soil that do not have access to dissolved carbon. Additionally, the incorporation of carbon from volatile organic compounds into soil identified a hitherto unrecognized mechanism for soil carbon sequestration.

Dedication

For my children, Arianna Echo McBride, and Gavin Riker McBride. I love you both!

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Contents

Chapter 1 – Introduction

Low Molecular Weight Carbon in Soil

Soil food webs are primarily controlled by the availability of carbon (C) resources (Thakur and Geisen 2019). The majority of C that enters soil is plant derived - e.g., leaf litter decomposition and root exudation (van Hees et al. 2005; Crow et al. 2009). These C compounds span a continuum of progressively more decomposed material: from plant and animal residues to monomeric C compounds (Lehmann and Kleber 2015). Soil microorganisms are involved in the degradation of all of these compounds, but they are only able to directly assimilate small biopolymers and monomers (Lehmann and Kleber 2015). This has lead researchers to recognize the importance of low molecular weight carbon compounds (LMWCC) from root exudates and leaf litter leachates (van Hees et al. 2005; Joly et al. 2016) on soil microbial communities and ecosystem functions.

Once LMWCCs enter soil they become microbially available and are rapidly consumed by soil organisms (van Hees et al. 2005; Strickland et al. 2012), where they create hotspots and hot moments (Kuzyakov and Blagodatskaya 2015). However, these compounds are largely confined to the rhizosphere where plants release their photosynthate, and where plant derived mucilage retains water allowing soil microbes to access dissolved LMWCC (Holz et al. 2018). In contrast, C availability for microbes in the bulk soil is limited by mass flow of water, and rewetting of soil that increases C accessibility (Kieft et al. 1987; Kuzyakov and Blagodatskaya 2015).

A large proportion of soil respiration originates from root exudation (Göttlicher et al. 2006; Högberg et al. 2008). Likewise, common root exudates have been found to affect soil microbial communities (Eilers et al. 2010) and reduce enzyme production

(Allison and Vitousek 2005), and many of the compounds in exudates have high carbon use efficiencies (Jones et al. 2018). Additionally, these readily assimilable compounds have been shown to be the primary precursor of mineral associated C (Cotrufo et al. 2013, 2015) and to increase nitrogen (N) competition by heterotrophic microorganisms (Verhagen and Laanbroek 1991; Verhagen et al. 1992). The importance of dissolved C compounds on microbial communities in soil ecosystems is clear, yet most of the organisms in the soil do not have access to that C leaving the majority of the soil microbial community dormant (Blagodatskaya and Kuzyakov 2013). However, VOCs are a class of LMWCC that is not limited by moisture connectivity and can rapidly traverse the soil matrix unlike dissolved compounds.

Ecology of Volatile Organic Compounds

Volatile organic compounds are small C containing compounds with low molecular mass and high vapor pressure. These characteristics allow VOCs to transition between liquid and vapor phase. There is extensive literature investigating the release of VOCs by plants and microorganisms as signaling or defensive compounds (Baldwin et al. 2002; Baldwin 2006; Bennett et al. 2012; Bitas et al. 2013). Recently, the importance of VOCs in soil biogeochemical cycling has also been identified. VOCs enter soil through a variety of mechanisms including atmospheric exchange, root emissions, by resident organisms, and decomposition of organic matter (Asensio et al. 2007a; Leff and Fierer 2008; Wenke et al. 2010; Bitas et al. 2013; Peñuelas et al. 2014). During the decomposition of leaf litter a variety of VOCs are produced in significant quantities (Gray et al. 2010; Ramirez et al. 2010; Peñuelas et al. 2014), primarily through microbial activity (Gray et al. 2010). Oxygenated VOCs such as methanol have been demonstrated

to be the most abundant VOCs produced during litter decomposition (Schink and Zeikus 1980; Niemenmaa et al. 2008; Gray et al. 2010; Ramirez et al. 2010).

The metabolism of VOCs, such as methanol is widely distributed through soil microorganisms including isolates from Proteobacteria, Verrucomicrobia, Firmicutes, and Actinobacteria. (Kolb 2009). Although it is not clear how much VOC is metabolized *in situ*, soil microorganisms do possess pathways to incorporate VOC-C into microbial biomass – e.g., the Wood-Ljungdahl pathway (Ragsdale and Pierce 2008), and methylglyoxalate pathway (Inoue and Kimura 1995) - and it appears that soil microbial biomass is directly impacted by VOC-C (Asensio et al. 2012). VOCs have also been shown to inhibit N transformations (Paavolainen et al. 1998; Smolander et al. 2006) suggesting a VOC mediated link between coupled C and N cycling.

These compounds are also of interest because they can diffuse thousands of times faster than DOC when in vapor phase. This allows VOCs to move through air filled pore space without reliance on water movement, possibly increasing their availability to distant soil microbes. However, VOC production and emissions has been shown to change with temperature and moisture (Warneke et al. 1999; Asensio et al. 2007b; Gray et al. 2014). Specifically, more VOC escapes dry soil than wet soil (Asensio et al. 2007b), and VOCs can diffuse a longer distance and more quickly when soil moisture level is low (Hiltpold and Turlings 2008). This balance between increasing the microbial population exposed to VOC, and the tendency for VOC to escape dry soils suggests an interesting interaction between VOCs and soil moisture that could affect VOC availability to soil microorganisms.

Dissertation Goals

This dissertation aims to determine the effects of volatile organic compounds on microbial communities and ecosystem functioning. The next four chapters will attempt to answer the following questions. 1) How do abundant VOCs affect microbial activity in soil. 2) How do VOCs affect N transformations and the microbes associated with microbial transformations? 3) How do VOCs affect microbial community composition? 4) Are VOCs from decomposing litter incorporated into soil C pools. We predict that VOCs will serve as an available source of C to soil microorganisms and thus increase their activity. Likewise, we predict that soil nitrification will decrease because the availability of VOC-C will increase heterotrophic demand for N and soil heterotrophs will outcompete nitrifiers for soil N. We expect that the soil VOCs will primarily enrich taxa, such as methylotrophs, that metabolize available VOCs. The enrichment of methylotrophs and other VOC metabolizers will coincide with VOC-C incorporation into microbial biomass, and passage into other soil C pools.

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Chapter 2- Volatile methanol and acetone additions increase labile soil carbon and inhibit nitrification

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Abstract:

Recent efforts to understand the contribution of low-molecular weight compounds to carbon dynamics in soil ecosystems has resulted in a framework that suggests that low-molecular weight, labile carbon compounds can be directly assimilated by microbial biomass before being stabilized on soil colloids. However, this model primarily focuses on dissolved organic matter inputs and overlooks the potential importance of volatile organic compounds (VOCs). Here we determined the effects of two VOCs commonly emitted from soil and decomposing leaf litter (methanol, and acetone) on soil respiratory dynamics during a 28-day lab experiment. At the end of the experiment we quantified carbon and nitrogen concentrations in dissolved organic matter, microbial biomass, particulate organic matter, mineral associated organic matter, the labile carbon pool, and

we quantified nitrifying microorganism abundance. Our results demonstrate that VOCs (i.e., methanol and acetone) increase soil respiration, contribute to labile soil C, and inhibit nitrification. Our VOC additions resulted in respiration spikes 4.1-5.5-fold greater than the control for acetone and methanol, respectively, though respiration returned back to control levels within 144 hours after additions. Our VOC additions resulted in a 1.6-1.7-fold increase in labile soil carbon, suggesting that litter-derived VOCs could enter soil C pools following microbial metabolism. Additionally, soils exposed to VOCs contained ~2.25-fold less total dissolved nitrogen, and ~34-220-fold less nitrate. Ammonia oxidizing archaea were ~1.5 fold less abundant in VOC treated soils than in the control. After VOC additions were ceased, nitrate levels increased at approximately the same rate in all treatments, suggesting an inhibitory effect of methanol and acetone on nitrifying microorganisms. These results indicate that common decomposition derived VOCs play an important yet under-recognized role in driving the formation of soil organic matter as well as increasing the immobilization of nitrogen in soil ecosystems.

Introduction

In soils, low molecular weight carbon compounds (LMWCC, e.g., glucose) drive C cycling (van Hees et al. 2005), are important controls of microbial structure and function (van Hees et al. 2005; Strickland et al. 2015), and form stable soil organic matter (SOM) (Cotrufo et al. 2013). Much of the research on LMWCC focuses on the role played by dissolved organic matter (DOM) such as root exudates and leaf litter leachates (van Hees et al. 2005; Bradford et al. 2013; Sokol et al. 2019), which are known drivers of soil microbial community composition (Eilers et al. 2010) and soil nutrient cycling (Mergel et al. 1998; Fisk et al. 2015). However, an entire class of LMWCCs have largely been ignored in relation to their effects on soil microbial communities and biogeochemistry: volatile organic compounds (VOCs).

VOCs enter the soil through various processes e.g., atmospheric deposition, root emissions, microbial release, non-enzymatic thermochemical Mailliard reactions (Warnecke 1999), and litter decomposition. Specifically, oxygenated VOCs (e.g., methanol and acetone) dominate emissions during decomposition of leaf litter (Schink and Zeikus 1980; Niemenmaa et al. 2008; Gray et al. 2010), and these VOC-C emissions can reach up to ~88% of CO_2-C emissions (Gray and Fierer 2012). Because VOCs can be taken up directly by soil microbes, dissolve in soils solution, and adsorb to colloidal and mineral surfaces, it is likely that gross VOC production is considerably higher than the emission rates observed during litter decomposition which can reach as high as 1,425 μg C-VOC g^{-1} dry litter h^{-1} (Ramirez et al. 2010, Peñuelas et al. 2014). Yet, it is currently difficult to find reliable estimates of VOC concentrations in soil air due to difficulties with these measurements - e.g., disrupting the soil matrix can alter VOC concentrations

(Peñuelas et al. 2014). However, it is likely that VOC concentrations could attain high levels. For instance, it is known that CO_2 concentrations in soil air can reach concentrations up to 1,000 times higher than instantaneous emissions (Pumpanen et al. 2003; Jassal et al. 2005; Flechard et al. 2007), which suggests that VOC concentrations in soil air, could expose soil microbes to a constantly elevated level of VOCs compared to VOC emission rates. Therefore, VOCs released from decomposing litter may play an under-recognized role in soil biogeochemical processes by acting as a major C source for microorganisms.

VOCs represent a labile C source that can diffuse freely, and rapidly, through the soil matrix, in the presence or absence of water. In pure culture, microorganisms metabolize a wide range of VOCs (Egli et al. 1980; Trudgill 1990; Salaspuro 1997; Alvarez et al. 2009), and VOCs have both stimulated and inhibited microbial activity in lab and field studies (Chuankun et al. 2004; Asensio et al. 2007; Owen et al. 2007; Ramirez et al. 2010). Specifically, methanol and acetone have been used as a C source for the cultivation of bacteria and fungi (Egli et al. 1980; Arfman et al. 1989; Kotani et al. 2007), as well as to inhibit microbial growth or microbial processes (Madkour et al. 1983; Güven et al. 2005). Suggestive of the potential importance of VOCs to soil processes is the fact that they are mineralized rapidly (Griebel and Owens 1972; Albers et al. 2018), and can serve as a C source for starved soil microorganisms (Schulz-Bohm et al. 2015). The amount of VOCs, and subsequently VOC-C, entering soils can also be significant. For example, Cleveland and Yavitt (1998) demonstrated that as much as 20 Tg of plant released isoprene is sequestered globally in the soil each year, and approximately 80% of VOCs released during leaf decomposition are absorbed by soil (Ramirez et al. 2010).

Such inputs of VOC-C may have similar effects on soil microbial activity and soil C cycling as the more widely studied effects of soluble LMWCCs (van Hees et al. 2005).

LMWCCs have previously been shown to affect soil N cycling processes by altering ratios of available C:N, which influence microbial N transformation rates due to stoichiometric demands of microbial biomass (Taylor and Townsend 2010). VOCs have also been shown to have direct and indirect effects on soil nitrogen (N) cycling processes such as nitrification (Smolander et al. 2012). One possible cause of these effects is that VOC consumption leads to increased N-demand and subsequent immobilization of N by heterotrophic soil microbes that outcompete nitrifiers for ammonium (NH_4) (Verhagen and Laanbroek 1991; Verhagen et al. 1992). Alternatively, VOCs may directly inhibit nitrification by interfering with ammonia oxidation enzymes (Hooper et al. 1997; McCarty 1999). However, the significance of VOCs on soil ecosystems is not well understood, thus the mechanism (or combination of mechanisms) that drives altered nitrification rates under VOC additions is unclear.

In this study, we performed a 28-day lab experiment with weekly application of three different VOC treatments: no VOC control, methanol addition, acetone addition. We chose these two VOCs because they account for large fractions of the VOCs emitted during litter decomposition, e.g., methanol accounted for ~90% of the VOC-C released during decomposition of maple leaves and acetone accounted for ~13% of the VOC-C released during pine litter decomposition (Ramirez et al. 2010). We address two questions (1) How do major litter derived VOCs (i.e., methanol and acetone) affect C mineralization, and microbial community structure and function? And, (2) do major litter derived VOCs affect the partitioning of N amongst soil fractions and N transformation

rates in soil? We predicted that because methanol and acetone are labile C sources, they would cause increased respiration, higher levels of labile soil C, and increased microbial biomass (Graaff et al. 2010; Wilson et al. 2018; Sokol and Bradford 2019). We also predicted decreased nitrification; specifically, since methanol and acetone are labile C sources they would increase competition for NH_4 between nitrifiers and heterotrophic microbes (Verhagen and Laanbroek 1991; Verhagen et al. 1992), or VOCs would directly inhibit nitrification (Hooper et al. 1997).

Materials and Methods

Experimental Design

We conducted a 28-day experiment, in which either methanol or acetone was added once a week (i.e., VOC added on day 0, 8, 15, 22; Figure 2.S 1) to examine the effect of VOCs on soil C and N cycling. At the conclusion of this experiment (day 28) we immediately subsampled the microcosms to quantify C and N concentrations in various soil fractions, and assay microbial community structure and function. We constructed microcosms by first adding 100 g (dry weight) of soil to a ~473 mL glass jar that had a 2 mL glass vial affixed to its interior. Soil for this microcosm experiment was collected by taking six individual A-horizon soil cores (8 cm diameter, 0-10 cm depth), allowing us to account for site heterogeneity, from Kentland Farm, VA (37.1987, -80.5833); Guernsey silt loam; *Pinus strobus* plant cover. Soil cores were composited, sieved (4.75 mm), homogenized, and stored at 4°C until microcosm construction. We chose a *P. strobus* stand given that soil communities associated with this plant cover tend to exhibit a relatively high degree of functional breadth (Strickland et al. 2009; Keiser et al. 2014),

and are likely exposed to an array of VOCs released during decomposition of *Pinus* spp. leaf litter (Leff and Fierer 2008; Gray et al. 2010; Gray and Fierer 2012). Microcosms were adjusted to 65% water-holding capacity (WHC) and maintained at this WHC and 20°C for the duration of the experiment.

Microcosms received one of three treatments and each treatment was replicated 3 times (n=3): **1)** control, no VOC addition, **2)** methanol addition (cas 67-56-1), or **3)** acetone addition (cas 67-64-1). Previous laboratory studies have found that VOC emissions from leaf litter decomposition expose soil to high levels of VOC-C, which could be variable for different VOCs: e.g., between ~967-1,392 µg VOC-C g^{-1} dry soil d^{-1} from methanol, and between ~62-108 µg VOC-C g^{-1} dry soil d^{-1} from acetone on average (Ramirez et al. 2010; Peñuelas et al. 2014). We performed a preliminary experiment with higher sampling frequency and two levels of VOC concentrations 216.7 µg VOC-C g^{-1} dry soil and 21.7 µg VOC-C g^{-1} dry soil in order to assess the effects of VOC addition rate on respiration dynamics, and to determine appropriate respiration sampling frequency. In this preliminary study we found that respiration levels peaked at 48 hours after VOC addition (Figure 2.S 2a), and that the high and low concentrations elicited similar respiration - the magnitude of the response was dose-dependent (Figure 2.S 2). Furthermore, the respiration results obtained during this preliminary work found the VOC (e.g., methanol and acetone) effect on respiration was undetectable one week after addition (Figure 2.S 2). For our primary experiment we added 216.7 µg VOC-C g^{-1} dry soil to the 2 mL vial affixed to the interior of our microcosms, once per week for 4 weeks (866.8 µg VOC-C g^{-1} dry soil for the entire 28-day experiment), an addition rate similar to that used in previous experiments (Paavolainen et al. 1998; Smolander et al. 2006).

The vials were permanently affixed to the interior of the glass jar, which prevented direct contact of the VOC and the soil – i.e., VOCs did not come into contact with soil until after volatilization. Immediately after VOC addition, jars were sealed for 24 h to allow the VOC to volatilize and diffuse through the soil. Due to physical limitations of VOC diffusion and volatilization, as well as rapid mineralization of VOCs by soil microbes (Albers et al. 2018) the soil air would not have been exposed to all of the VOC at one time. We used a static incubation procedure to measure CO_2 production rates two times each week, first at 48 h after VOC addition and again at 72 h or 144 h after VOC addition. Before each measurement, lids were removed and the headspace was allowed to equilibrate with the atmosphere for ~15 minutes. We then sealed the microcosms with lids fitted with butyl septa. An initial 5mL headspace sub-sample was measured to determine CO_2 using an infrared gas analyzer (Li-7000; Li-Cor Biosciences, Lincoln, Nebraska, USA), and a second 5 mL subsample was measured after a 24 h incubation period. We subtracted the initial concentration of headspace CO_2 from the CO_2 produced after incubation and divided by hours incubated to calculate respiration rate (CO_2-C mg^{-1} dry weight soil h^{-1}).

Effects of VOCs on Soil C and N concentrations

After the 28-day experiment, we subsampled each microcosm to assess soil C and N concentrations and soil microbial biomass. For soil C and N concentrations, we quantified mineralizable-C, particulate organic matter (POM) and mineral-associated C and N, dissolved organic C and N (DOC and DON), and inorganic N (NH_4 and NO_3) concentrations. For microbial biomass, we determined both the active microbial biomass and total biomass C and N.

For mineralizable-C – an estimate of bioavailable soil C that we expected to increase if VOCs represent a significant C source – a static incubation method (sensu: Fierer et al., 2005) was employed. We used this method to estimate the concentration of labile-C in the soil at the end of the 28-day experiment (i.e., post-VOC additions); using the assumption that the microbially available C was dominated by labile C sources (Knorr et al. 2005). Briefly, 6 g soil (dry weight) was subsampled from the experimental microcosms and incubated in a 50 mL centrifuge tube, fit with a butyl septum. We then measured respiration using an Infrared Gas Analyzer (IRGA; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA) at nine time points during the incubation: 2, 7, 10, 16, 24, 32, 41, 45, 52-days. Total mineralizable-C at the end of the 28-day experiment was determined via integration across this time period. VOC additions did not occur during this assay.

We determined particulate organic matter (POM) and mineral-associated C and N following Paul et al. (2001). Briefly, air-dried soil was dispersed with sodium hexametaphosphate (18 h of shaking), and then passed through a 53 μm sieve. POM (material > 53 μm) is expected to be a faster cycling pool of organic matter, whereas, the mineral-associated (material < 53 μm) pool is expected to be slower (Schlesinger and Lichter 2001). Although changes in POM were not expected since POM is an indicator of plant derived inputs, changes in the mineral-associated C and N could indicate VOCs were incorporated into microbial biomass (Grandy and Neff 2008).

Dissolved organic C (DOC), and total dissolved N as well as microbial biomass C and N were determined by simultaneous chloroform fumigation extraction (Fierer and Schimel 2003). Extracts were analyzed for DOC and total dissolved N with an Elementar

Variocube TOC/TN (Elementar Americas Inc, Mt. Laurel, NJ, USA). To estimate active microbial biomass we used substrate induced respiration (Wardle and Ghani 1995), following the method outlined by West et al. (1986). We determined soil NO_3 and NH_4 concentrations using a Lachat QuikChem flow injection analyzer (Hach Company, Loveland, CO, USA). Briefly, 10 g soil from each microcosm was placed into 175 mL Nalgene bottles. Immediately after adding soil to the Nalgene bottles, N was extracted using 50 mL of 2M KCl. Dissolved organic N was calculated by subtracting inorganic N concentration from the total dissolved N concentration.

Effects of VOCs on microbial community structure and function

At the end of the 28-day experiment we assayed changes in microbial community function using fluorometric enzyme assays and catabolic response profiles (Degens and Harris, 1997; Osburn et al., 2018; Saiya-Cork et al., 2002; see materials and methods in Online Resource 1). We also performed an N mineralization assay, to determine if VOC-C additions had a long-term effect on soil N transformations. As in the previous section, 10 g soil from each microcosm was placed into 175 mL Nalgene bottles. Those bottles were allowed to incubate, without VOC addition, at 20°C for 28-days with soil maintained at 65 % WHC. At the end of the 28-day incubation period KCl extraction was performed again and extracts were analyzed using a Lachat QuikChem flow injection analyzer (Hach Company, Loveland, CO, USA). Net N mineralization was calculated by subtracting initial (NH_4 + NO_3) from final (NH_4 + NO_3), and net nitrification was calculated by subtracting initial NO_3 from final NO_3 (Goodale and Aber 2001).

We assayed community structure by quantifying bacteria, fungi, and ammonia-oxidizing bacteria and archaea using qPCR. Prior to qPCR, DNA was extracted from

~0.25 g of fresh soil using the DNeasy PowerSoil kit (Qiagen, Valencia, CA, USA) and extracts were quantified using a Qubit fluorometer (Thermo Fisher Inc., Waltham, MA, USA). Total bacterial abundance and total fungal abundance were estimated by amplification of the 16S rRNA gene and the internal transcriber spacer (ITS) region, respectively. We estimated the abundance of bacteria, and fungi to determine if the VOC treatments affected microbial abundance and/or led to major functional shifts in microbial composition (Fierer et al., 2005b). For 16S rRNA quantification, we used the primer set EUB 518/EUB 338, while for ITS we used the primers ITS1f /5.8s (Fierer et al., 2005b). In order to determine VOC effects on the nitrifier community, we quantified ammonia-oxidizing bacteria (AOB) using the primer pair amoA-1f and amoA-2r (Rotthauwe et al. 1997), and ammonia-oxidizing archaea (AOA) using the primer pair Arch-amoAF and Arch-amoAR (Francis et al., 2005). Each qPCR assay (16S, ITS, AOA, and AOB) contained 10 µl SsoAdvanced Universal SYBR Green Supermix (Bio-rad, Hercules, CA, USA). For 16S and ITS, we also added 0.5 µm forward and reverse primer, 2 µl DNA template diluted to < 3 ng/ µL and nuclease-free H_2O to 20 µl. For AOA and AOB we added 0.25 µM forward and reverse primer, 2 µl undiluted DNA template, and nuclease-free H_2O to 20 µl. Thermal cycling conditions for 16S and ITS were 3 min at 98°C followed by 40 cycles of 15 s at 94°C, 30 s at 55°C and 30 s at 72°C .Thermal cycling conditions for AOA were 3 min at 98°C followed by 40 cycles of 15 s at 94°C, 30 s at 53°C and 60 s at 72°C. Thermal cycling conditions were identical for AOB except for an annealing temperature of 55°C and a 78°C plate read following the extension step. All qPCR reactions were performed in triplicate.

Statistical Analysis

All statistical analyses were performed in R version 3.5.0 (R Core Development Team, 2017). In order to assess respiratory dynamics after each VOC addition, and to determine if there was any interaction between VOC treatment and time, we used a linear mixed effects model with the *lme4* package (Bates et al., 2015). In order to avoid confounding interactions between VOC treatment and time we converted all respiration measurement times from the full 28-day experiment (Figure 2.S 1) to hours since last VOC addition, producing a single average 144-hour respiration time series – i.e., Day 3 and Day 9 were both converted to 48 hours since last VOC addition which were on day 1 and 7 respectively. Treatment and hours since VOC addition were considered fixed effects while each replicate, and the discrete VOC additions were considered random effects. Cumulative respiration was calculated by integrating beneath the 144 h time series curves. The *emmeans* package was used to make pairwise comparisons between fixed effects (Lenth 2018).

We used ANOVA to compare treatment differences for all C and N concentrations, fluxes and all quantified genes. In order to determine if abundance of AOA/AOB was predictive of the resulting NO_3 in soil, the relationship between AOA/AOB and NO_3 was measured using a regression of Log_{10} [AOA/AOB] with Log_{10} [NO_3] (from the post-respiration incubation KCl extraction).

Results

Respiration dynamics during VOC Addition experiment

During the initial 28-day experiment, we observed significant differences in respiration among VOC addition treatments ($\chi^2_{(2)} = 167.7$; $p < 0.001$) and among times

after VOC addition ($\chi^2_{(2)}$ = 245.3; $p < 0.001$; Figure 2.1A), as well as a significant treatment by time interaction ($\chi^2_{(4)}$= 123; $p < 0.001$; Figure 2.1A). The VOC pulses resulted in an initial spike in respiration that peaked at 48 h after addition and was ~5.5 and ~4.1 fold greater than the control for methanol and acetone, respectively. The pulse in respiration rapidly decreased and was statistically indistinguishable from the control after 144 hours (Figure 2.1A), similar to our observation in the pilot incubation (Figure 2.S 2). Although the pulse was transient, cumulative weekly respiration was significantly higher than the control in both VOC treatments, with methanol and acetone additions associated with a ~2.7 and ~2-fold increase in cumulative respiration, respectively ($F_{(2)}$ = 59.32; $p < 0.05$; Figure 2.1B).

Carbon and Nitrogen concentrations, and Microbial community structure and function

At the end of the 28-day experiment, we measured C and N concentrations, and microbial community structure and function. We found no treatment effects on the DOC concentration ($F_{(2)}$ = 2.37; p = 0.17; Table 2.1). Microbial biomass C ($F_{(2)}$ = 0.58; p = 0.59), microbial biomass N ($F_{(2)}$ = 0.17; p = 0.84), and microbial biomass C:N ($F_{(2)}$ = 3.23; p = 0.11) also remained unchanged between the VOC treatments and the control (Table 2.1). Likewise, the active microbial fraction determined by substrate induced respiration ($F_{(2)}$ = 1.75; p = 0.25) remained unchanged between treatments. However, as determined after the 28-day experiment labile C was significantly higher in the VOC treatments ($F_{(2)}$ = 88.02; $p < 0.001$), with methanol amended soils 1.7-fold higher and acetone amended soils 1.6-fold higher than controls (Figure 2.1C).

Control soils had significantly higher total dissolved N (~2.25-fold) than VOC amended soils ($F_{(2)} = 41.62$; $p < 0.001$; Figure 2.2A). The dominant N fraction in the total dissolved N pool of the control soil was NO_3, which was ~30-200 times greater than the acetone and methanol treatments, respectively ($F_{(2)} = 17.52$; $p < 0.01$; Figure 2.2B). The majority of N in the VOC treatments was DON which was ~100-fold greater than DON concentration in control soils ($F_{(2)} = 50.52$; $p < 0.001$; Figure 2.2A). We found no significant differences in POM-C ($F_{(2)} = 2.57$; $p = 0.16$), POM-N ($F_{(2)} = 0.84$; $p = 0.48$), MIN-C ($F_{(2)} = 2.24$; $p = 0.19$), or MIN-N ($F_{(2)} = 2.06$; $p = 0.21$) between the VOC treatments and the control (Table 2.1).

Microbial enzyme activity was unaltered (Table 2.S 1), and catabolic response to 5 of 6 substrates indicated no statistical differences between treatments, while the response to cellulose was significantly lower than the control in both VOC treatments (Table 2.S .2). We quantified fungal (ITS) and bacterial (16S) abundance in the soil to determine if VOC addition changed microbial abundance or resulted in a shift in fungal versus bacterial dominance. ITS ($F_{(2)} = 0.08$; $p = 0.92$) and 16S ($F_{(2)} = 0.17$; $p = 0.85$) gene copies, as well as the ratio of ITS:16S gene copies ($F_{(2)} = 2.45$; $p = 0.17$; Table 2.1) were not significantly different between treatments. We quantified AOA and AOB to determine if VOC additions affected the abundance of nitrifying microorganisms. AOA was ~1.5-1.6 fold greater in the control than in the soils treated with VOCs ($F_{(2)} = 6.57$; $p = 0.03$; Figure 2.3A), while no significant difference was noted for AOB abundance ($F_{(2)} = 1.38$; $p = 0.32$; Figure 2.3B). AOA gene copy abundance had a significant positive relationship with soil NO_3 concentrations ($F_{(1,7)} = 68.02$; Adj. $R^2 = 0.89$; $p < 0.001$;

Figure 2.3C). There was no significant relationship evident between AOB gene abundance and NO_3.

Effects on Nitrogen transformation rates

At the end of the 28-day experiment with VOC additions, we observed low concentrations of NO_3 in microcosms receiving VOCs (Figure 2.2A). Once VOC additions were ceased and the soils incubated an additional 28-days without addition of VOCs, there was a rebound in nitrification rates, with the soils exposed to acetone and methanol having a net increase in NO_3 of ~34 and 220-fold, respectively (Figure 2.2C). That is, soils previously treated with methanol or acetone had nitrification rates equivalent to the control and in the case of the methanol treatment a marginally greater rate of nitrification after ceasing VOC additions ($F_{(2)} = 4.5$; $p = 0.06$) (Figure 2.2D). In the VOC treatments, NO_3 at the end of the N mineralization assay (Figure 2.2C) was similar to NO_3 in the control immediately post VOC additions (~27 µg NO_3–N gdw^{-1}).

Discussion

Methanol and Acetone increase respiration and labile C, but do not affect soil C fractions

Previous research has indicated that VOCs are a C source for soil microorganisms (Schulz-Bohm et al. 2015; Albers et al. 2018), and can even affect microbial biomass (Asensio et al. 2012), and N transformation rates (Paavolainen et al. 1998; Smolander et al. 2006). In this experiment, the addition of VOC substrates (methanol, or acetone) caused an immediate increase in soil respiration (Figure 2.1A & 2.S 2). This result is

similar to Albers et al. (2018), who found that methanol and several other VOCs are rapidly mineralized by soil communities. This rapid spike in respiration after methanol and acetone addition indicates that some litter-derived VOCs serve as an energy source for the soil microbiota (Griebel and Owens 1972; Ramirez et al. 2010; Schulz-Bohm et al. 2015). Acetone addition resulted in a ~27% lower mineralization rate than methanol addition, which could indicate that acetone supports a higher carbon use efficiency than methanol, which is also supported by the lower, yet statistically insignificant, level of labile, mineralizable C in the acetone treatment (Figure 2.1c). This may be because acetone metabolism can support both aerobic and anaerobic microbial growth (Taylor et al. 1980; Ensign et al. 1998) through an acetone monooxygenase pathway that produces propionate or 1-propanol (Boumba et al. 2008), and intermediates that can enter the methylglyoxal pathway which ultimately produces pyruvate (Inoue and Kimura 1995). Conversely, methanol is primarily used as a microbial energy source that is metabolized first to formaldehyde and formate then to CO2 (Kramshøj et al. 2018), however, C from methanol could enter biomass as formate through the Wood-Ljungdahl pathway (Ragsdale and Pierce 2008).

After the initial spike, respiration proceeded to decrease rapidly (Figure 2.1A), 144 h after each weekly VOC addition, respiration associated with both methanol and acetone treated soils were statistically indistinguishable from the control soil (Figure 2.1A). The rapid increase and decrease in respiration suggests that methanol and acetone only stimulate microbial activity transiently. It is possible methanol and acetone are altering respiration dynamics by priming decomposition of soil organic matter (Kuzyakov et al. 2000). However, we observed no change in POM-C, mineral-C or DOC (Table 2.1),

indicating that priming likely did not play a role in C dynamics associated with our VOC additions. Although the initial increase in respiration was short lived, methanol and acetone amended soils contained higher labile C (Figure 2.1C) - as determined by a 52-day carbon mineralization assay which occurred after cessation of VOC additions - indicating that methanol and acetone additions may have led to an increase in the labile C concentration, possibly by preserving native soil C or potentially through increased turnover of soil microorganisms due to increased microbial activity. This is particular important since a microbial pipeline has been proposed, where assimilated labile C is stabilized in the mineral soil and can increase SOM stocks over longer timescales (Schmidt et al. 2011; Cotrufo et al. 2013).

Previous studies have suggested that the increase in microbial respiration after the addition of VOC is due to the antimicrobial effects of certain VOCs, specifically monoterpenes (Asensio et al. 2012). Although methanol and acetone may have antimicrobial effects (Inoue et al. 2004; Bitas et al. 2013), they did not seem to have measurable negative effects on microbial biomass, active microbial biomass (i.e., substrate induced respiration - biomass; Table 2.1), or microbial gene copies (16S and ITS; Table 2.1). This suggests that the rapid increase in respiration is likely due to an increase in microbial activity rather than VOC fumigation and decomposition of microbial necromass. The dramatic increase of DON in the VOC treated soils (Figure 2.2a) potentially contradicts this reasoning; however, the increased DON could also be explained by the concomitant decrease in AOA which are known to mineralize DON (Offre et al. 2009). We propose that litter-derived VOCs act as a C source that can ultimately result in increased labile soil C stocks. Further, it is likely that when litter-

derived VOCs enter the soil they create hotspots of resource availability similar to that previously observed for dissolved labile C inputs to soil, such as root exudates (Kuzyakov and Blagodatskaya 2015). Additionally, soil VOC concentrations can be periodically elevated due to VOC pulses that occur after rewetting of dry soils (Rossabi et al. 2018), resulting in temporal resource hotspots associated with VOC emissions.

Methanol and Acetone affect distribution of N fractions, reduce total soil N and the abundance of nitrifiers

At the completion of the VOC addition experiment, we measured N concentrations to determine the potential effect of methanol and acetone on soil N cycling. We expected a reduction in inorganic-N concentrations, driven by increased heterotrophic N demand induced by increased labile-C availability (Bremner and McCarty 1988; Ma et al. 2015). We also expected reductions in NO_3 concentrations either due to lower nitrification rates because of increased heterotrophic demand for NH4, or as a result of direct inhibition of nitrification by VOCs (Paavolainen et al. 1998; Smolander et al. 2006). Overall, soils treated with methanol and acetone had significantly less total dissolved N than soil that did not receive VOC addition (Figure 2.2A). This may indicate that N was being immobilized by the soil microbial biomass in response to increased labile C derived from VOCs. While total dissolved N was lower in the soils treated with VOCs, there was also a striking difference in the distribution of the total dissolved N concentrations among NO_3, NH4, and DON. In the control soils, total dissolved N was dominated by NO_3 with very low DON and NH4, while the total dissolved N concentration for soils treated with VOCs was dominated by DON and very

little inorganic N (i.e., NO_3 and NH4; Figure 2.2A). This indicates that nitrification was inhibited by methanol and acetone treatment. NH4 was the least abundant N species we measured in the soil, constituting between ~2 and 7% of total dissolved nitrogen across all treatments. We posit that the fate of NH4 in this study is dependent on the presence or absence of VOC. Because, NO_3 is high in the control, and AOA abundance is higher in the control than the VOC treated soils, we suggest that NH4 is likely being rapidly converted to NO_3 by nitrifiers in control soils. Conversely, in the VOC treated soils NO_3 and total dissolved nitrogen is low. This suggests that that NH4 has been assimilated into heterotrophic biomass due to increased N-demand, thus preventing the formation of NO_3. Under such conditions, one possible explanation is that nitrifiers will be outcompeted for NH4 (Verhagen and Laanbroek 1991; Verhagen et al. 1992), potentially leading to a shift in the extractable N concentration similar to what we observed. Alternately, or in addition, is the potential for methanol and acetone to function as direct inhibitors of the ammonia monooxygenase (AMO) enzyme. The AMO enzyme has been shown to be nonspecific for ammonium and directly binds to methanol, and other products in the oxidation pathway of methanol, including formaldehyde and formic acid (Hooper et al. 1997; McCarty 1999). The AMO enzyme can also bind to some of the metabolic products of acetone, though it does not directly bind to acetone (Hooper et al. 1997; McCarty 1999; Kotani et al. 2007). These studies suggest that direct nitrifier inhibition is a possible explanation of the reduced NO_3 with VOC additions in this study.

In order to clarify the potential mechanism driving differences in the extractable N concentration due to VOC additions, we quantified (via qPCR) both ammonia oxidizing archaea (AOA), and ammonia oxidizing bacteria (AOB). AOA abundance was ~1.5-1.6-

fold less in the VOC treated soils than in the control, and while there was no significant difference in AOB abundance a similar pattern was observed (Figure 2.3A). There was also a strong relationship between AOA abundance and NO_3 concentration (Figure 2.3C). It is unclear why volatile methanol and acetone would inhibit AOA to a greater degree than AOB. However, these results suggest that the variation in nitrification response that has been observed in previous VOC studies (Smolander et al. 2006; Ramirez et al. 2010) is potentially driven by the abundance of AOA in the soil.

We performed a net N mineralization/nitrification assay in order to determine the potential for nitrification to rebound after cessation of VOC additions and found that net nitrification rates in VOC addition soils were equivalent to or, marginally greater than nitrification in the control soils (Figure 2.2D). If competition for NH_4 were the primary driver of nitrification rates, we would expect nitrification to remain suppressed after ceasing VOC addition due to higher labile C (i.e., higher NH_4 competition) in VOC amended soils (Figure 2.1C). Therefore, we expect that direct nitrifier amoA enzyme inhibition, not NH_4 competition, is the primary driver of reduced nitrification in this study. However, the inhibitory effect is transient. Although AOA abundance was ~1.5-1.6 fold lower following VOC additions, this did not prevent nitrification rates from rebounding, suggesting that the functional potential of the nitrifier community remained intact. This indicates that the added VOCs simultaneously suppressed nitrification while stimulating heterotrophs, similar to results from studies using DOC (Kuzyakov and Blagodatskaya, 2015; Ma et al., 2015). Further research (e.g., 15N tracer studies) that clearly identifies the potential mechanism or mechanisms of VOC-mediated effects on soil N transformations is necessary.

Conclusion

Our results show that litter derived VOCs such as methanol and acetone play a potentially under-represented role in soil C and N cycling. Although we used methanol and acetone as representative VOCs, it is important to note that VOCs produced during leaf litter decomposition are composed of many individual compounds suggesting that combinations of VOCs could result in non-additive effects. It will be important for future studies to better understand the dynamics of VOC production and consumption in the soil ecosystem. For example, we found that methanol and acetone increased soil respiration even in the absence of a subsequent increase in total or active microbial biomass; however, we were unable to determine to what extent C from those compounds were entering microbial biomass or whether any of that C is stabilized in the soil matrix. Since methanol and acetone are major constituents of VOCs produced during litter decomposition (Ramirez et al. 2010), our results suggest that the majority of litter-derived VOC-C is bioavailable to soil microbial communities. This indicates that VOCs may function similarly to root exudates by providing the underlying soil community with a C subsidy that could ultimately be stabilized on soil colloids (Cotrufo et al. 2013). If this is the case, VOC metabolism would represent a previously unrecognized carbon sequestration pathway. Furthermore, the effect of methanol and acetone on soil N concentrations and nitrifier abundance suggests VOCs mediate relationships between soil heterotrophs and nitrifiers which could further our understanding of N dynamics in natural and managed systems. Overall, these compounds do not require an aqueous medium to move within the soil matrix it is possible that volatile compounds play an

important role in driving soil C and N cycling in bulk soil, similar to the role of root exudates in driving soil C and N cycling in the rhizosphere.

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Figures

Figure 2.1

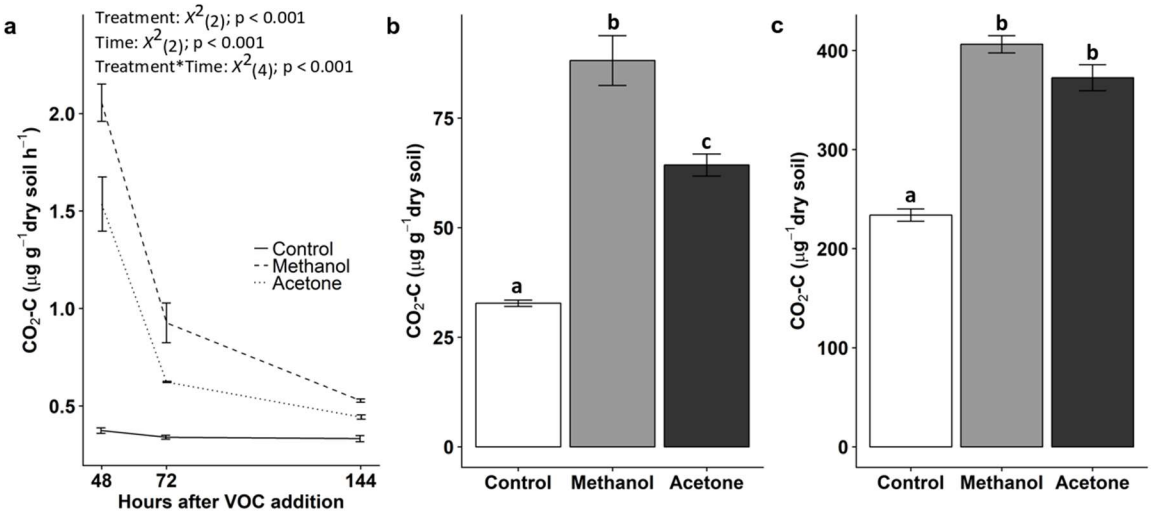

Figure 2.1

Figure 2.1

Average 144 h respiration dynamics following VOC additions – based off of 4 weekly VOC additions across 28-days – **a**, cumulative weekly respiration **b**, and labile C obtained from a 52-day C-mineralization assay **c**. Cumulative respiration was ~2-2.7 fold greater in VOC treated soils **b**, and labile C was 1.6-1.7 fold greater **c**. Significant treatment differences are denoted by different letters (α=0.05) for both **b** and **c**. Shown are means ± 1 standard error for **a**, **b**, and **c**

Figure 2.2

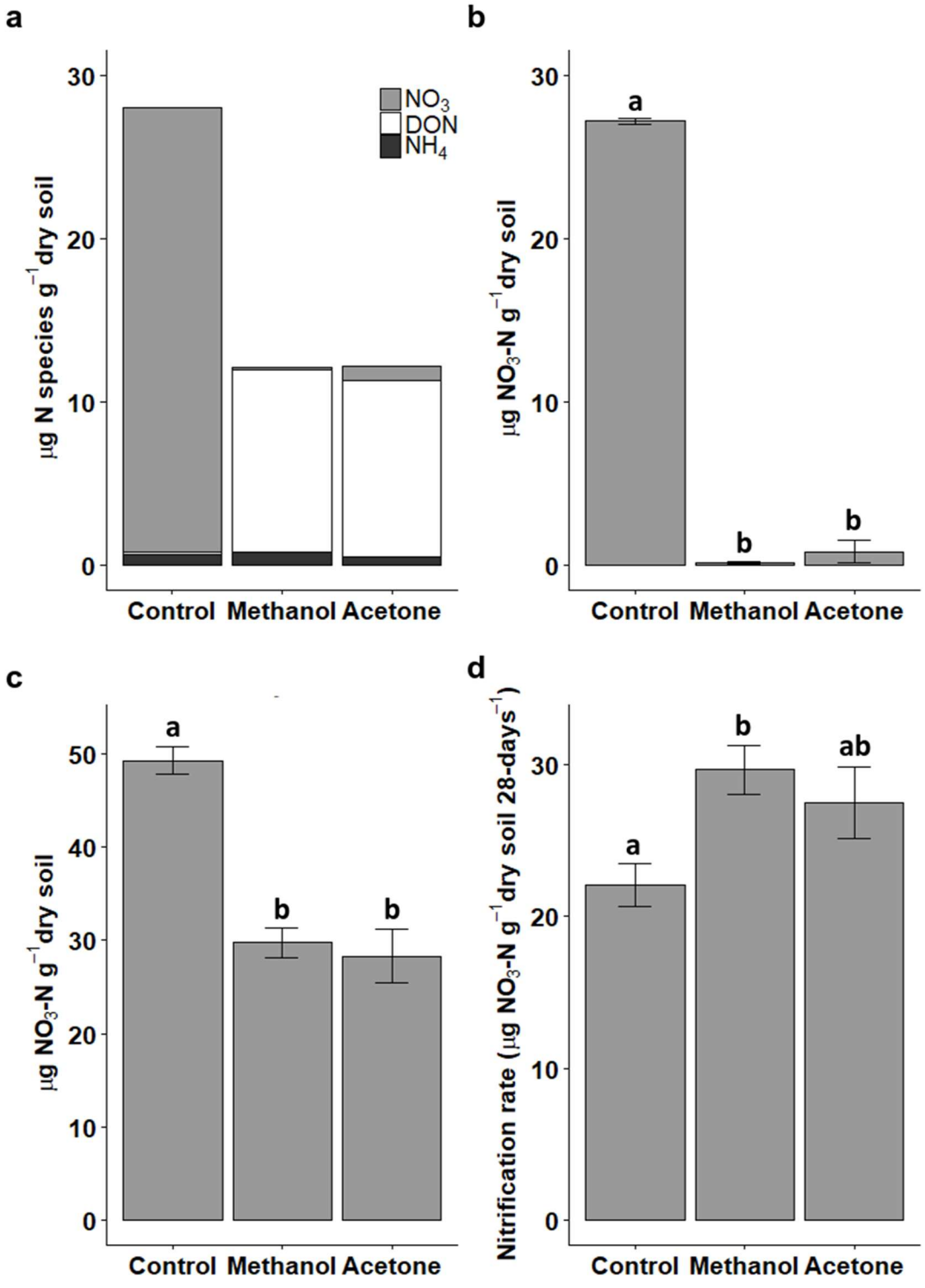

Figure 2.2

Figure 2.2

N fractions **a**, soil NO_3-N measured immediately after the 28-day experiment with VOC additions **b**, soil NO_3-N after a 28-day nitrification assay performed after the end of the experiment without the addition of VOCs **c**, and the nitrification rate during the 28-day nitrification assay, without VOC additions **d**. At the end of the initial 28-day experiment, the VOC treated soils had ~30-200 fold lower NO_3-N concentration compared to the control **b**. After the 28-day nitrification assay, NO_3-N levels increased in all treatments, and although the control had higher total NO_3-N (C), nitrification rate was marginally higher in methanol amended soils **d**. Pairwise differences denoted by letter; α=0.05 except for (D) α=0.10. Shown are means ± 1 standard error.

Figure 2.3

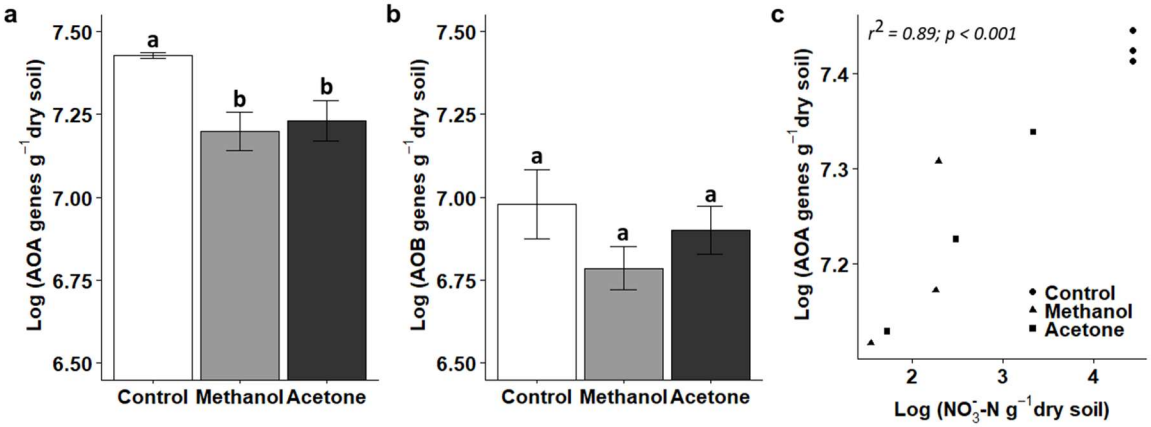

Figure 2.3

Figure 2.3

Log AOA gene copies **a**, Log AOB gene copies **b**, and the relationship between log AOA gene copies and log NO_3^--N **c**. There were 1.5-1.6 fewer AOA gene copies in the VOC treated soils at the end of the 28-day experiment than in the control soils **a**, while AOB gene copies were not significantly different between treatments **b**, as determined by ANOVA (α=0.05). Shown are means ± 1 standard error. Log AOA and log NO_3-N had a significant positive correlation, as determined by regression analysis (α=0.05) **c.**

Tables

Table 2.1

Treatment	DOC	POM-C	POM–N	MIN-C	MIN-N	MB-C	MB-N	SIR	ITS	16S
Control	93±5.3	6.96±0.42	0.33±0.03	26.68±0.71	2.70±0.06	59±8.6	11.5±2.1	0.43 ±0.01	5.82±0.09	8.07±0.05
Methanol	102±0.8	7.10±0.11	0.37±0.02	28.70±0.81	2.90±0.07	63±8.4	10.6±2.1	0.46 ±0.02	5.80±0.05	8.04±0.07
Acetone	93±1.7	6.33±0.08	0.33±0.02	28.75±0.83	2.86±0.10	71±6.8	10.0±1.1	0.51 ±0.05	5.77±0.11	8.09±0.08

Table 2.1

Table 2.1

Soil chemical and microbial data collected at the end of the 28-day experiment; dissolved organic carbon (µg C g^{-1} dry soil) (DOC), Particulate organic matter (POM) C (mg C g^{-1} dry soil) and N (mg N g^{-1} dry soil), mineral associated (MIN) C (mg C g^{-1} dry soil) and N (mg N g^{-1} dry soil), Microbial biomass (MB) C (µg C g^{-1} dry soil) and N (µg N g^{-1} dry soil), substrate induced respiration (SIR; µg C g^{-1} dry soil h^{-1}), fungal log abundance gdw^{-1} (ITS), bacterial log abundance g^{-1} dry soil (16S).

Supplementary Methods

Pilot 12-day Lab experiment

In order to determine the concentration of VOC to add to our microcosms we set up a pilot experiment with two concentrations of VOC additions. Microcosms were constructed and maintained the same as in the main 28-day experiment. Once set up, microcosms were then subjected to one of five treatments: **1)** control, no VOC addition, **2)** addition of methanol (cas 67-56-1) 1.75 mmol VOC-C $week^{-1}$, or **3)** addition of acetone (cas 67-64-1) 1.75 mmol VOC-C $week^{-1}$ **4)** addition of methanol (cas 67-56-1) 0.175 mmol VOC-C $week^{-1}$, or **5)** addition of acetone (cas 67-64-1) 0.175 mmol VOC-C $week^{-1}$. VOCs were added to the appropriate microcosm by pipetting the VOC weekly into a small vial (1 mL) affixed to the interior of the 473.2 mL glass jar. Immediately after VOC additions, each jar was sealed with a lid for 24 h to allow for the VOC compound to volatilize and diffuse through the soil. We measured respiration 24 h after VOC addition, and several times during the week. Once microcosms were capped, an initial 5mL sub-sample of headspace was sampled and CO_2 concentration was determined using an infrared gas analyzer (IRGA; Li-Cor; Model LI-7000, Li-Cor Biosciences, Lincoln, Nebraska, USA), and this was repeated after a 24 h incubation. This allowed us to calculate the respiration rate for each microcosm. This procedure (VOC addition followed by respiration estimates) was repeated weekly for 2 weeks.

Functional assays performed at the end of the 28-day experiment

We measured catabolic response profiles, and extracellular enzyme activity as estimates of community function. To measure catabolic response profiles we used 6 substrates (glucose, glycine, oxalic acid, cellulose, chitin, water), following the method developed by Degens and Harris (1997) using a modified fluorometric enzyme assay for 5 hydrolytic enzymes (acid

phosphatase (AP), β-1,4-*N*-acetylglucosaminidase (NAG), β-1,4-glucosidase (βG), cellobiohydrase (CBH)); Saiya-Cork et al. (2002). Briefly, we homogenized ~0.25 g of field moist soil in 120 mL of pH-adjusted buffer, then added 200 µl aliquots to a black 96-well microplate containing fluorescently labelled substrate 4-methylumbelliferone (MUB). We used a 10 µM MUB standard on each plate, and each assay was performed with eight technical replicates of each assay. We measured fluorescence using a Tecan infinite M200 microplate to determine reader (Tecan Group ltd, Mannedorf, Switzerland) with excitation and emission wavelengths of 365 nm and 450 nm, respectively.

Supplementary Figures

Figure 2.S 1

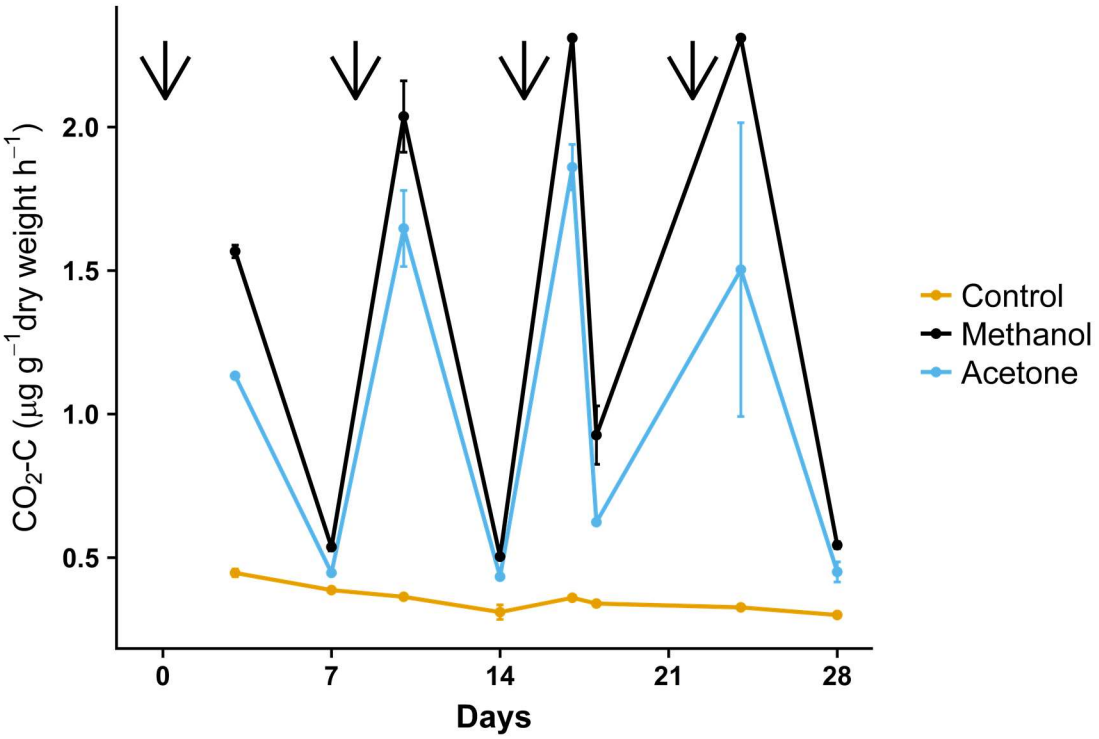

Figure 2.S 1

Figure 2.S 1

Full 28-day experiment. Arrows indicate the four times VOC was added to the microcosms.

Shown are ±1 standard error

Figure 2.S 2

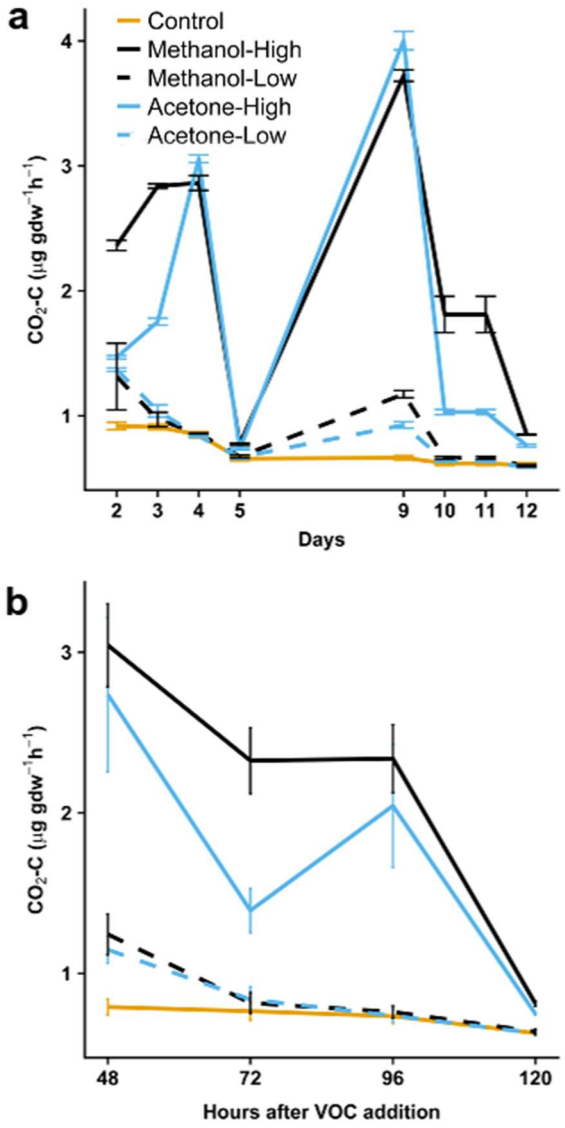

Figure 2.S 2

Figure 2.S 2

Time series of pilot VOC addition experiment. The full 12 days of respiration measurements are shown – VOC additions were made on day 0 and day 7 **a**. The time series was converted to time since VOC addition and each overlapping time point was averaged – e.g., day 2 and day 9 were both 48 hours since last VOC addition **b**. Shown are ± 1 standard error

Supplementary Tables

Table 2.S 1

Treatment	AP	NAG	βG	CBH
Control	8129 ±889[a]	4859 ±1336[a]	700 ±136[a]	3786 ±25[a]
Methanol	9414 ±661[a]	5686 ±1948[a]	770 ±121[a]	4419 ±238[a]
Acetone	8734 ±505[a]	4498 ±1423[a]	657 ±186[a]	3519 ±639[a]

Table 2.S 1

Table 2.S 1

Extracellular Enzyme Activity. Differences between treatments are denoted by letter, α=0.10.

Displayed are mean nmol g^{-1} dry soil h^{-1} ± 1 standard error

Table 2.S 2

Treatment	Glucose	Glycine	Oxalic Acid	Chitin	Cellulose	Water
Control	0.57 ±0.12[a]	0.23 ±0.01[a]	0.36 ±0.02[a]	0.24 ±0.01[a]	0.33 ±0.01[a]	0.17 ±0.01[a]
Methanol	0.41 ±0.03[a]	0.26 ±0.02[a]	0.32 ±0.03[a]	0.25 ±0.01[a]	0.28 ±0.01[b]	0.17 ±0.02[a]
Acetone	0.45 ±0.01[a]	0.27 ±0.03[a]	0.34 ±0.05[a]	0.28 ±0.03[a]	0.27 ±0.02[b]	0.29 ±0.13[a]

Table 2.S 2

Table 2.S 2

Catabolic Response Profile. Differences between treatments are denoted by letter, α=0.10.

Displayed are mean $\mu g\ C\ g^{-1}$ dry soil $h^{-1} \pm 1$ standard error

Supplementary Works Cited

1. Degens BP, Harris JA (1997) Development of a physiological approach to measuring the catabolic diversity of soil microbial communities. Soil Biol Biochem 29:1309–1320. doi: 10.1016/S0038-0717(97)00076-X

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Chapter 3-The effect of volatile organic compounds and dissolved organic carbon on soil chemistry and microbial communities

Authors: Steven G. McBride

Abstract

Soil microbial communities are exposed to a variety of carbon (C) compounds that affect biogeochemical processes, and community composition. Current research has put considerable focus on dissolved organic C (DOC) from root exudates and leaf litter leachates, which are an important C source for soil microbes. Recently, volatile organic compounds (VOCs) have emerged as another important C source in soil. Like DOC, VOCs can affect microbial community composition, and soil biogeochemistry. Unlike DOC, VOCs can diffuse through air filled pore space, suggesting that VOCs may be important C sources in bulk soil where exudates and leachates are less abundant, and in dry soils that have minimal water connectivity. In order to compare the effects of DOC and VOCs on soil chemistry and microbial communities under different moisture regimes, we performed a 28-day microcosm 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 oxalic acid), and two volatile compounds (methanol, and α-pinene). At the end of the experiment we measured soil respiration, DOC, and total dissolved nitrogen (N), and characterized the microbial community using marker gene sequencing. Respiration increased under each C amendment, and with increasing moisture. K_2SO_4 extractable DOC largely did not respond to C amendment.Total dissolved N increased with moisture but was suppressed by α-pinene at low moisture, and methanol at high moisture. We found statistically significant separation of communities based on

an interaction between soil moisture and C amendment. Abundant taxa mostly responded to glucose and oxalic acid in wetter treatments, α-pinene primarily in drier treatments, and methanol more dispersed across all moisture treatments. Notably, further examination of these communities revealed that the methanol amendment resulted in greater relative abundance of Proteobacteria at all moisture levels (12-23%), driven largely by an increase in the relative abundance of the family Methylobacteriaceae. This study suggests that VOCs may have an equivalent or greater effect on microbial communities than DOC. Since VOCs are likely more transient in nature than DOC - i.e., they are present during decomposition of litter but can rapidly diffuse through the soil matrix – it is possible that VOCs create areas of increased microbial activity and biogeochmical processing which lead to blooms or inhibition of specific taxa.

Introduction

Soil microbial activity and growth is primarily limited by carbon (C) availability. Dissolved organic C (DOC) - e.g., root exudates and leaf litter leachates - are important C sources for soil microorganisms (Soong et al.; van Hees et al. 2005). However, due to limitation of C movement through soil, exudates and leachates are primarily limited to the rhizosphere (0.5-4 mm from the root), and mass flow of C through preferential flow paths (Kuzyakov and Blagodatskaya 2015; Kuzyakov and Razavi 2019). This lack of C availability is proposed to be the main driver of microbial dormancy in soil (Blagodatskaya and Kuzyakov 2013). When C is available in high quantities such as the rhizosphere it generates hotspots of microbial activity, and when C becomes available in bulk soil it creates short term increases in activity where soil microorganism rapidly use the available substrate before returning to their normal state of low activity (Kuzyakov and Blagodatskaya 2015). Recent studies have shown that volatile organic compounds (VOCs) such as methanol (McBride et al. 2019) and monoterpenes (Asensio et al. 2012) also increase soil microbial activity. Unlike DOC, these compounds rapidly diffuse through air filled pore spaces, potentially providing C to microbes distant from its source and eliminating the need for water connectivity.

Components of root exudates, such as carbohydrates, amino acids, and organic acids are known to be readily available to soil microbes increasing microbial activity (Papp et al. 2020), but have been shown to have minimal impact on microbial community structure and C metabolism (Strickland et al. 2015). However, rhizosphere stimulation did show changes in microbial community structure and enzyme function (Eilers et al. 2010; Lucas et al. 2020). Since the diffusion of root exudates is so slow (10^{-7} – 10^{-5} cm^2 s^{-1}) and they are rapidly metabolized in the rhizosphere, the spatial extent of rhizodeposited DOC is limited. This is exemplified by the low levels of DOC in bulk soil compared to the rhizosphere (Wang and Zabowski 1998)

Therefore, DOC availability in the bulk soil is largely limited by mass flow of water, driven by soil structure (Kuzyakov and Blagodatskaya 2015). This lack of available C drives microbial dormancy in the bulk soil, and limits activity to times and places when and where C availability increases.

VOCs have the ability to reach dormant soil microbes in the bulk soil without water flow because they can diffuse rapidly through air-filled pore spaces. These compounds enter the soil in quantity through a variety of mechanisms including soil-atmosphere exchange, root exudation, and leaf litter decomposition (Asensio et al. 2007; Leff and Fierer 2008; Wenke et al. 2010; Peñuelas et al. 2014). Indeed, under ideal conditions decomposing maple leaves can release up to 1.4 mg VOC-C g^{-1} dry litter h^{-1} (Ramirez et al. 2010). When scaled up to the ecosystem level this is on par with the amount of DOC through *Pinus taeda* root exudation (Phillips et al. 2011). During litter decomposition methanol accounts for the majority of VOC-C being released from most litters, however, some species such as *Pinus* spp., and *Eucalyptus* spp. release a sizeable proportion of monoterpenes (Gray et al. 2010). Methanol and monoterpenes have both been shown to serve as a microbial C substrate (Radajewski et al.; Madyastha et al. 1977). However, one major difference is their ability to diffuse through the soil matrix. Methanol is able to readily diffuse through both soil air and water, while monoterpenes are only able to diffuse through soil air as they are mostly insoluble in water indicating that their movement through soil would be limited by increased soil moisture.

Here, we used a microcosm approach to determine the effect of DOC and VOC on soil microbial community structure 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 oxalic acid), and two volatile compounds (methanol, and α-pinene). We incubated these soils in the lab for 28-days before analyzing their microbial community structure using target gene sequencing. We predicted that the two dissolved carbon compounds (glucose and oxalic acid) would have little effect on soil communities under low moisture content and would affect the relative abundance of taxa at high moisture content. This is because these compounds require water connectivity to diffuse through the soil matrix. Because methanol is both miscible in water and able to enter vapor phase, we predicted it would affect microbial community structure by enriching methylotrophs at all moisture levels. However, since α-pinene is hydrophobic we predicted it would only affect communities at low moisture content.

Methods

Experimental Design

We collected six individual A-horizon soil cores (8 cm diameter, 0-10 cm depth) from Kentland Farm, VA (37.1987, -80.5833); Guernsey silt loam; *Pinus strobus* plant cover. 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, then placing a 0.2 mL conical tube in the center of the soil. Soil was then allowed to dry down to 25% of water holding capacity (WHC). We then adjusted WHC to 5 levels 25%, 35%, 45%, 60%, 70%. All tubes were then incubated for two weeks at 20°C and 100% humidity, with weekly moisture adjustments.

Respiration, DOC, and total dissolved nitrogen

At the end of the initial two-week incubation, we began weekly amendments of four C sources – 2 volatile organic compounds (methanol, and α-pinene), 2 dissolved organic compounds (glucose, and oxalic acid) – at a rate of 120 μg C g^{-1} dry soil. This rate is similar to

that of previous studies (McBride et al. 2019; Asensio et al. 2012). Additionally, we had a no C control which was only water adjusted. This resulted in 25 treatments with five levels of moisture, and 5 levels of C addition for a total of 125 experimental units (n=5). Weekly VOC-C amendments were made by adding liquid phase methanol or α-pinene into the 0.2 mL conical tube to force vapor phase transport of VOCs into the soil. Weekly DOC amendments were added directly to the soil. Immediately after C addition, the conical tubes were sealed, and we used a static incubation procedure to measure CO_2 production (McBride et al. 2019). 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. We subtracted the initial concentration of headspace CO_2 from the CO_2 produced after incubation and divided by hours incubated to calculate respiration rate (CO_2-C mg^{-1} dry weight soil h^{-1}). Additional measurements in between C additions were made after flushing microcosms with CO_2-free air for three minutes, then measuring headspace CO_2 after ~24 h. At the end of the 28-day experiment, we subsampled each microcosm to assess soil DOC and total dissolved nitrogen (TDN) concentrations. For soil C and N concentrations, we quantified DOC and TDN with an Elementar Variocube TOC/TN (Elementar Americas Inc, Mt. Laurel, NJ, USA).

Bacterial Community assessment

We assessed bacterial community structure via marker gene sequencing. DNA was extracted from each soil sample using the Qiagen© PowerSoil kit (Qiagen, Hilden, Germany), according to the manufacturer's protocols. We amplified the 16S ribosomal marker gene using the 515F/806R primer pair in a 2 step PCR (Caporaso et al. 2012). After completing first round of PCR, sequences were cleaned 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^{TM} 96-well plates (Invitrogen, Carlsbad, CA, USA) to clean and normalize samples. We pooled equimolar amounts of DNA, 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 determine if there was any contamination.

The University of Idaho's Genomic Resource Core used the program dbcAmplicons (Uribe-Convers et al. 2016) to demultiplex raw sequences, remove barcodes, and remove primers from sequences. We then used the DADA2 pipeline (Callahan et al. 2016) to process paired sequences; we trimmed to uniform lengths, dereplicated, and denoised using the 'dada' function, accounting for errors through the model generated with the 'learnErrors' command. DADA2 is designed to resolve amplicon sequence variants (ASVs) from Illumina sequence data without sequence clustering. Paired sequences were merged and chimeras were removed *de novo* using dada2. Taxonomy assignments were determined using the naïve Bayesian classifier against the Silva reference database (ver. 132, Quast et al. 2013). To account for differences in sequencing depths, we removed samples with low coverage then rarefied samples to 17085 sequences per sample.

Statistical analyses

The effect of moisture, C amendment, and their interaction on cumulative microcosm respiration, soil DOC and TDN pools, and abundant phyla were investigated using analysis of variance (ANOVA), the residuals of the model were analyzed for normality using the Shapiro-Wilk test (Shapiro and Wilk 1965). When the residuals did not meet normality we used a generalized

linear model (GLM), with a gamma distribution and log link. When there was no significant interaction 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 interactions, we determined pairwise differences between C treatments within moisture levels, and between moisture levels within C treatments using the Tukey post-hoc test in the *emmeans* package (Lenth et al. 2020). Microbial community structures (i.e., prokaryotic communities) were analyzed using Bray-Curtis distances in a permutational MANOVA (perMANOVA) and visualized using non-metric multidimensional scaling (NMDS) in the *vegan* package (Oksanen et al. 2019). Significant treatment effects were considered at $P < 0.05$, and marginal significance was considered at $P < 0.10$. Differentially abundant ASVs were determined using edgeR (Chen et al. 2020).

Results

Soil Respiration, DOC and total dissolved N

Soil respiration significantly responded to the interaction between C amendment and moisture ($X^2_{16} = 67.98$; $P < 0.001$; Figure 1A). Unsurprisingly, respiration increased under each C amendment at all moisture levels. Respiration peaked at 60% WHC for all C treatments, which was 150% (control), 81% (glucose), 106% (oxalic acid), 95% (methanol), and 70% (α-pinene) higher than the lowest respiration rates at 25% WHC (Figure 1A). TDN concentrations were also affected by an interaction between C and moisture level ($X^2_{16} = 41.48$; $P < 0.001$; Figure 1B). All C treatments follow similar patterns; TDN increased steadily between 35% WHC and 60% WHC before decreasing between 60% WHC and 70% WHC. α-pinene had the lowest TDN under dry conditions and highest at 60% WHC (Figure 1B). There was a marginal C amendment effect on

DOC (X_4^2 = 8.85; *P* = 0.06; Figure 2), driven by DOC in methanol amended soils being 11% lower than in glucose (*P* = 0.09) amended soils, 10% lower than oxalic acid (*P* = 0.12) and α-pinene (*P* = 0.14) amended soils, and 5% lower than the control (*P* = 0.86; Figure 2).

Summary of Prokaryotic communities

We used NMDS with Bray-Curtis distance to visualize the bacterial communities, and found clearseparation of communities based on both C and moisture treatments (PERMANOVA: F_{16} = 1.41, *P* = 0.001; Figure 3A). Aggregated across all samples, 36 prokaryotic phyla were detected with 12 phyla each accounting for greater than 1% of sequences. The phylum Proteobacteria had the highest relative abundance, accounting for ~41% of sequences, followed by Acidobacteria 17%, and Bacteroidetes 10% (Figure 3B).

Individual carbon and moisture effects on abundant phyla

Actinobacteria, Bacteroidetes, Chloroflexi, Thaumarchaeota, and Verrucomicrobia exhibited significant responses to C treatments and moisture treatments with no interactions (Figure 4A & B). Planctomycetes only responded to C (Figure 4A), and Gemmatimonadetes only responded to moisture (Figure 4B). Actinobacteria response to C (χ_4^2 = 22.03; *P* < 0.001) was driven by a 26% increase under glucose (*P* = 0.11), and oxalic acid (*P* = 0.04). While methanol (*P* = 0.80), and α-pinene (*P* = 0.94) did not affect Actinobacteria relative abundance compared to the no C addition, relative abundance in the methanol treatment was 31% less than oxalic acid (*P* < 0.001) and glucose (*P* = 0.003). Actinobacteria response to moisture (χ_4^2 = 58.20; *P* < 0.001) was largely driven by the 25% WHC treatment having 32-45% lower relative abundance than all other moisture treatments (all *P* < 0.05l). Bacteroidetes (F_4 = 2.25; *P* = 0.069), and Verrucomicrobia (F_4 = 2.03; *P* = 0.096) both significantly decreased under methanol amendment: Verrucomicrobia 12% (*P* = 0.06), Bacteroidetes 16% (*P* = 0.06). All other

C amendments were intermediate between the control and methanol. However, Bacteroidetes (F_4 = 3.48; P = 0.011), and Verrucomicrobia (F_4 = 2.03; P = 0.096) had contrasting responses to moisture. Bacteroidetes were least abundant at 25% WHC, and had significantly higher relative abundance at 45% (P = 0.08) and 60% WHC (P = 0.011); Verrucomicrobia were 12-19% more abundant at 25% WHC than the other moisture treatments (all $P < 0.05$). Planctomycetes (F_4 = 4.34; P = 0.003) responded in a similar way to C amendment as Bacteroidetes and Verrucomicrobia; however, they displayed no response to moisture (F_4 = 0.45; P = 0.77). In addition to decreasing 19% under methanol amendment (P = 0.001), they also decreased 16% under oxalic acid amendment (P = 0.051). Chloroflexi response to C treatment (F_4 = 2.48; P = 0.049) was driven by the methanol treatment being 13% lower than the oxalic acid treatments (P = 0.03). Chloroflexi (F_4 = 4.96; P = 0.001) also responded negatively with increasing moisture where in this instance the 25% WHC treatment was 9-17% more abundant than the other moisture treatments. As with each of the previous phyla, Thaumarchaeota (F_4 = 4.25; P = 0.003) responded negatively to methanol amendment, decreasing in abundance by 20% (P = 0.08). Additionally, the relative abundance of Thaumarchaeota was highest in oxalic acid treatment: 27% less than methanol (P = 0.005) and 23% more than α-pinene (P = 0.03). Additionally, Thaumarchaeota (F_4 = 11.50; $P < 0.001$) responded negatively to increasing moisture, decreasing 21-41% under moisture treatments greater than 25% WHC. Gemmatimonadetes only responded to moisture treatments (F_4 = 9.51; $P < 0.001$) and not C treatments (F_4 = 0.82; P = 0.52) as the relative abundance of Gemmatimonadetes peaked at 35-45% WHC and was lowest at 25% WHC.

C and moisture interactive effects on abundant Phyla

There was a significant interaction between C and moisture in Acidobacteria (F_{16} = 1.65; P = 0.073), and was primarily driven by differences in response to individual C treatments across moistures as the only significant difference between C treatments within moistures was that there was a 23% decrease in relative abundance at 70% WHC under methanol amendment. While there were no significant effects within the control and oxalic acid across moisture treatments, there were effects on glucose, methanol and α-pinene, all of which had highest relative abundance at 35% WHC. Under glucose (P = 0.014) treatments Acidobacteria were 24%, more abundant at 35% WHC than their counterparts at 60% WHC. At 35% WHC and under α-pinene treatment Acidobacteria were 30% more abundant than the 25% WHC treatment (P = 0.084), and 18% more abundant than the 70% WHC treatment (P < 0.001). While under methanol treatment both the 25% WHC (P = 0.042), and 35% WHC (P = 0.004) treatments were more abundant than the 70% WHC treatment. Proteobacteria (χ^2_{16} = 30.48; P = 0.016) were major responders to C amendment within and across moisture levels. At 25% WHC methanol (P = 0.09) and α-pinene (P < 0.001) increased Proteobacteria relative abundance 16% and 28%, and were significantly more abundant than all other C treatments (all P < 0.10). Additionally, methanol treatment lead to 13% increase in relative abundance at 35% WHC (P = 0.088), and 20% at 70% WHC (P = 0.011). Also, of particular interest is the apparent moisture effect on glucose and α-pinene. Glucose treatments increased relative abundance of Proteobacteria between 25% WHC and both 60% WHC (19%; P = 0.013), and 70% WHC (16%; P = 0.058), while α-pinene treatments decreased relative abundance 15% between 25% WHC and 60%WHC (P = 0.054). The interaction in Firmicutes (χ^2_{16} = 28.45; P = 0.028), was largely driven by response to α-pinene. Under α-pinene amendment Firmicutes were at lowest abundance at 35% WHC, which was 75% less than at 45% WHC (P = 0.027), and 72% less than at 70% WHC (P =

0.039). Additionally, at 35% WHC, glucose (P = 0.045), oxalic acid (P = 0.008), and methanol (P = 0.049), treatments Firmicutes were 71%, 78%, and 71% more abundant than in the α-pinene treatment. The oxalic acid treatment (P = 0.021), increased Firmicutes relative abundance 293%. Generally, relative abundance of Firmicutes increased with increased moisture for all of the C addition treatments; however in the no C control relative abundance was highest at 60% and 25% WHC without any C amendment. Nitrospirae (X^2_{16} = 55.01; P < 0.001), and Rokubacteria (F_{16} =2.29; P = 0.007), had similar interactive responses to the moisture and C treatments. At 25% WHC, α-pinene decreased relative abundance Nitrospirae 39% (P < 0.001), and Rokubacteria 38% (P = 0.079). Likewise, Nitrospirae, and Rokubacteria each increased in relative abundance under α-pinene amendment with increasing moisture. At 70% WHC, methanol amendment decreased Nitrospirae 34% (P = 0.002), and Rokubacteria 57% (P = 0.001).

Differentially Abundant ASVs under C amendment

There were 140 distinct ASVs from 12 phyla, that were identified as differentially abundant by edgeR. Aggregated across all samples differentially abundant Proteobacteria comprised 73%, Actinobacteria 12%, and Bacteroidetes 5% of all differentially abundant ASVs. There were 85 ASVs from the Proteobacteria, spanning 22 identified families, with the most responsive families being Methylophilaceae (14 ASVs), Burkholderiaceae (13 ASVs), and Sphingomonadaceae (8 ASVs). The Methylophilaceae responded positively to VOC amendment – especially methanol - and negatively to glucose and oxalic acid amendment. The Burkholderiaceae were split between negative and positive responders under all C amendments. While the Sphingomonadaceae ASVs mostly responded positively to C amendment, especially under glucose and α-pinene addition.

Discussion

Soil respiration responded significantly to the addition of each C compound, which is expected since soils are generally C limited (Soong et al.; van Hees et al. 2005). Surprisingly, methanol increased respiration the most at all moisture levels except 70% WHC (Figure 1A). This respiratory response could be due to soil microbes using methanol primarily as an energy source instead of building biomass (Kramshøj et al. 2018), whereas compounds such as glucose have very high C use efficiencies (Geyer et al. 2016). DOC concentrations did not change from the control in any of the C treatments (Figure 2). This lines up with previous studies investigating methanol additions (McBride et al. 2019). The lack of response to DOC additions (glucose and oxalic acid) may be due to the rapid use of those compounds, or a lack of sensitivity in our measurements. As in a previous study (McBride et al. 2019), TDN decreased between the control and methanol treatment under higher moisture levels (Figure 1B). Additionally, α-pinene decreased TDN as well. Monoterpenes have been previously been shown to affect N transformations (Paavolainen et al. 1998; Smolander et al. 2012). However, glucose was often intermediate between the control and these VOCs. This indicates that C, in general, is driving down TDN levels due to heterotrophic competition for N (Verhagen and Laanbroek 1991; Verhagen et al. 1992).

Both the type of C addition and moisture affected microbial community composition (Figure 2). Visualization of communities using NMDS show a clear separation of communities which is driven by both C addition and moisture level (Figure 2A). While the 25% moisture treatments are distinct from the other moisture levels, the moisture effect is most apparent under VOC addition. This suggests that VOCs may have a more prominent role in structuring microbial communities at low moisture levels. Indeed, The transport of monoterpenes like α-pinene through soil, has been shown to be impeded by increasing moisture saturation (van Roon

et al. 2005). Methanol and α-pinene, like other VOCs, can diffuse through air filled pore space 10^3 to 10^4 times faster than through soil (e.g., methanol diffusion rate in water is 0.15 cm^2 s^{-1} and 1.64 x 10-5 cm^2 s^{-1}) water which may allow VOCs to reach more soil microorganisms while in vapor phase and under dry soil conditions than DOC.

Unsurprisingly, 11 of the 12 most abundant phyla were significantly affected by moisture content, or an interaction between moisture content and C amendment. The phyla Actinobacteria, Chlorflexi, Thaumarchaeota, and Verrucomicrobia, all had highest relative abundance at 25% WHC. Actinobacteria and Chloroflexi are known to have higher relative abundance at lower moisture levels (Ren et al. 2018; Banerjee et al. 2018; Yang et al. 2019). It has been suggested that Actinobacteria have a preparedness strategy – i.e., ready to increase activity – for dealing with a reduction in soil moisture availability, while the Chloroflexi use resistant life-strategy, maintaining the same level of activity under reduced moisture (Barnard et al. 2013). Thaumarchaeota contain the ammonia oxidizing archaea and have been shown to thrive in marine environments (Karner et al. 2001), but also in dry soils (Li et al. 2017). Verrucomicrobia have been shown to have a weak positive relationship with soil moisture (Buckley and Schmidt 2001), which is the opposite of our results. It is possible that moisture preference is not conserved at the phyla level for Verrucomicrobia and future studies should investigate Verrucomicrobia moisture preference at lower taxonomic levels. The phylum Gemmatimonadetes had highest relative abundance at the lower end of our study but were lowest at 25% WHC, which is contrary to previous studies that have shown the relative abundance of Gemmatimonadetes highest at the lowest moisture levels (DeBruyn et al. 2011). The effect of moisture on soil microbial communities is well documented (Brockett et al. 2012; Guenet et al.

2012; Yang et al. 2019). Our results reinforce the notion that moisture is a major driver of microbial community composition, and suggests that microbial niche space is multidimensional.

Verrucomicrobia, Bacteriodetes, and Planctomycetes were all most abundant under no C addition, suggesting that these organisms could fit within the ecological classification of oligotrophy. Additionally, Verrucomicrobia, Bacteriodetes, Planctomycetes, and Thaumarchaeota had significantly lower relative abundance under methanol amendment than in the control, likewise Chloroflexi and Actinobacteria had lowest relative abundance with methanol amendment, yet not statistically significant. This suggests that methanol either inhibits these taxa or that other taxa are thriving under methanol amendment. Thaumarchaeota were highest under oxalic acid amendment and Thaumarchaeota are known to do well under low pH conditions. Although we did not measure soil pH at the end of the experiment it is possible that oxalic acid increased soil acidity (Nicol et al. 2008; Martens-Habbena et al. 2009; Li et al. 2017). Our Thaumarchaeota result matches previous work that showed a reduction in ammonia oxidizing archaea under methanol amendment (McBride et al 2019). This further strengthens the link between VOCs and nitrification inhibition that has been previously shown (Paavolainen et al. 1998; Smolander et al. 2012; McBride and Strickland 2019).

Several phyla exhibited significant C by moisture interaction including Firmicutes, Nitrospirae, Rokubacteria, Proteobacteria, and Acidobacteria. The Acidobacteria generally did not do well with C addition, however at 35% WHC the Acidobacteria responded positively to C addition, possibly indicating a moisture optimum for these organisms. The Nitrospirae and Rokubacteria both generally increased with increasing moisture, but, did best at high moisture without C amendment. The Firmicutes and Proteobacteria both did well in moister soils, and both responded positively to methanol at 70% WHC. However, Proteobacteria also responded

positively to methanol at 25%, 45%, and 60%. Likewise, Proteobacteria had a strong positive response to α-pinene at low moisture. The effect of α-pinene only at low moisture may be a result of the inability of α-pinene to diffuse quickly through water due to its general insolubility in water. This suggests that many Proteobacteria may have the ability to metabolize VOCs.

Of the differentially abundant ASVs, 73% were Proteobacteria. This is in line with previous studies that suggest this phylum is composed of copiotrophs that are able to quickly metabolize simple C compounds (Fierer et al. 2007). The three most common families were Methylophilaceae, Burkholderiaceae, and Sphingomonadaceae.

Conclusion

We found that there was an interaction between C source and soil moisture on microbial community structure. Both DOC treatments had the largest effect on microbial community structure at higher moisture levels, the hydrophobic VOC α-pinene affected the community the most at low moisture, and methanol affected community structure at all moisture levels. This reinforces our hypothesis that VOCs may play an important role as a C source in bulk soil and in dry soils. Possibly, due to their fast diffusion in air filled pore space, VOCs are able to increase microbial activity even at lower moisture levels, which may lead to blooms in taxa able to use these substrates. Methanol elicited the largest phyla level response, causing a decrease in relative abundance of Actinobacteria, Bacteroidetes, Chloroflexi, Planctomycetes, Thaumarchaeota, and Verrucomicrobia, across all moistures, and Acidobacteria, Nitrospirae, and Rokubacteria at high moisture; however, Proteobacteria relative abundance increased across moisture levels, and the Proteobacteria comprised a disproportionate percentage of differentially abundant ASVs. The role of VOCs on soil microbial taxa is becoming clear that it extends beyond simply inhibiting some taxa, but that these compounds are significant sources of C for some organisms.

Figures

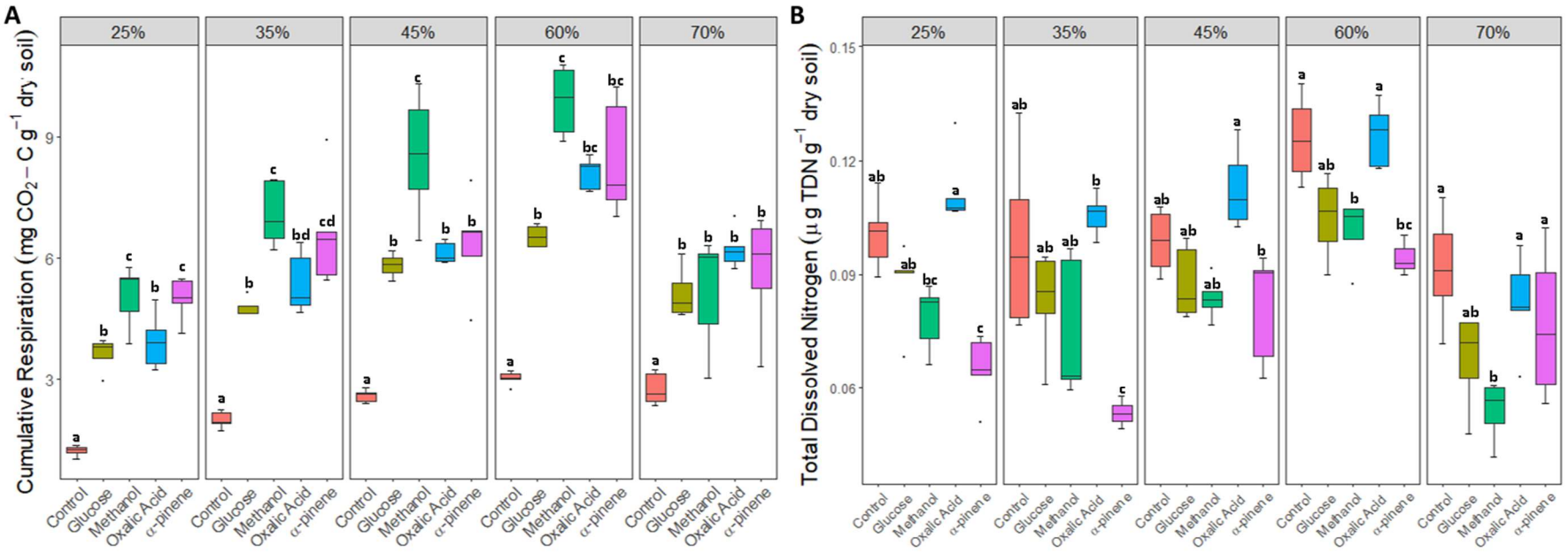

Figure 3.1

Figure 3.1

Cumulative respiration (A), and Total dissolved nitrogen (B) within each moisture regime. Letters represent significant differences between carbon treatments within a moisture regime. α = 0.10.

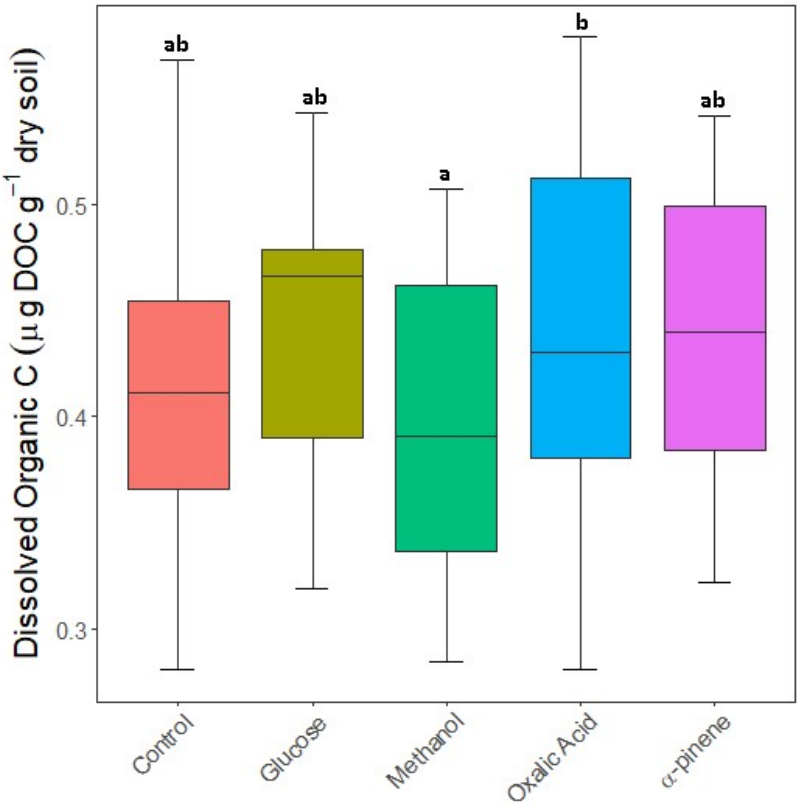

Figure 2

Figure 3.2

Dissolved organic carbon. Letters represent significant difference between carbon treatments averaged across moisture treatments. $\alpha = 0.10$.

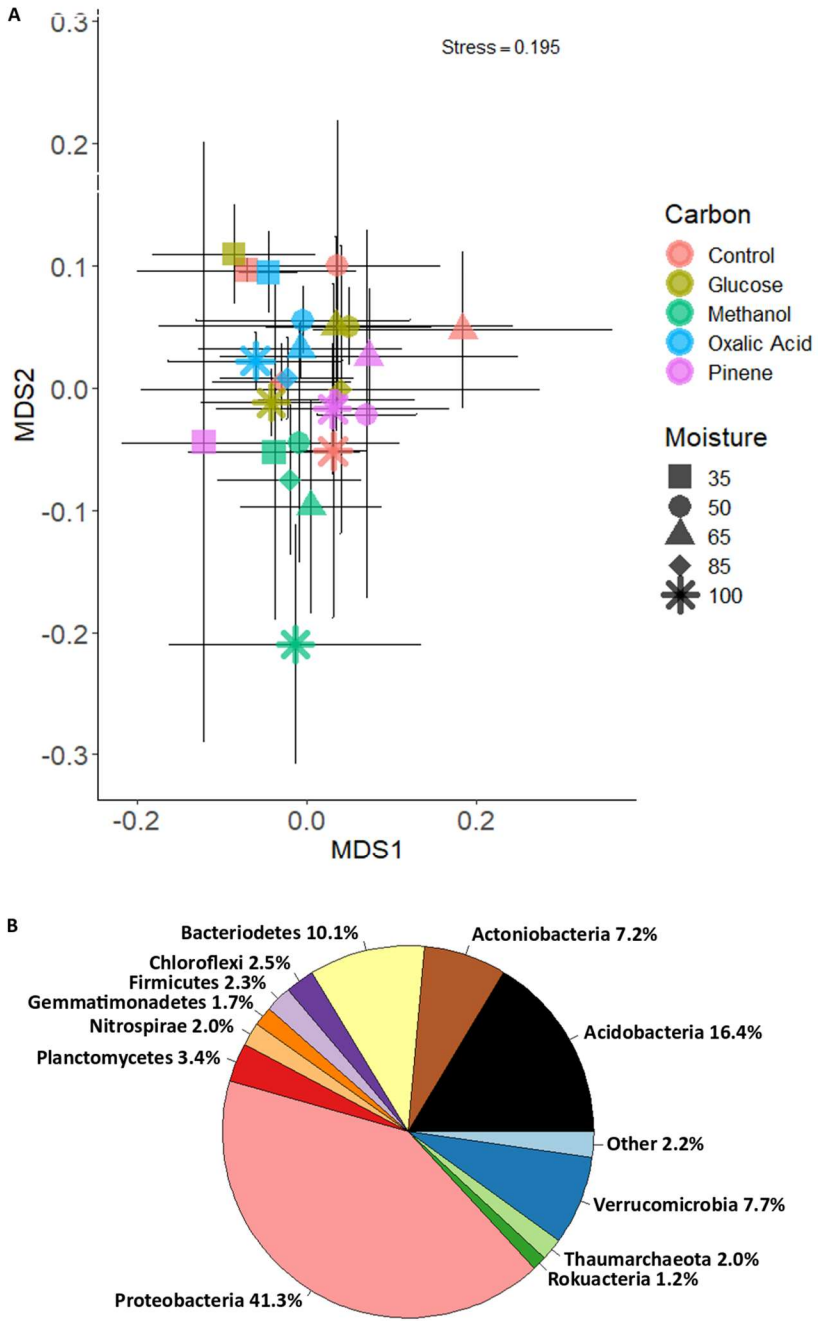

Figure 3.3

Figure 3.3

Non-metric multidimensional scaling of Bray-Curtis matrix (A), and Aggregated percentage of each phyla (B).

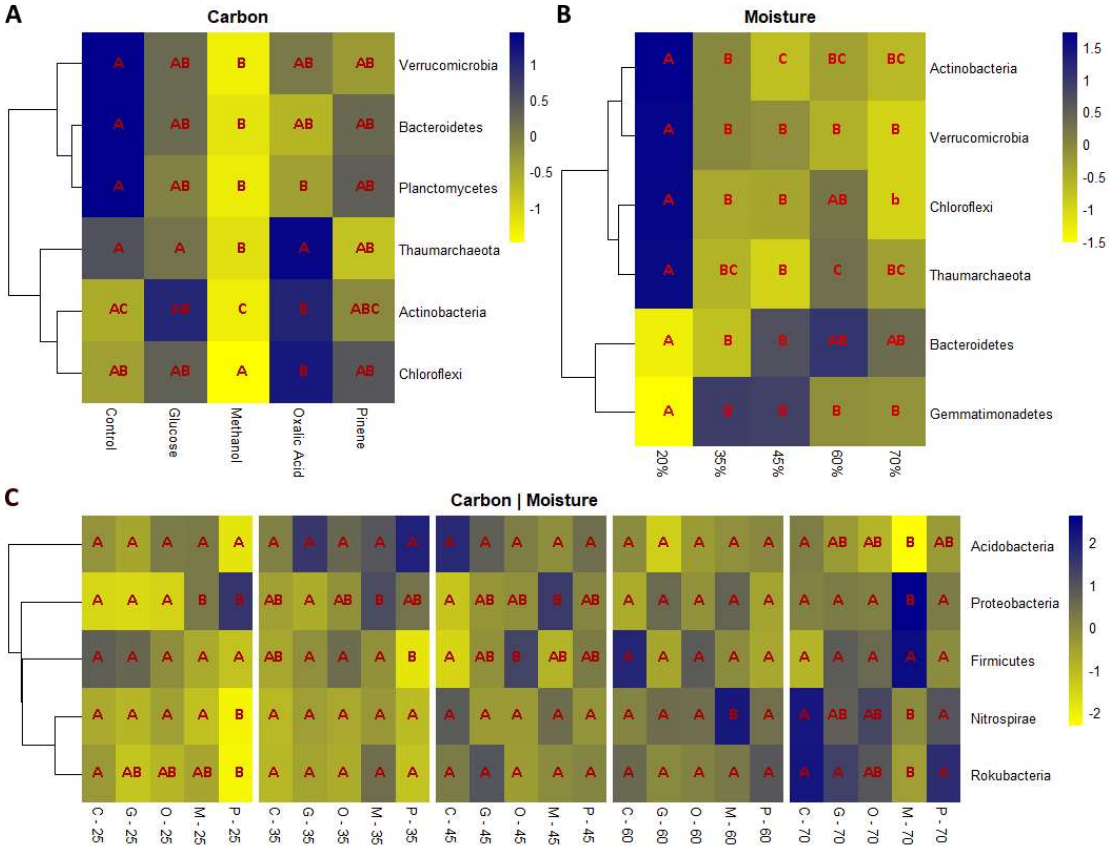

Figure 3.4

Figure 3.4

Phyla with significant carbon effects, letters denote difference between carbon treatments averaged across moisture treatments (A), moisture effects, letters denote difference between moisture treatments averaged across carbon (B), and carbon by moisture

interactions, letters denotes significant differences between carbon treatments within moisture treatments (C). The color bar represents scaled Z-scores for each bacterial phylum. α =0.10.

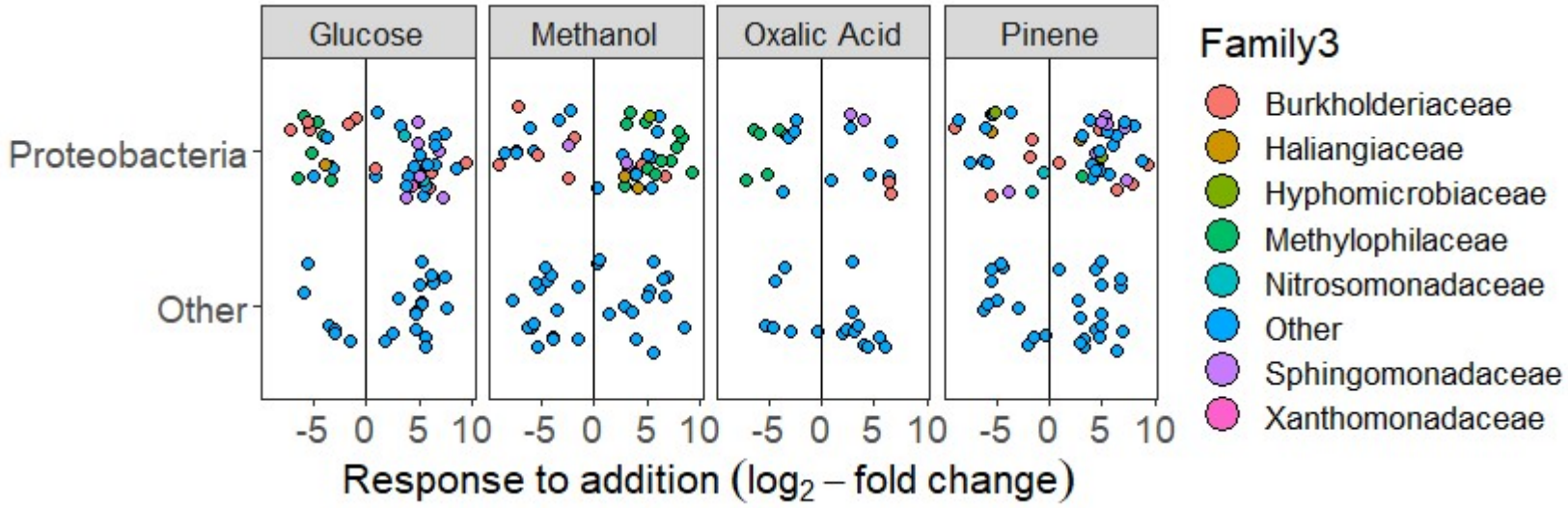

Figure 5

Figure 3.5

Differentially abundant ASVs separated into differentially abundant Proteobacteria and all other phyla. Proteobacteria phyla that comprised less than 2% of all Proteobacteria were grouped into the family “other”.

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Chapter 4- 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 has primarily focused on the leaching of soluble carbon from litter to soil 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-day 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.

Introduction

Leaf litter decomposition is thought to contribute to soil organic matter (SOM) formation primarily through two pathways 1) microbial assimilation of high quality, usually water soluble carbon (C) (e.g., leaf litter leachates), and 2) physical incorporation of plant litter directly into underlying mineral soil horizons (Kalbitz and Kaiser 2003, Cotrufo et al. 2015, Bradford et al. 2016, Sokol and Bradford 2019). In these pathways, high quality C is rapidly decomposed then assimilated into microbial biomass before stabilizing in the slow cycling mineral associated organic matter (MAOM), while plant structural materials are physically incorporated into soil, forming particulate organic matter (POM; Cotrufo et al. 2013, Cotrufo et al. 2015, Bradford et al. 2013). However, this focus largely overlooks the potential of litter-derived volatile organic compounds (VOC) to shape soil microbial communities and soil biogeochemical processes. Terrestrial vegetation releases a significant quantity of chemically diverse volatile compounds (Kesselmeier et al. 2002, Isidorov et al. 2016), e.g., alcohols, carbonyls, and monoterpenes. Given that VOCs 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 ultimately contribute to SOM formation.

VOC production during litter decomposition is primarily driven by microbial activities (Gray et al. 2010). While variable, the total quantities of VOCs released during litter decomposition can be surprisingly high, occasionally exceeding 100 μmol VOC-C g litter^{-1} h^{-1} (Ramirez et al. 2009), with some litter types emitting VOCs at rates that approach those of CO_2-C from litter decomposition (Gray et al. 2010). Due to their abundance, litter-derived VOCs could represent an important, rarely considered, source of organic C to underlying soils – e.g., we conservatively estimate that VOC emissions from *Pinus* litters (3-11 g of VOC-C m^{-2} y^{-1} (Gray et al. 2010)) are similar to reported rates of root exudate C inputs from *Pinus taeda* (9 g m^{-}

^{2} y^{-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. 2009). Beyond C dynamics, VOCs impact nutrient dynamics - e.g., methanol and acetone have been shown to affect nitrogen (N) transformations (McBride et al. 2019). VOCs can also alter microbial community dynamics by limiting or promoting the growth of specific microbial taxa, e.g., methylotrophic bacteria (Wheatley 2002, Gray et al. 2015).

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. By using ^{13}C-labelled leaf litter, we tracked litter-derived VOC-C into several soil C pools (i.e., dissolved organic C [DOC], microbial biomass C [MBC], POM-C, and MAOM-C throughout a 99-day incubation period. If litter-derived VOC-C represents a significant contribution to soil C pools, then we expect to observe enrichment of the ^{13}C tracer in the measured pools. We harvested soils at the end of the incubation and used cultivation-independent molecular approaches to determine if the litter-derived VOCs alter the composition of soil bacterial and fungal communities. Together our study confirms that VOCs emitted during litter decomposition can alter soil microbial communities and C dynamics, highlighting the importance of an understudied mechanism by which decomposing litter influences underlying soils.

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. The soil was sourced from a single site near Blacksburg, VA, USA (37.20, -80.59): soils are fine, mixed, semiactive, mesic Typic Hapludults; 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, 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 weight equivalent soil:99 mL 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. Soil in the large jar and litter in the small jar were maintained at 65 and 50% water holding capacity, respectively, at 20ºC throughout the 99-day experiment. 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 reps x 3 litter types), 12 'litter-only' microcosms (4 reps x 3 litter types), and 4 'soil-only' microcosms (4 soil reps).

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-day 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-day experiment, we destructively harvested each microcosm containing soil and determined MBC, 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 (see below). For MBC and DOC, we conducted a modified chloroform fumigation extraction (Fierer and Schimel 2003).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, CO, 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.

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, CA, 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:

$$atom\% = R_{std}\left(\frac{\delta^{13}C_{sample}}{1000}+1\right)/\left(1+R_{std}\left(\frac{\delta^{13}C_{sample}}{1000}+1\right)\right)$$

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

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).

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

where C_{pool} is the total amount of C in a given pool, atom%$^{13}C_{VOC\ exposed}$ is the atom%^{13}C value of a given pool after exposure to litter-derived VOCs, atom%$^{13}C_{soil}$ 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}Clitter is the atom%^{13}C value of the actual litter. The percent of litter-derived C associated with a given C pool was determined as $100 \times (C_{voc\ derived} / C_{pool})$.

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-day 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 the Appendix S1. In total, 4,422 bacterial and archaeal 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 at https://figshare.com/s/e913dbad66eb74d8904d.

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 Core Development Team). Differences between litter species and the soil-only control were determined via analysis of variance (ANOVA). Pairwise treatment comparisons were assessed via Tukey HSD. 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 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., 2017). 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, α=0.05 (uncorrected p-value) using the R package 'mctoolsr' (https://github.com/leffj/mctoolsr/) - omitting rare taxa with relative abundances less than 0.025.

Results

Contribution of litter-derived VOCs to soil C pools

Litter-derived VOCs contributed appreciably to all measured soil C pools (Figure 1A). Across all leaf litter species, litter-derived VOCs accounted for between 4.06% and 0.44% of the C in the MBC pool (Figure 1A). 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 (Figure 1B; $F_{2,9}$=10.4; P<0.01). For the DOC pool, litter-derived VOCs accounted for between 1.41% and 0.32% of (Figure 1A). Although litter-derived VOCs contributed to the DOC pool, no significant differences between litter types were observed (Figure 1C; $F_{2,9}$ = 0.59; P=0.32). For POM C, litter-derived VOCs accounted for between 0.31% and 0.04% of the C in this pool (Figure 1A). As with the DOC pool, while litter-derived VOCs contributed to the POM C pool, no differences between litter species were observed (Figure 1D; $F_{2,9}$ = 1.3; P=0.32). For MAOM C, litter-derived VOCs accounted for between 0.29% and 0.11% of the C in this pool (Figure 1A). 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 (Figure 1E; $F_{2,9}$ = 5.95; P<0.05).

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. Generally, differences in soil communities exposed to switchgrass and tulip poplar litter VOCs were more similar to each other and greater than differences in communities exposed to eucalyptus litter VOCs. For instance, bacterial and archaeal diversity differed across litter treatments (Figure 4.2; $F_{3,12}$=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 (Figure 2; $F_{3,12}$=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 (Figure 2). 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 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).

Total soil C and N pools

Exposure to litter-derived VOCs had relatively little effect on total MBC, DOC, POM C, MAOM C, or total soil C (Appendix S1: Table S1; *P>0.05* in all cases). However, as noted above, litter-derived VOC-C was observed in all of these pools (see *Contribution of litter-derived VOCs to soil C pools*). This seeming discrepancy is due to the fact that we were quantifying changes in relatively large soil C pools over a short time period, which necessitates the use of a C tracer to observe appreciable change in soil C pool sizes. For soil N pools, we observed no effect of exposures to litter-derived VOCs on MAOM N or POM N, but did observe a slight effect of litter treatment on total soil N ($F_{3,12}$ = 3.02; *P*=0.07), where switchgrass and

tulip poplar treatments had higher N than the soil and eucalyptus treatments. Likely because of these slight differences in N, we observed significant effects of litter-derived VOCs on POM ($F_{3,12}$ = 29.9; P<0.001), MAOM ($F_{3,12}$ = 5.96; P<0.01), and total ($F_{3,12}$ = 55.02; P<0.001) C:N ratios. For POM and total pools, this difference in C:N ratios could be attributed to a greater C:N ratio for the 'soil-only' microcosms and the soils incubated with the eucalyptus litter compared to the soils exposed to the VOCs from the decomposing switchgrass and tulip poplar litters. For the C:N ratio of the MAOM fraction, this difference was due to a greater C:N associated with soils exposed to tulip poplar VOCs compared to those soils exposed to the eucalyptus and switchgrass VOCs. Finally, we observed significant differences in inorganic N pool sizes (NO_3^- and NH_4^+). For NO_3^- ($F_{3,12}$ = 162.8; P<0.0001), we observed the greatest concentrations in soils exposed to both switchgrass and tulip poplar VOCs, and the lowest concentrations in soil exposed to eucalyptus VOCs and the soil only control. For NH_4^+ ($\chi^2_{3,12}$ = 169.25; P<0.0001), we observed the greatest concentration in soil exposed to switchgrass VOCs, soils from all other treatments were lower.

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 microbial biomass C 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 versus soluble low molecular weight C (i.e., glucose) to soil C pools we note several examples where the contribution 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 0.7-7.59% 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 high of 4.06% to a low of 0.44% depending on the litter type (Figure 1). While 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 0.32-1.4%. This is considerably less than is attributed to root exudate C (Giesler et al. 2007), however, this is likely due to the fact that 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 – i.e., 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.

This 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). While 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. Here we observe that the leaf litter with the lowest mineralization rate (Appendix S1: Fig. S2), eucalyptus, was associated with a greater contribution of VOC-C to both MBC, and MAOM C. This is likely due to 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. While we did not directly 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, including cyclic and acyclic compounds, that have been shown to have antimicrobial properties, as well as inhibit microbial processes such as methane oxidation and denitrification (Amaral et al. 1998, Trombetta et al. 2005). While we would expect a more pronounced effect of increased monoterpene concentration we were unable to measure these compounds so they may not have reached high enough concentration to induce a measurable allelopathic effect on the bacteria. Propanal and acetone are structural isomers that can be produced through a variety of pathways that include non-enzymatic Maillard reactions (Warneke et al. 1999), as well as fermentation of sugars and oxidation of lipids (Beesch 1952, Marco et al. 2006). Furthermore, while 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 composition of the bacterial and fungal communities shifted in response to exposure to VOCs emitted from the decomposing litters, with these community shifts most pronounced in soils exposed to the switchgrass and tulip poplar litters (Figure 2). 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). 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, Appendix S1: 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 (Figure 1), we hypothesize that these VOCs are serving as growth-promoting labile C substrates to support the growth of particular taxa. 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's 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 we show that these processes are not necessary for litters to influence C dynamics and SOM formation in underlying soil horizons. With this study, we show that 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 show that soil microorganisms are consuming litter VOCs. 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. However, since VOCs are not constrained by diffusion in water or mass flow paths, VOCs can clearly serve as an important C source in bulk soils, similar to the role of root exudate C inputs to rhizosphere soils.

Acknowledgements

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Figures

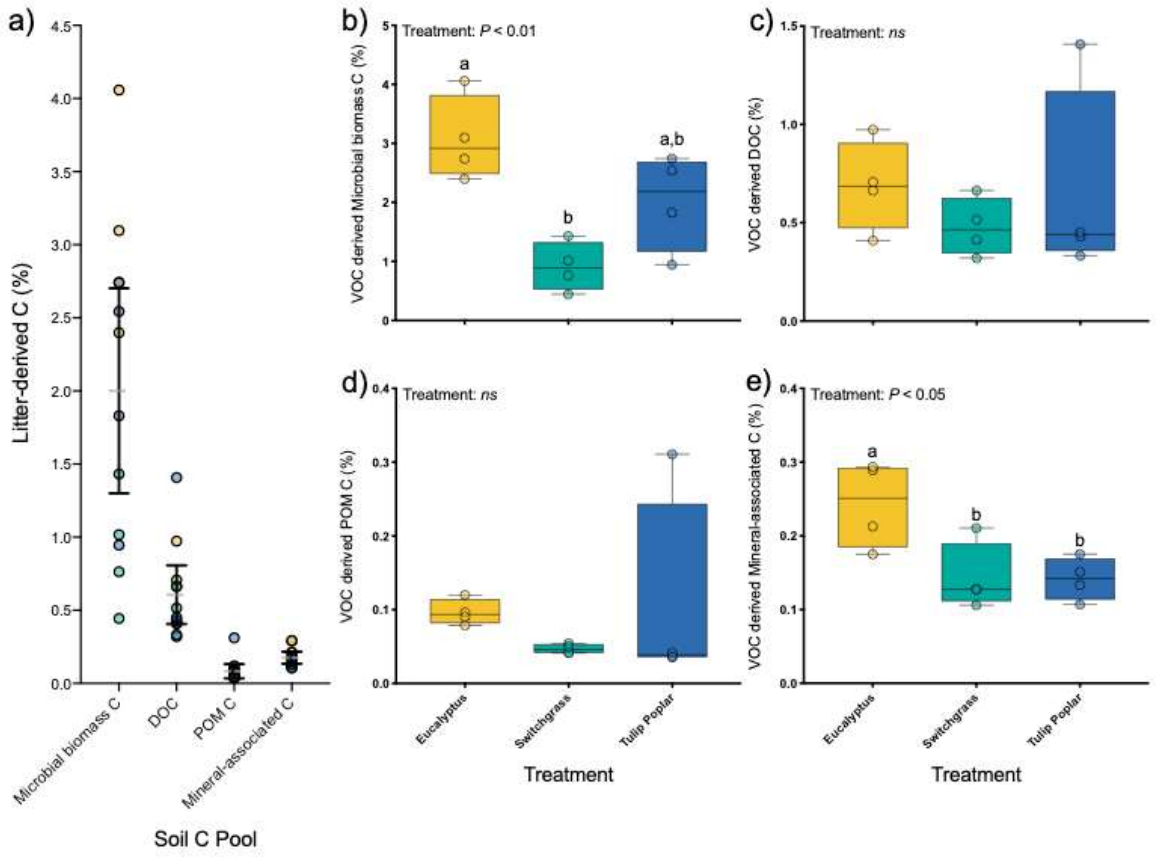

Figure 4.1

Figure 4.1

The contribution of litter-derived VOCs to measured soil C pools assessed after a 99-day microcosm experiment. a) Across all species, litter-derived VOCs contributed significant C to the measured pools. Shown is the mean and 95% C.I. for each soil C pool. If the confidence interval 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, switchgass, tulip poplar) associated with b) microbial biomass C, c) dissolved organic C (DOC), d), and particulate organic matter (POM) C e) mineral associated

soil C. For both microbial biomass C and mineral associated soil C the contribution of VOC-C was dependent on the litter type in question. Different letters indicate significant pair-wise treatment differences between litter treatments.

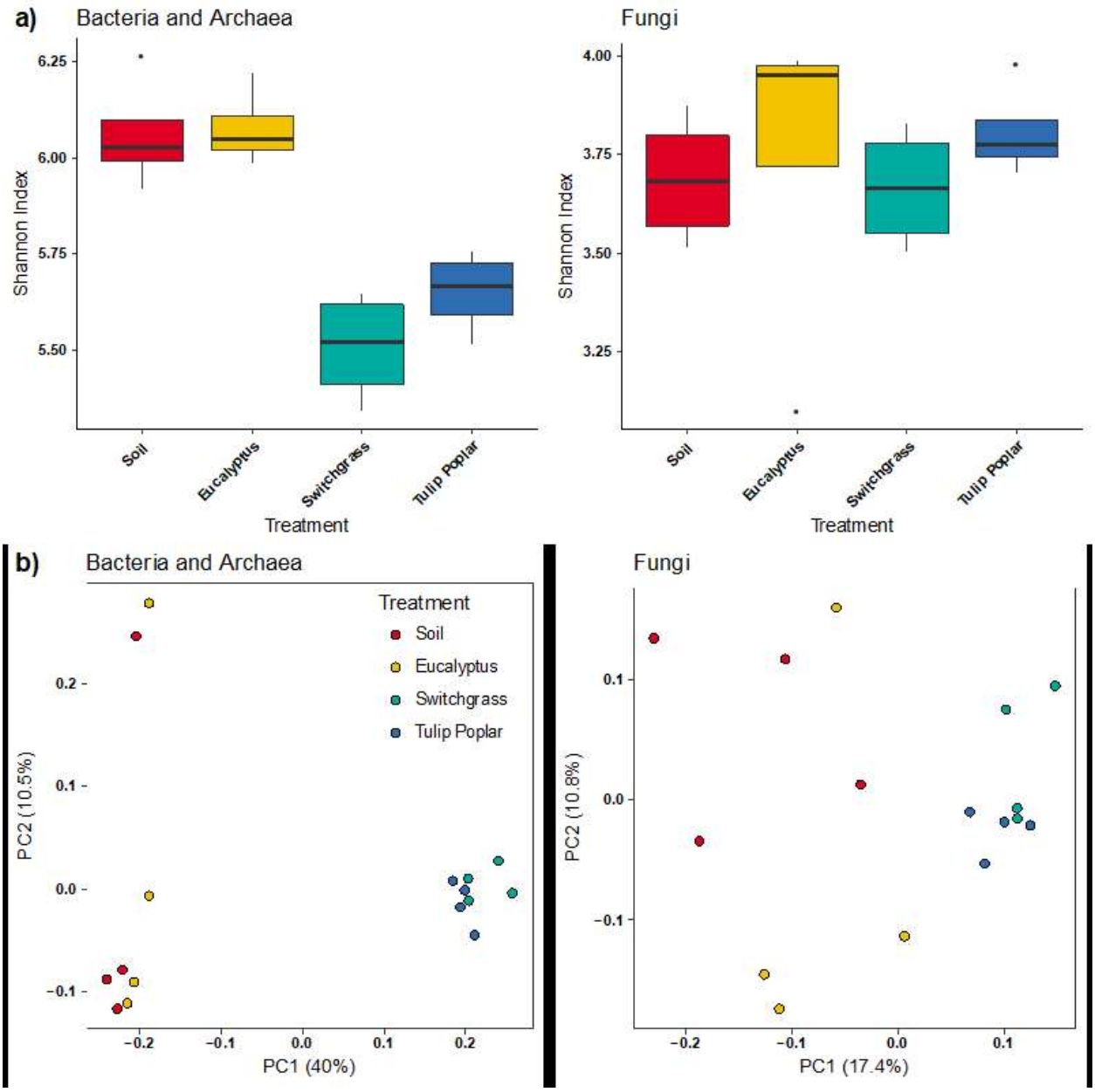

Figure 4.2

Figure 4.2

Richness and species composition of soil microbial communities exposed to litter-derived VOCs. a) Box and whisker plots show microbial richness estimates for each litter treatment. Bacterial and archaeal diversity differs 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; R2 = 0.486, P = 0.001). Fungal community composition also differs across treatments (PERMANOVA; R2 = 0.283, P = 0.001).

Supplementary Methods

Litter CO_2 production and Soil C and N pools

We used a modified static chamber method for measuring CO2 emission rates (Fierer et al 2003). Headspace was sampled weekly across the experiment by taking an initial and final (24 h after the initial sample) 5mL headspace sample. Concentrations of CO_2 were determined using an infrared gas analyzer (IRGA, Li-Cor Biosciences, Lincoln, NE, USA, Model: LI-7000). Notably the IRGA only captures a portion of $^{13}CO_2$ (Stephens et al. 2011), thus these analyses were conducted using unlabeled litter samples. Cumulative litter CO_2 production was determined via integration across the entire 99-day experiment.

In order to determine particulate organic matter (POM) and mineral associated organic matter (MAOM) carbon (C) and nitrogen (N), soil samples (4.5 g of air-dried soil) were dispersed in sodium hexametaphosphate (30 mL sample) via shaking (18 h) and then passed through a 53 μm sieve. Material <53 μm is considered mineral-associated and material >53 μm is considered POM associated. Mineral-associated and POM material were ball-milled after drying (105ºC), and percent C and N were determined with an elemental analyzer (Costech, Valencia, CA, USA, Model: ECS 4010).

In order to measure microbial biomass C (MBC) and dissolved organic C (DOC), paired chloroform fumigated and unfumigated samples were shaken with 0.5 M K_2SO_4 for 4 h, filtered, and DOC concentrations determined using a TOC/TN analyzer (Elementar Americas Inc, Mt. Laurel, NJ, USA, Model: Vario cube). MBC was estimated as the flush of DOC following fumigation. Raw MBC values are reported with no correction factors applied.

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

First, genomic DNA was extracted from each of the 16 soil samples representing 4 microcosm treatments using the DNeasy PowerSoil kit (Qiagen, Germantown, MD, USA) and amplified in triplicate, with both DNA extraction blanks and no-template PCR negative controls included to assess potential contamination. Pooled and multiplexed amplicons were sequenced on a single run on the Illumina MiSeq platform using 2 x 150 bp paired-end chemistry at the BioFrontiers Sequencing Facility at the University of Colorado Boulder (Boulder, CO, USA). After raw sequences were demultiplexed, primer sequences were removed using the program cutadapt (Martin 2011). We used the R package dada2 v1.8 to infer exact amplicon sequence variants (ESVs) (Callahan et al. 2016). Briefly, forward and reverse reads were quality filtered using default parameters (16S rRNA gene sequences were trimmed to 150 bp while ITS sequences were not trimmed) and dereplicated. ESVs were inferred using error rates learned from the quality filtered sequences, denoised paired reads were merged, and chimeric sequences were removed. Taxonomy was assigned to ESVs using the naïve Bayesian classifier method (Wang et al. 2007) implemented in dada2 and trained on the SILVA reference database (silva_nr_v132_train_set.fa.gz) (Quast et al. 2013) for bacterial and archaeal sequences and the UNITE ITS reference database (sh_general_release_dynamic_s_01.12.2017.fasta) (Nilsson et al. 2018) for fungal sequences. For the 16S rRNA gene sequence dataset, we removed chloroplast and mitchondrial reads and reads with no taxonomic assignment. For the fungal ITS sequence dataset, we removed eukaryotic Rhizaria reads and reads with no taxonomic assignment. Low coverage samples with less than 10,000 reads were removed, and remaining samples were rarefied to 10,000 reads. For each replicated soil sample, sequencing reads were pooled and reflect mean read counts.

Supplementary Results

Litter mineralization results:

Litter CO_2 production exhibited typical dynamics with an initial rapid increase in CO_2 followed by a decline in CO_2 through the course of the experiment (Figure S1). Switchgrass litter tended to exhibit the greatest CO_2 production rates followed by tulip poplar. Eucalyptus litter tended to have the lowest CO_2 production rates. This generalization was verified by the observation of significant differences in cumulative CO_2 production between litter treatments ($F_{3,12}$ = 343.6; P<0.001). Specifically, the greatest cumulative CO_2 production was observed for switchgrass, followed by tulip poplar, and then eucalyptus. Notably cumulative CO_2 production was 1.4 and 1.9-fold greater for switchgrass compared to tulip poplar and eucalyptus, respectively. The 'soil-only' microcosms had CO_2 production rates that were ~9-fold lower than production rates observed for any of the 'litter-only' microcosms.

Supplementary Figures

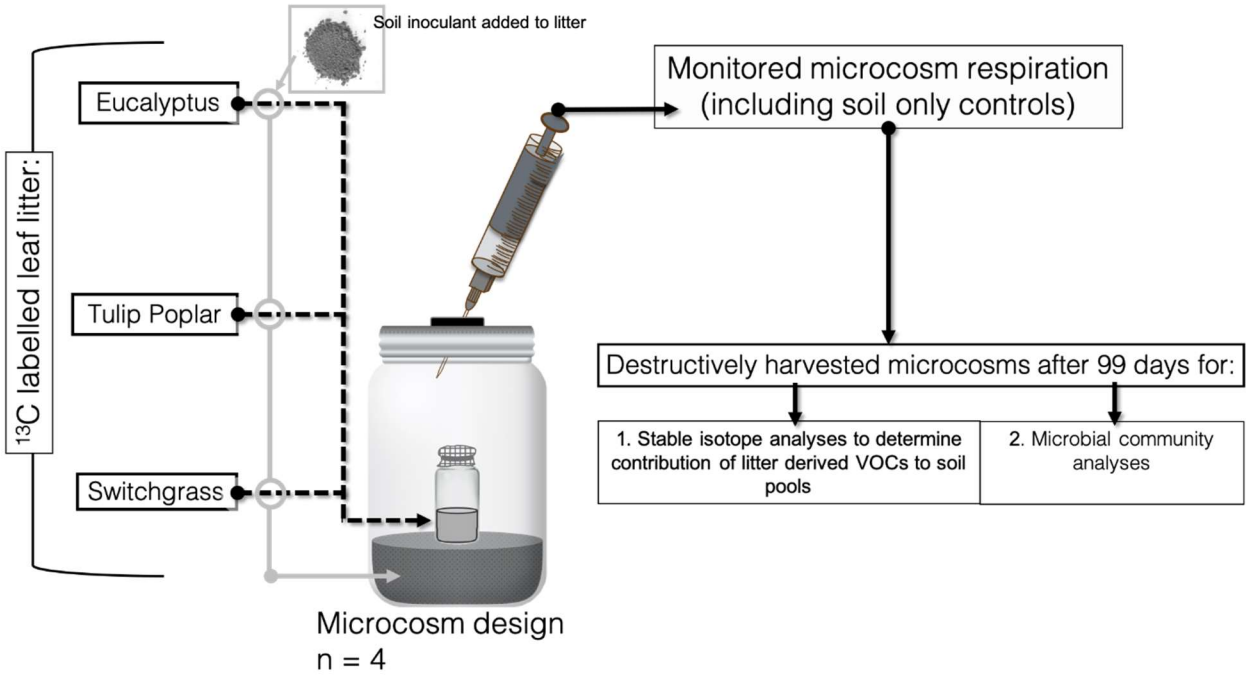

Figure 4.S 1

Figure 4.S 1

Experimental design to assess the contribution of litter-derived VOC-C to soil carbon pools, and their effect on microbial community composition. Note that the ^{13}C-labelled leaf litter shared the same headspace with the underlying soil but was not in direct contact. At the end of 99 days microcosms were destructively harvested to determine the effect of litter-derived VOCs.

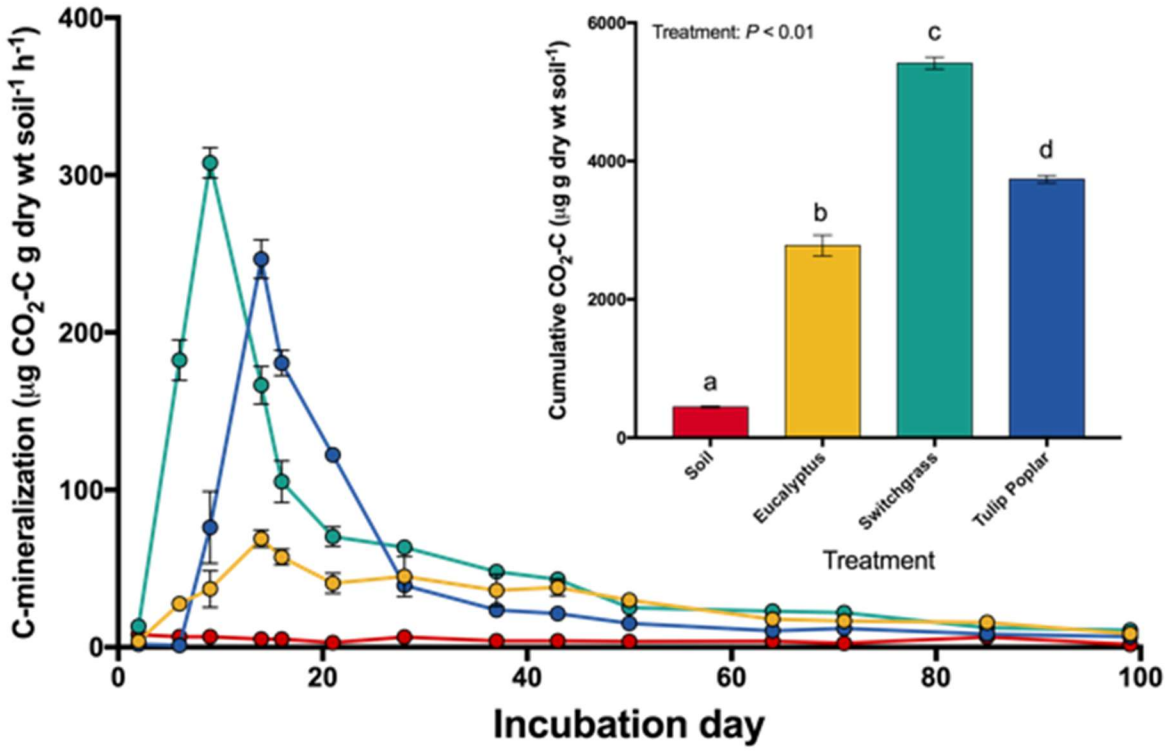

Figure 4.S 2

Figure 4.S 2

C-mineralization dynamics and cumulative C-mineralization associated with the three litters (i.e., eucalyptus, switchgass, tulip poplar) and the soil only control. C-mineralization dynamics are plotted across the time course of the experiment. Note that for the litters an initial increase in mineralization was observed followed by a general decrease in mineralization rates. The inset shows cumulative C-mineralization, determined via integration, for the entire time course of the experiment. Different letters indicate significant pair-wise treatment differences. All symbols represent the mean ± 1 S.E.

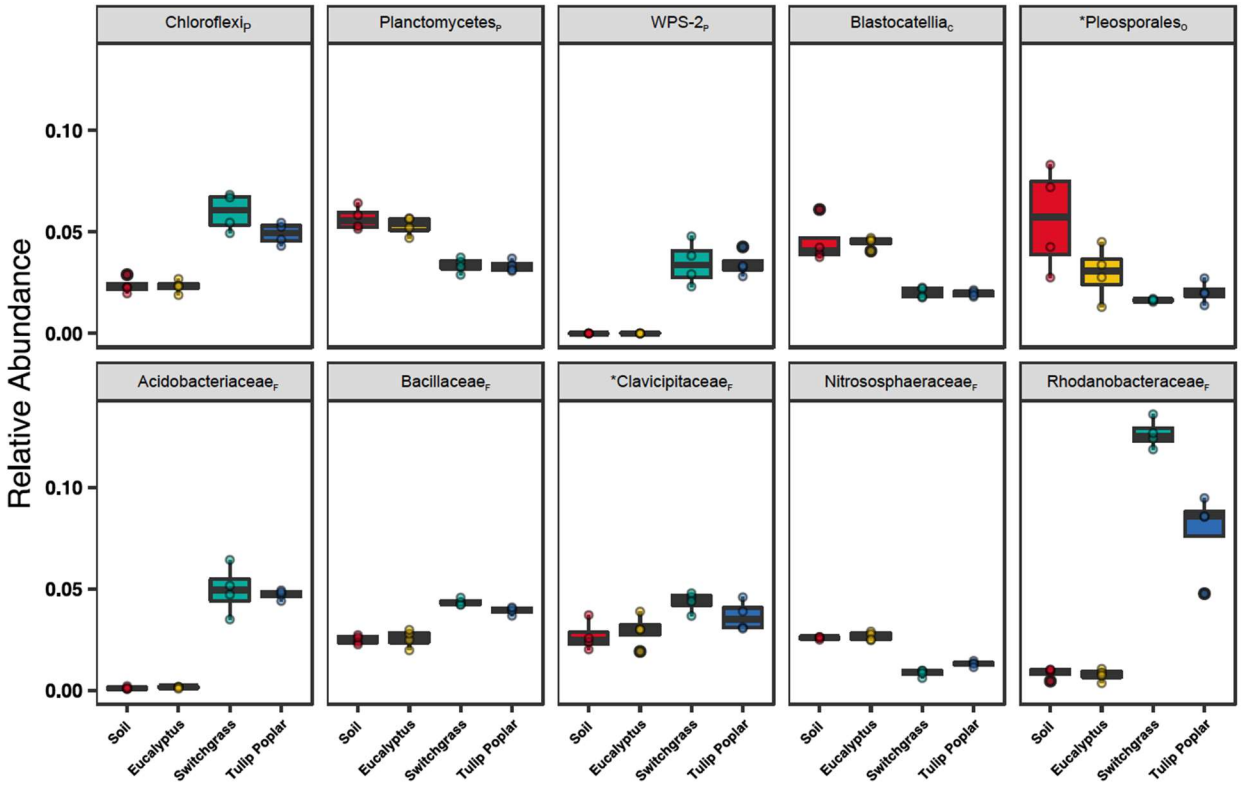

Figure 4.S 3

Figure 4.S 3

Soil microbial taxa that were differentially abundant across the litter treatments. Box and whisker plots show select taxa whose relative abundances differ between litter treatments (KW-test, uncorrected *P*-value < 0.05). The level of taxonomic classification is indicated by the subscript (i.e., P, phylum; C, class; O, Order; F, Family). Asterisks indicate fungal taxa. See Table S2 for relative abundances and KW-test results for all taxonomic groups.

Supplementary Tables

Table 4.S 1

Effect of litter decomposition derived VOCs on bulk soil C and N pools. Significant treatment effects are denoted by bold *P*-values, letters indicate significant pair-wise differences. Also shown are marginally significant results (i.e., *P<0.10*) denoted by the exact *P*-value.

Treatment	Total Soil C (mg g dry wt soil^{-1})	Total Soil N (mg g dry wt soil^{-1})	Total Soil C:N	POM C (mg g dry wt soil^{-1})	POM N (mg g dry wt soil^{-1})	POM C:N	Mineral C (mg g dry wt soil^{-1})	Mineral N (mg g dry wt soil^{-1})	Mineral C:N	DOC (mg g dry wt soil^{-1})	Microbial biomass C (mg g dry wt soil^{-1})	NO_3^- (µg g dry wt soil^{-1})	NH_4^+ (µg g dry wt soil^{-1})
Soil	14.99 ± 0.36	1.48 ± 0.02	8.95 ± 0.11^{a}	4.42 ± 0.31	0.30 ± 0.02	10.14 ± 0.15^{a}	10.58 ± 0.20	1.18 ± 0.02	14.95 ± 0.19^{a,b}	0.13 ± 0.00	0.05 ± 0.01	129.4 ± 10.7^{a}	7.7 ± 1.6^{a}
Eucalyptus	15.01 ± 0.18	1.51 ± 0.01	8.84 ± 0.08^{a}	4.50 ± 0.16	0.32 ± 0.01	9.97 ± 0.10^{a}	10.51 ± 0.17	1.19 ± 0.02	14.27 ± 0.15^{b}	0.15 ± 0.02	0.07 ± 0.02	134.2 ± 9.9^{a}	5.9 ± 1.3^{a}
Switchgrass	15.68 ± 1.13	1.82 ± 0.16	7.47 ± 0.11^{b}	4.52 ± 0.15	0.32 ± 0.01	8.67 ± 0.14^{b}	11.16 ± 1.10	1.50 ± 0.16	14.24 ± 0.25^{b}	0.12 ± 0.00	0.09 ± 0.01	442.3 ± 17.2^{b}	131.2 ± 17.4^{b}
Tulip Poplar	14.82 ± 0.67	1.62 ± 0.08	7.57 ± 0.12^{b}	4.95 ± 0.18	0.32 ± 0.01	9.14 ± 0.12^{b}	9.88 ± 0.82	1.30 ± 0.10	15.42 ± 0.32^{a}	0.15 ± 0.00	0.05 ± 0.01	393.8 ± 13.1^{b}	22.6 ± 4.5^{a}
***P*-value**	*ns*	*P = 0.07*	***P<0.001***	*ns*	*ns*	***P<0.001***	*ns*	*ns*	***P<0.01***	*ns*	*ns*	***P<0.0001***	***P<0.0001***

Table 4.S 2

Differentially abundant microbial taxa as determined by Kruskal-Wallis (KW) test. Table reports the *P*-values (pvals) and the *P*-values with Bonferroni correction (pvals Bon) for taxa whose abundance differs between litter treatments. Relative abundance for each taxa is the average across treatment replicates.

Taxonomy	KW-test		Relative Abundance			
	pvals	pvalsBon	Eucalyptus	Soil	Switch- grass	Tulip Poplar
p__WPS-2	0.00	0.04	0.00	0.00	0.03	0.03
p__Thaumarchaeota	0.01	0.05	0.03	0.03	0.01	0.01
p__Chloroflexi	0.01	0.06	0.02	0.02	0.06	0.05
p__Planctomycetes	0.01	0.08	0.05	0.06	0.03	0.03
p__Proteobacteria	0.03	0.28	0.41	0.41	0.39	0.37
p__Verrucomicrobia	0.05	0.43	0.11	0.11	0.08	0.11
p__Actinobacteria	0.13	1.13	0.09	0.08	0.11	0.12
p__Firmicutes	0.25	2.23	0.05	0.05	0.06	0.06
p__Acidobacteria	0.63	5.65	0.17	0.17	0.16	0.16
p__WPS-2; NA	0.00	0.07	0.00	0.00	0.03	0.03
p__Thaumarchaeota; c__Nitrososphaeria	0.01	0.07	0.03	0.03	0.01	0.01
p__Proteobacteria; c__ αproteobacteria	0.01	0.07	0.19	0.19	0.15	0.17
p__Chloroflexi; c__Ktedonobacteria	0.01	0.09	0.00	0.00	0.04	0.03
p__Planctomycetes; c__Planctomycetacia	0.01	0.12	0.03	0.04	0.02	0.02
p__Proteobacteria; c__Deltaproteobacteria	0.01	0.13	0.05	0.05	0.03	0.04
p__Acidobacteria; c__Blastocatellia_(Subgroup_4)	0.01	0.13	0.04	0.05	0.02	0.02
p__Acidobacteria; c__Subgroup_6	0.01	0.14	0.07	0.07	0.04	0.05
p__Acidobacteria; c__Acidobacteriia	0.01	0.14	0.03	0.03	0.08	0.08
p__Actinobacteria; c__Actinobacteria	0.03	0.35	0.05	0.05	0.07	0.07

p__Proteobacteria; c__Gammaproteobacteria	0.04	0.58	0.16	0.16	0.21	0.17
p__Verrucomicrobia; c__Verrucomicrobiae	0.05	0.67	0.11	0.11	0.08	0.11
p__Actinobacteria; c__Thermoleophilia	0.17	2.40	0.03	0.03	0.04	0.04
p__Firmicutes; c__Bacilli	0.31	4.28	0.05	0.05	0.06	0.06
p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales	0.00	0.05	0.02	0.02	0.13	0.08
p__WPS-2; NA; NA	0.00	0.05	0.00	0.00	0.03	0.03
p__Chloroflexi; c__Ktedonobacteria; o__Ktedonobacterales	0.00	0.05	0.00	0.00	0.04	0.03
p__Thaumarchaeota; c__Nitrososphaeria; o__Nitrososphaerales	0.01	0.05	0.03	0.03	0.01	0.01
p__Proteobacteria; c__ αproteobacteria; o__Rhizobiales	0.01	0.06	0.14	0.14	0.11	0.11
p__Proteobacteria; c__Gammaproteobacteria; o__Betaproteobacteriales	0.01	0.08	0.08	0.07	0.04	0.04
p__Acidobacteria; c__Acidobacteriia; o__Acidobacteriales	0.01	0.09	0.01	0.01	0.06	0.06
p__Acidobacteria; c__Subgroup_6; NA	0.01	0.10	0.07	0.07	0.04	0.05
p__Verrucomicrobia; c__Verrucomicrobiae; o__Chthoniobacterales	0.05	0.48	0.10	0.09	0.08	0.10
p__Firmicutes; c__Bacilli; o__Bacillales	0.31	3.06	0.05	0.05	0.06	0.06
p__WPS-2; NA; NA; NA	0.00	0.05	0.00	0.00	0.03	0.03
p__Firmicutes; c__Bacilli; o__Bacillales; f__Bacillaceae	0.01	0.05	0.03	0.02	0.04	0.04
p__Proteobacteria; c__Gammaproteobacteria; o__Xanthomonadales; f__Rhodanobacteraceae	0.01	0.05	0.01	0.01	0.13	0.08
p__Thaumarchaeota; c__Nitrososphaeria; o__Nitrososphaerales; f__Nitrososphaeraceae	0.01	0.05	0.03	0.03	0.01	0.01
p__Proteobacteria; c__Gammaproteobacteria; o__Betaproteobacteriales; f__Burkholderiaceae	0.01	0.08	0.03	0.02	0.01	0.01
p__Acidobacteria; c__Acidobacteriia; o__Acidobacteriales; f__Acidobacteriaceae_(Subgroup_1)	0.01	0.09	0.00	0.00	0.05	0.05
p__Acidobacteria; c__Subgroup_6; NA; NA	0.01	0.10	0.07	0.07	0.04	0.05
p__Proteobacteria; c__ αproteobacteria; o__Rhizobiales; f__Xanthobacteraceae	0.03	0.34	0.08	0.08	0.07	0.08
p__Verrucomicrobia; c__Verrucomicrobiae; o__Chthoniobacterales; f__Chthoniobacteraceae	0.06	0.56	0.09	0.08	0.07	0.10
p__Proteobacteria; c__Gammaproteobacteria; o__Betaproteobacteriales; f__Nitrosomonadaceae	0.35	3.53	0.02	0.03	0.02	0.02
NA	0.35	1.41	0.05	0.04	0.04	0.04
p__Basidiomycota	0.38	1.53	0.07	0.10	0.07	0.08
p__Mortierellomycota	0.54	2.18	0.51	0.52	0.58	0.54
p__Ascomycota	0.59	2.36	0.36	0.34	0.30	0.32

p__Ascomycota; c__Dothideomycetes	0.04	0.26	0.04	0.07	0.02	0.02
p__Basidiomycota; c__Agaricomycetes	0.33	2.32	0.05	0.09	0.05	0.06
NA; NA	0.35	2.46	0.05	0.04	0.04	0.04
p__Ascomycota; c__Eurotiomycetes	0.52	3.62	0.06	0.06	0.05	0.06
p__Mortierellomycota; c__Mortierellomycetes	0.54	3.81	0.51	0.52	0.58	0.54
p__Ascomycota; c__Leotiomycetes	0.59	4.13	0.04	0.02	0.02	0.03
p__Ascomycota; c__Sordariomycetes	0.59	4.16	0.19	0.17	0.19	0.18
p__Ascomycota; c__Dothideomycetes; o__Pleosporales	0.04	0.39	0.03	0.06	0.02	0.02
p__Ascomycota; c__Sordariomycetes; o__Hypocreales	0.09	0.84	0.10	0.08	0.11	0.10
p__Basidiomycota; c__Agaricomycetes; o__Auriculariales	0.13	1.21	0.00	0.03	0.00	0.00
p__Ascomycota; c__Eurotiomycetes; o__Chaetothyriales	0.25	2.23	0.06	0.06	0.05	0.06
p__Basidiomycota; c__Agaricomycetes; o__Agaricales	0.25	2.23	0.02	0.03	0.03	0.02
NA; NA; NA	0.35	3.17	0.05	0.04	0.04	0.04
p__Ascomycota; c__Leotiomycetes; o__Helotiales	0.54	4.90	0.04	0.02	0.02	0.02
p__Mortierellomycota; c__Mortierellomycetes; o__Mortierellales	0.54	4.90	0.51	0.52	0.58	0.54
p__Ascomycota; c__Sordariomycetes; o__Sordariales	0.97	8.73	0.07	0.07	0.06	0.06
p__Ascomycota; c__Dothideomycetes; o__Pleosporales; f__Cucurbitariaceae	0.01	0.07	0.01	0.04	0.00	0.00
p__Ascomycota; c__Sordariomycetes; o__Hypocreales; f__Clavicipitaceae	0.04	0.32	0.03	0.03	0.04	0.04
p__Ascomycota; c__Sordariomycetes; o__Hypocreales; f__Nectriaceae	0.05	0.41	0.04	0.03	0.03	0.04
p__Ascomycota; c__Eurotiomycetes; o__Chaetothyriales; f__Herpotrichiellaceae	0.13	1.08	0.04	0.04	0.04	0.05
p__Basidiomycota; c__Agaricomycetes; o__Auriculariales; NA	0.14	1.11	0.00	0.03	0.00	0.00
NA; NA; NA; NA	0.35	2.82	0.05	0.04	0.04	0.04
p__Mortierellomycota; c__Mortierellomycetes; o__Mortierellales; f__Mortierellaceae	0.54	4.35	0.51	0.52	0.58	0.54
p__Ascomycota; c__Sordariomycetes; o__Sordariales; f__Chaetomiaceae	0.98	7.85	0.06	0.06	0.05	0.05

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Chapter 5 – Summary and Synthesis

Conclusions

The importance of low molecular weight carbon compounds on soil microbial communities and ecosystem function has been well established (van Hees et al. 2005; Eilers et al. 2010; Cotrufo et al. 2013; Sokol and Bradford 2019). Common root exudates (e.g., carbohydrates, amino acids, and organic acids) can increase the relative abundance of certain taxa in soils (Eilers et al. 2010). Additionally, Early in decomposition litter leachates are the primary litter C source stabilized through mineral associations (Cotrufo et al. 2015). While the importance of these low molecular weight C compounds is quite apparent, it is also clear that previous research has come to this consensus while largely focused on dissolved organic carbon, at the expense of volatile organic compounds (VOCs). In an effort to close this gap, this dissertation addresses three areas where the role of VOCs is important to soil microbial communities and ecosystem functioning: the effect of VOCs on nitrogen transformations, soil microbial community structure, and the incorporation of VOCs into soil organic matter pools.

In Chapter 2, the results suggest methanol and acetone (common litter VOCs) are important to soil C and N cycling. Specifically, microbial activity, as indicated by respiration rate, increased 2-2.7 times under VOC amendment. Also interestingly, the increase in microbial activity coincided with a significant decline in NO_3^- concentrations, where the unamended soil had 30-200 times more NO_3^- than the VOC amended soils. Likewise, the ammonia oxidizing archaea decreased in abundance. It has previously been established that VOCs (i.e., monoterpenes) can inhibit nitrification (Paavolainen et al. 1998; Smolander et al. 2006), and that microbially available C compounds increase competition between heterotrophic microbes and nitrifiers (Verhagen and Laanbroek 1991; Verhagen et al. 1992). However, there is also a possibility that the ammonia monooxygenase enzyme is competitively inhibited when methanol

enters the active site of the enzyme where it can be oxidized to formaldehyde (Voysey and Wood 1987; Hooper et al. 1997). Unfortunately, this study was unable to disentangle the mechanism of inhibition. However, it does suggest that during decomposition of leaf litter, where methanol emissions can be quite high, N transformations are inhibited. Furthermore, since these compounds do not require an aqueous medium to traverse soil pores they could play an important role in driving soil C and N cycling in bulk soil.

In Chapter 3, the role of VOCs was compared to that of DOC on microbial community structure under differing moisture regimes. Since vapor phase volatile compounds can diffuse much faster than dissolved compounds it was proposed that VOCs would have a larger effect than DOC at low moisture. We found that this is the case for several major phyla, including Proteobacteria, Nitrospirae, and Rokubacteria, which all responded significantly to α-pinene at 25% field capacity. Acidobacteria, Proteobacteria, Nitrospirae, and Rokubacteria all significantly responded to methanol treatment at some of the higher moisture levels. We were surprised to find that while some phyla from glucose treated soils sometimes differed from other C amendments, glucose never had a significant effect compared to the no C control. Similarly, oxalic acid treatment only differed from the no C control in Firmicutes at 45% field capacity. This result contradicts that of Eilers et al. (2010), but are in line with Strickland et al. (2015). The nature of compositional data makes it difficult to determine if our result is due to shifts in abundance of the measured phyla or in other phyla. Regardless of the particular mechanism affecting microbial community response to C source, this study suggests that VOCs play an important role in dry soils. This further indicates that VOCs may be important in bulk soil where DOC is low, and its movement is largely governed by mass flow.

In Chapter 4, we moved from direct addition of volatile compounds to investigating the role of the full volatolome of decomposing leaf litter. We allowed the litter from each of three plant species (*Eucalyptus* spp., *Liriodendron tulipifera, and Panicum virgatum*) to decompose individually while sharing headspace with mineral soil. We show that litter VOCs alter soil bacterial and fungal communities, and that VOC-C enters all measured SOM pools. Our results suggest that like other low molecular weight C compounds, VOCs are being consumed by soil microorganisms and likely being stabilized through mineral associations after microbial turnover. This chapter further supports the idea that VOCs can replace root exudates and leaf litter leachates as a readily available C source, and due to their ability to rapidly diffuse through air filled pore spaces they may be filling this role in bulk soils.

Future Directions

This work either focused on the effects of a few VOCs or on the entire volatile profile of decomposing litter. Since decomposing litters produce many more VOCs (Leff and Fierer 2008; Gray et al. 2010) than we directly assessed, future studies should continue to investigate the role of individual VOCs on soil communities and ecosystem function. The studies in this dissertation were also limited by the use of microcosms. While it is known that variation in climatic factors affect the emission and uptake of VOCs by soils (Asensio et al. 2007), it is unclear how these factors affect the microbial communities ability to use these compounds. One possibility is that under warm conditions litter VOCs largely diffuse into the atmosphere and are less likely to enter the soil. Furthermore, there are open questions regarding the role of VOCs on nitrification inhibition. One possibility would be to pair C additions with ^{15}N pool dilution assays to attempt to tease out the mechanism of nitrification inhibition. Lastly, I propose that VOCs act similarly

to root exudates and litter leachates (Figure 5.1). In this model VOC-C is consumed by soil microbes before being respired or incorporated into microbial biomass. Once in microbial biomass the C can then being stabilized through mineral associations or incorporation into POM after microbial turnover. However, to confirm this hypothesis future studies will need to both account for abiotic stabilization of C in the soil mineral fraction and include higher resolution sampling of C pools.

Figures

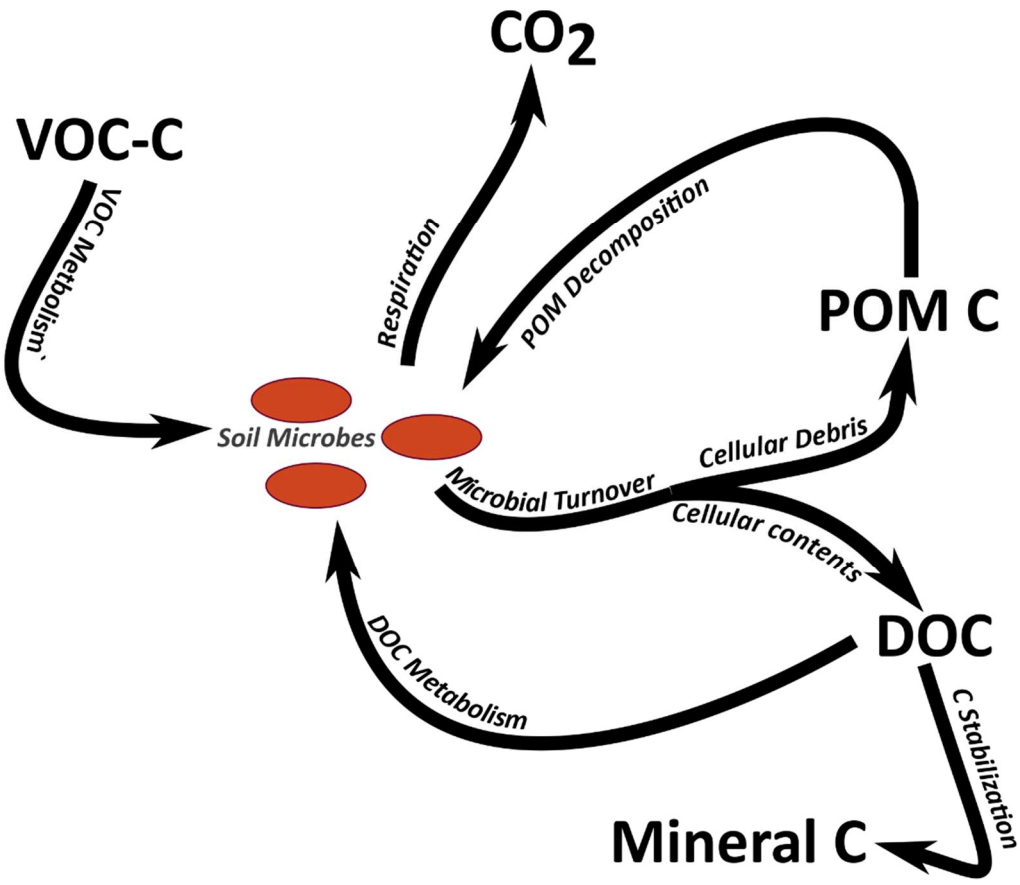

Figure 5.1

Figure 5.1

A proposed mechanism for the biotic control of VOC-C movement through major soil C pools. In this model, VOC-C is first metabolized by soil microbes before being respired or incorporated in microbial biomass. After Microbial turnover cellular debris of larger microbes like fungi could enter the particulate organic matter (POM)-C pool, while cytoplasmic contents would enter the dissolved organic carbon (DOC) pool. From there the C could either be stabilized in the mineral or POM soil fractions or be metabolized by soil microbes.

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