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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 h 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.

Keywords Ammonia oxidation · Carbon flux · Nitrification inhibition · Nitrogen transformations · qPCR · Volatile organic compounds (VOC)

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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 (Warneke et al. 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 $\sim$ 88% of CO_2–C emissions (Gray and Fierer 2012). Because VOCs can be taken up directly by soil microbes, dissolve in soil 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 1425 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 attain high levels. For instance, it is known that CO_2 concentrations in soil air can reach concentrations up to 1000 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, or 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 (de 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; Fig ESM-1.1) 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 three 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 and 1392 μg VOC–C g^{-1} dry soil day^{-1} from methanol, and between ~ 62 and 108 μg VOC–C g^{-1} dry soil day^{-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 h after VOC addition (Fig. ESM-1.2a), and that the high and low concentrations elicited similar respiration—the magnitude of the response was dose-dependent (Fig. ESM-1.2). 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 (Fig. ESM-1.2). 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 $\sim$ 15 min. We then sealed the microcosms with lids fitted with butyl septa. 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), and a second 5 mL sub-sample 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. 2005a) 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-day. 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 2 M 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-day 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/5.8 s (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 (Fig. ESM-1.1) to hours since last VOC addition, producing a single average 144-h respiration time series—i.e. Day 3 and day 9 were both converted to 48 h 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 et al. 2019).

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 ($\chi^2_{(2)} = 245.3$; $p < 0.001$; Fig. 1a), as well as a significant treatment by time interaction ($\chi^2_{(4)} = 123$; $p < 0.001$; Fig. 1a). The VOC pulses resulted in an initial spike in

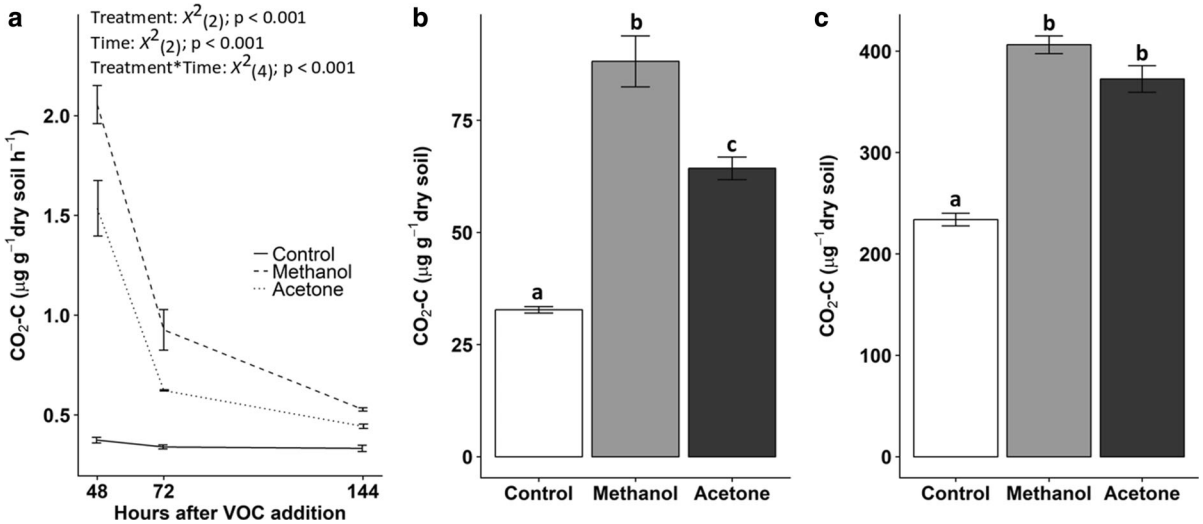

Fig. 1 Average 144 h respiration dynamics following VOC additions—based off of 4 weekly VOC additions across 28-days—(**a**) cumulative weekly respiration (**b**) and labile C obtained from a 52-day C-mineralization assay (**c**). Cumulative respiration was ~ 2–2.7 fold greater in VOC treated soils (**b**),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

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 h (Fig. 1a), similar to our observation in the pilot incubation (Fig. ESM-1.2). 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 ~ twofold increase in cumulative respiration, respectively ($F_{(2)} = 59.32$; $p < 0.05$; Fig. 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 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 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 (Fig. 1c).

Control soils had significantly higher total dissolved N (~ 2.25-fold) than VOC amended soils ($F_{(2)} = 41.62$; $p < 0.001$; Fig. 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 ($F_{(2)} = 17.52$; $p < 0.01$; Fig. 2b). The majority of N in the VOC treatments was DON which was ~ 100-fold greater than DON concentration in control soils ($F_{(2)} = 50.52$; $p < 0.001$; Fig. 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 1).

Microbial enzyme activity was unaltered (Table ESM-1.1), 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 ESM-1.2). We quantified fungal (ITS) and bacterial (16S) abundance in the soil to

Table 1 Soil chemical and microbial data collected at the end of the 28-day experiment

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

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)

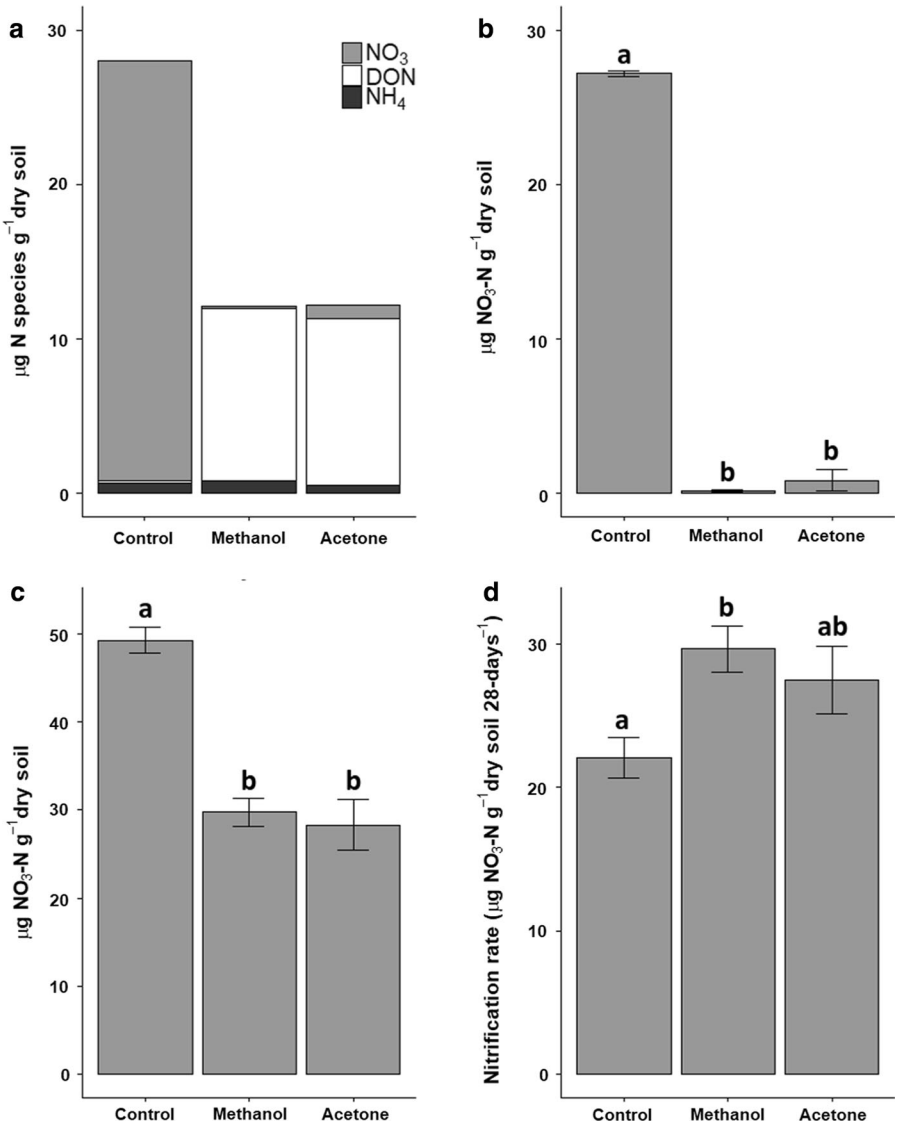

Fig. 2 N fractions (**a**), soil NO_3-N measured immediately after the 28-day experiment with VOC additions (**b**), soil NO_3–N after a 28-day nitrification assay performed after the end of the experiment without the addition of VOCs **c**, and the nitrification rate during the 28-day nitrification 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; $\alpha = 0.05$ except for (D) $\alpha = 0.10$. Shown are means ± 1 standard error

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 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 ($F_{(2)} = 6.57$; $p = 0.03$; Fig. 3a), while no significant difference was noted for AOB abundance ($F_{(2)} = 1.38$; $p = 0.32$; Fig. 3b). AOA gene copy abundance had a significant positive relationship with soil NO_3 concentrations ($F_{(1,7)} = 68.02$; Adj. $R^2 = 0.89$; $p < 0.001$; Fig. 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 (Fig. 2a). Once VOC additions were ceased and the soils incubated an additional 28-day without addition of VOCs, there was

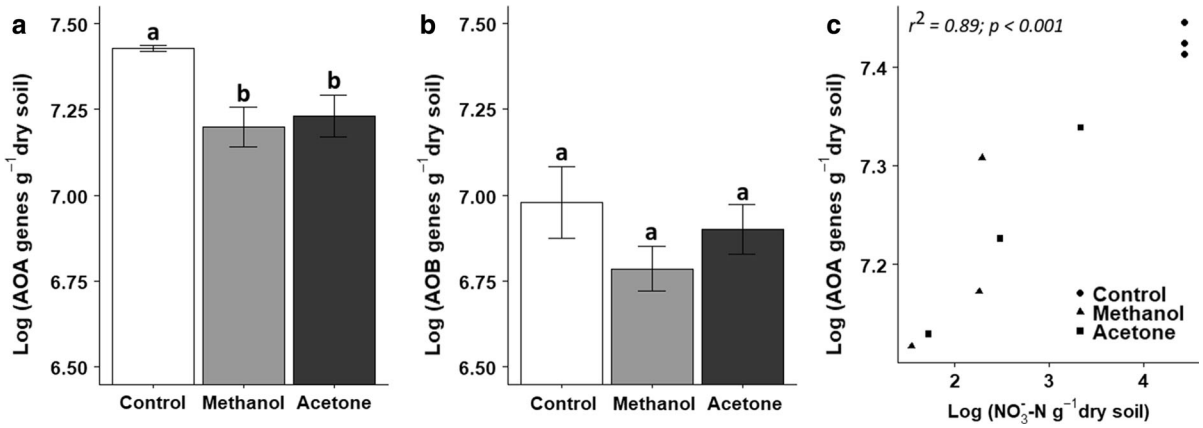

Fig. 3 Log AOA gene copies (**a**), Log AOB gene copies (**b**), and the relationship between log AOA gene copies and log NO_3^-–N **c**. There were 1.5–1.6 fewer AOA gene copies in the VOC treated soils at the end of the 28-day experiment than in the control soils (**a**), while AOB gene copies were not significantly different between treatments (**b**), as determined by ANOVA ($\alpha = 0.05$). Shown are means $\pm$ 1 standard error. Log AOA and log NO_3–N had a significant positive correlation, as determined by regression analysis ($\alpha = 0.05$) (**c**)

a rebound in nitrification rates, with the soils exposed to acetone and methanol having a net increase in NO_3 of $\sim$ 34 and 220-fold, respectively (Fig. 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 ($F_{(2)} = 4.5$; $p = 0.06$) (Fig. 2d). In the VOC treatments, NO_3 at the end of the N mineralization assay (Fig. 2c) was similar to NO_3 in the control immediately post VOC additions ($\sim$ 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 (Fig. 1a; ESM-1.2). 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 $\sim$ 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 (Fig. 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 CO_2 (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 (Fig. 1a), 144 h after each weekly VOC addition, respiration associated with both methanol and acetone treated soils were statistically indistinguishable from the control soil (Fig. 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 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 (Fig. 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 1), or microbial gene copies (16S and ITS; Table 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 (Fig. 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 NH_4, 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 (Fig. 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, NH_4, and DON. In the control soils, total dissolved N was dominated by NO_3 with very low DON and NH_4, 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 NH_4; Fig. 2a). This indicates that nitrification was inhibited by methanol and acetone treatment. NH_4 was the least abundant N species we measured in the soil, constituting between $\sim$ 2 and 7% of total dissolved nitrogen across all treatments. We posit that the fate of NH_4 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 NH_4 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 NH_4 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 NH_4 (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 $\sim$ 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 (Fig. 3a). There was also a strong relationship between AOA abundance and NO_3 concentration (Fig. 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 (Fig. 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 (Fig. 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 $\sim$ 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. ^{15}N 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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