Soil Biology & Biochemistry 40 (2008) 1629–1636

Contents lists available at ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Volatile organic compound (VOC) emissions from soil and litter samples

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ARTICLE INFO

Article history:
Received 30 October 2007
Received in revised form 17 January 2008
Accepted 29 January 2008
Available online 7 May 2008

Keywords:
Volatile organic compounds
Soil carbon
Soil microorganisms, Monoterpenes
Furfural

ABSTRACT

The production of nonmethane volatile organic compounds (VOCs) by soil microbes is likely to have an important influence on soil ecology and terrestrial biogeochemistry. However, soil VOC production has received relatively little attention, and we do not know how the emissions of microbially-produced VOCs vary across soil and litter types. We collected 40 root-free soil and litter samples from a diverse array of ecosystem types and conducted laboratory incubations in order to compare the types and quantities of VOCs emitted. VOC production rates were higher in litter samples than in soil samples, and the rates were correlated with microbial biomass and CO_2 production levels. On average, the litter samples produced more types of VOCs than the soil samples with litters emitting a number of VOCs (including terpenoids) that were not generally emitted from the soil samples. Across all of the samples, we identified 100 VOCs, and more than 70% of these compounds could not be positively identified by GC/MS analyses. Of those VOCs that could be identified, furfural and similar furan compounds were noteworthy in that they were emitted in large amounts from nearly every sample examined. Other identifiable VOCs produced across a range of soil and litter samples included propanoic and butanoic acids, which are known products of microbial fermentation. Together these results suggest a need for additional research examining the specific factors influencing VOC emissions from soil and the identification of specific VOCs emitted from soil and litter as many of these compounds are likely to have important effects on belowground ecology.

1. Introduction

Microorganisms can produce a wide variety of volatile organic compounds (VOCs), and a significant portion of the VOCs released from soil and litter appear to be of microbial origin (Isidorov and Jdanova, 2002; Stahl and Parkin, 1996; Stotzky and Schenck, 1976). However, surprisingly few studies have investigated nonmethane VOC production by soil bacteria and fungi. The vast majority of studies examining VOC efflux from terrestrial ecosystems have focused on the production of VOCs, including isoprene, terpenes, and alkenes, by plants (Kesselmeier and Staudt, 1999). As a result, we know relatively little about the types and quantities of VOCs released by soil microbes and how VOC production varies across soil types.

The production of VOCs by soil microorganisms is likely to have an important influence on atmospheric chemistry, soil processes, and biotic interactions in soil. VOCs, particularly those of biogenic origin, can alter atmospheric photochemistry by reducing hydroxyl (OH) concentrations, increasing tropospheric ozone (O_3), and stimulating the production of organic nitrates in the atmosphere (Monson and Holland, 2001). The presence of specific VOCs in the soil atmosphere can also alter the rates of microbial processes including: nitrification (Bending and Lincoln, 2000; Paavolainen et al., 1998; Ward et al., 1997; Wheatley et al., 1996), nitrogen mineralization (Smolander et al., 2006; White, 1994), denitrification, and methane oxidation (Amaral et al., 1998). VOCs may also regulate microbial interactions by inhibiting or stimulating the growth and activity of soil fungi and bacteria (Chuankun et al., 2004; Mackie and Wheatley, 1999; Wheatley, 2002; Xu et al., 2004). In particular, VOCs produced by soil bacteria have been shown to suppress the growth of specific fungal phytopathogens in soil (Bending and Lincoln, 1999; Ezra and Strobel, 2003; Fernando et al., 2005; Kai et al., 2007). Recent studies suggest that microbially-produced VOCs may also be able to directly influence rates of plant growth (Farag et al., 2006; Ryu et al., 2003).

Although plants are probably the dominant source of biogenic VOCs in terrestrial ecosystems (Monson and Holland, 2001), high rates of VOC production by soil and litter microbes have been observed in a number of studies (Isidorov and Jdanova, 2002; Stahl and Parkin, 1996; Stotzky and Schenck, 1976). Soil bacteria and fungi are capable of producing a diverse array of VOCs, including many that are not commonly produced by plants (Stahl and Parkin,

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doi:10.1016/j.soilbio.2008.01.018

1996; Stotzky and Schenck, 1976; Wheatley, 2002), and soil microbes may be an important source of certain types of VOCs in terrestrial ecosystems (Dimmer et al., 2001; Harper, 1985; Hellen et al., 2006; Schade and Goldstein, 2001; Watling and Harper, 1998). Likewise, soil microbes can readily consume a wide range of VOCs and may represent an important sink for VOCs in terrestrial ecosystems (Cleveland and Yavitt, 1998; Owen et al., 2007; Smolander et al., 2006; Yoo and Day, 2002).

There is some evidence suggesting that the microbes found in different soils and litters produce distinct types and quantities of VOCs (Isidorov and Jdanova, 2002; Smolander et al., 2006; Stahl and Parkin, 1996). Such differences could be driven by changes in a number of soil characteristics, including microbial community composition, microbial biomass, carbon substrate characteristics, redox status, nutrient availability, and moisture status (Asensio et al., 2007; Brinton, 1998; Larsen and Frisvad, 1995; Mayrhofer et al., 2006; Scholler et al., 2002; Wheatley et al., 1997). Since nonmethane VOC production by soil microorganisms has not been well studied, we are currently unable to predict how specific soil biotic and abiotic characteristics will influence the types and quantities of VOCs released from soils. We designed this study to address this knowledge gap and gain a more integrated understanding of how VOC production varies across soil and ecosystem types. We incubated 40 distinct root-free soil and litter types under controlled conditions and measured the net rates of VOC production and the chemical diversity of the VOCs released.

2. Materials and methods

2.1. Sample collection

A total of 28 soil samples and 12 litter samples were collected from a wide range of ecosystem types within the United States, including arid deserts, temperate forests, and alpine tundra among others (Table 1). The goal was to collect soil and litter samples that represent a broad range of biotic and abiotic characteristics. Soil samples were collected from individual plots of 1–5 m^2 in size using a hand trowel to remove the top 5 cm of mineral soil after removing any coarse debris and litter material. All of the soil and litter samples were collected between June and August of 2006. Samples were shipped to the University of Colorado at Boulder within a few days of collection where the soil samples were sieved to 2 mm and the litter samples were chopped into 5 mm pieces and thoroughly homogenized. All samples were stored at 4 °C for 1–2 weeks prior to conducting the analyses.

2.2. Sample characterization

We measured the following characteristics of each soil and litter sample: gravimetric moisture content, water holding capacity (WHC), pH, carbon mineralization rate (CO_2 production), microbial biomass, percent carbon (C), and percent nitrogen (N). Gravimetric soil moisture was measured after drying the soils and litters at 120 °C for 48 h. To measure soil moisture at 100% of WHC, soils and litters were saturated with deionized water and allowed to drain for 2 h. Soil and litter pH was measured in deionized water (2:1 ratio of water to sample volume) after a 30 min equilibration period. A static incubation procedure similar to that described in Fierer et al. (2003a) was used to measure potential rates of microbial CO_2 production. Briefly, triplicate soil and litter samples were adjusted to 50% of WHC to normalize water availability between samples, and samples were equilibrated for 5 days at 21 °C. After the equilibration period, the samples were incubated at 25 °C for 2.5–24 h in glass vials sealed with gas-tight caps fitted with silicone septa. Headspace CO_2 concentrations were then measured on an infrared gas analyzer (CA-10a, Sable Systems Inc., Las Vegas, NV, USA). In all cases, headspace CO_2 concentrations did not exceed 1% during the course of the incubation period. Microbial biomass was measured in triplicate subsamples using the substrate-induced respiration (SIR) procedure described in Fierer et al. (2003b). After samples had equilibrated for 5 days under conditions identical to those described above, yeast solution (12 g autolyzed yeast extract L^{-1}) was added to individual glass vials containing approximately 8 g of soil and an equal volume of litter, and the gas-tight vials were shaken continuously for a 3 h period. Microbial biomass was inferred from the measured rate of CO_2 production over the 3 h period and therefore microbial biomass is reported as a respiration rate (Stenstrom et al., 1998). C and N concentrations were measured on ground litter and soil samples using a Carlo-Erba CHN Analyzer (Fisons EA-1108). For the soil samples, silt and clay percentages were determined using the method described by Kettler et al. (2001).

Table 1
General information on soil and litter sample sites

Samples	Site description	Latitude (°N)	Longitude (°W)	Dominant flora
1 (L)	Montane forest, Boulder County, CO	40.0	105.3	*Pinus ponderosa*, *Pseudotsuga menziesii*
2	Riparian forest, Boulder County, CO	40.0	105.3	*Pinus ponderosa*, *Pseudotsuga menziesii*, *Picea pungens*
3	Alpine meadow, Boulder County, CO	40.1	105.6	*Deschampsia caespitosa*, *Geum rossii*
4	Sub-alpine forest, Boulder County, CO	40.1	105.6	*Abies lasiocarpa*, *Picea englemannii*
5 (L)	Grassland, Boulder County, CO	40.1	105.2	*Yucca* sp.
6 (L)	Deciduous forest, NH	43.7	72.2	*Acer saccharum*
7 (L)	Deciduous forest, NH	43.7	72.2	*Tsuga canadensis*
8 (L)	Grassland, LBJ Wildflower Ctr., Austin, TX	30.2	97.9	*Bothriochloa ischaemum*
9 (L)	Desert scrub, Sevilleta, NM (LTER site)	34.4	106.5	*Juniperus* sp.
10 (L)	Grassland, Sevilleta, NM (LTER site)	34.4	106.7	*Bouteloua* sp.
11 (L)	Desert scrub, Sevilleta, NM (LTER site)	34.3	106.7	*Larrea tridentate*
12 (L)	Rosemary scrub, Archbold Biological Station, FL	27.2	81.4	*Ceratiola ericoides*
13	Cultivated land, Calhoun Exp. Forest, SC	34.5	81.6	*Triticum* sp., *Zea mays*
14 (L)	Pasture, Calhoun Exp. Forest, SC	34.6	81.7	*Cynodon* sp.
15 (L)	Pine forest, Calhoun Exp. Forest, SC	34.6	81.7	*Pinus taeda*
16 (L)	Hardwood forest, Calhoun Exp. Forest, SC	34.6	81.7	*Quercus* sp., *Carya* sp.
17–19	Pine forests, Calhoun Exp. Forest, SC	34.6	81.7	*Pinus taeda*
20–22	Cultivated fields, Calhoun Exp. Forest, SC	34.6	81.7	*Triticum* sp., *Zea mays*
23–25	Pasture fields, Calhoun Exp. Forest, SC	34.6	81.7	*Cynodon* sp.
26–28	Hardwood forests, Calhoun Exp. Forest, SC	34.6	81.7	*Quercus* sp., *Carya* sp.

All samples were collected in the United States. Samples 1–16 were collected in July 2006, and samples 17–28 were collected in September 2006. (L) indicates that a litter sample was collected from the same site. Samples 17–28 were collected from the same general area (the Calhoun Exp. Forest in South Carolina) as samples 13–16, but not at the same locations.

2.3. VOC collection and identification

To compare the types of VOCs produced from each of the samples and the relative rates of VOC production, we used a procedure modified from that described in Stahl and Parkin (1996). Approximately 200 g of each soil and equivalent volumes of each litter were weighed into Erlenmeyer flasks and the samples were adjusted to 50% of WHC and equilibrated for 5 days at 21 °C. After the equilibration period, VOCs were collected over a 4-h period by placing samples in 1 L glass vacuum flasks with VOC-free air pulled through each flask at a rate of 11 L min^{-1} for the entire 4-h period. VOCs were removed from the intake air by fitting the opening of each flask with a cork stopper holding a 25 cm glass column (5 mm i.d.) filled with activated charcoal. Air exiting the flask was pulled through a glass tube containing 20 mg of 80/100 mesh Super Q (Alltech Inc.) to trap the VOCs produced by the sample. Flasks without soil or litter samples were used as experimental controls ("blanks") to determine background levels of VOC contamination. With this method, we measured rates of VOC production under controlled conditions so we could effectively compare relative rates of VOC production between samples. However, it is important to recognize that the measured rates do not necessarily reflect field rates as all samples were homogenized and incubated at near-optimal moisture contents at a constant temperature. Likewise, we did not include a sterile control and therefore we were not able to distinguish between biotic and abiotic contributions to soil VOC emissions.

Following the VOC collection period, the glass tubes containing the Super Q were rinsed with 0.5 ml of n-hexane to remove the VOCs and the hexane solution was analyzed by gas chromatography/mass spectrometry (GC/MS). To quantify intra-sample variability in VOC production, we analyzed triplicate subsamples from two of the soil samples and one of the litter samples. VOCs in the n-hexane solutions were identified and quantified using an Agilent 6890 N GC (Santa Clara, CA, USA) coupled with an Agilent 5975 inert mass selective detector with an ion source of 70.0 eV at 230 °C. Helium was used as the carrier gas at a flow rate of 1.0 ml min^{-1}, the injector temperature was set at 260 °C, and a 0.25 mm i.d. 30 m EC-Wax glass capillary column (Alltech Associates Inc.) was installed in the GC. Oven conditions included an isothermal hold at 60 °C for 5 min, followed by a ramp of 10 °C min^{-1} to 250 °C. One microliter of each sample was injected in the splitless mode. Our analytical techniques did not allow us to quantify emissions of C_1 and C_2 compounds or VOCs with retention times greater than 25 min. The identification of compounds was based on a comparison of their mass spectra with those in the NIST/EPA/NIH mass spectral library (Version 2.0d) and chromatograms were integrated with ChemStation software version D.02.00.275 (Agilent Technologies, Santa Clara, CA). Only those VOCs not found in the experimental control ("blank") samples were considered in this study. In all cases, the soil and litter samples yielded total VOC quantities (sum of all peak areas) that were more than 10 times higher than the "blank" samples.

2.4. Data analysis

To compare soil and litter samples with respect to the types of VOCs released, the program PRIMER 5 for Windows (Primer-E Ltd., Plymouth, UK) was used with relative peak heights (size of each individual peak divided by the sum of the heights for all peaks combined) as the input data. Relative peak heights were used for these analyses to effectively compare samples based on the chemical diversity of VOCs released and not to explicitly quantify differences in total VOC production rates between samples. The relative peak heights were log-transformed, and a Euclidean distance metric was used to estimate a distance between each pair of samples based on the proportional abundances of VOCs released from each sample. We used a multi-dimensional scaling (MDS) approach to condense the multivariate VOC data in a comprehensible number of dimensions and visualize the relative degree of similarity among samples. The MDS ordination technique is well-suited for this type of data since it makes few assumptions about the form of the data, and it does not assume that the variables are linearly related to one another (Clarke and Warwick, 2001). To determine if soil and litter samples produced distinct types of VOCs, we used an analysis of similarity (ANOSIM) procedure within the program PRIMER. To examine correlations between measured sample characteristics and VOC types, we conducted Mantel tests (Clarke and Warwick, 2001) to determine if specific soil or litter characteristics could be used to predict the apparent differences in types of VOCs released from the samples.

We used SYSTAT (2004) to evaluate correlations between total VOC production rates and the measured characteristics of the soil and litter samples. Values not normally distributed were log-transformed prior to running statistical analyses. Total VOC production rates were quantified by summing the areas of all peaks considered from each sample's chromatogram. Since many of the VOCs could not be identified (see details below), we were not able to calculate VOC production rates in terms of molecular mass units or molar equivalents, and our reported rates are only used to compare the relative rates of VOC production across the samples.

3. Results

3.1. Factors affecting VOC production rates

The net production of VOCs was significantly higher from litter samples than from soil samples ($P = 0.004$); on average, the litter samples released VOCs at rates one order of magnitude higher than the soil samples (Fig. 1). Across all samples (both litter and soil samples together), VOC production rates were significantly correlated with measured CO_2 production rates and microbial biomass (Fig. 1). However, it is worth noting that these relationships are primarily driven by the large differences in CO_2 production rates and microbial biomass between litter and soil samples (Fig. 1).

If we exclude litter samples from the analyses and examine the soil samples alone, we find that a number of soil characteristics are significantly correlated with net VOC production rates despite the high degree of variability in the abiotic and biotic characteristics of the collected soil samples. Across the 28 soil samples, organic C content ($r = 0.64$), microbial biomass ($r = 0.59$), CO_2 production rate ($r = 0.59$), and nitrogen content ($r = 0.56$) were all significantly correlated with net VOC production rates ($P < 0.01$ in all cases). If we examine only the 12 litter samples, we find no significant correlations between the measured litter characteristics and net VOC production rates ($P > 0.4$ in all cases).

3.2. Identities of VOCs produced

The soil and litter samples produced a large number of unique VOCs representing many distinct types of compounds (Tables 2 and 3). Litters emitted a greater diversity of VOC compounds (87 in total) than the soil samples (36 in total) with the average litter sample producing more individual types of VOCs than the average soil sample (10 and 5 unique VOCs, respectively, Table 3). Of the 100 unique types of VOCs emitted from the 40 soil and litter samples, 13 VOCs were only found in the soil samples and 64 VOCs were only found in the 12 litter samples. A large proportion of the VOCs emitted from the soil and litter samples could not be positively identified by GC/MS analyses. Specifically, more than 70% of the VOCs emitted from the samples could not be identified with a high degree of certainty as the compounds had matches with a quality

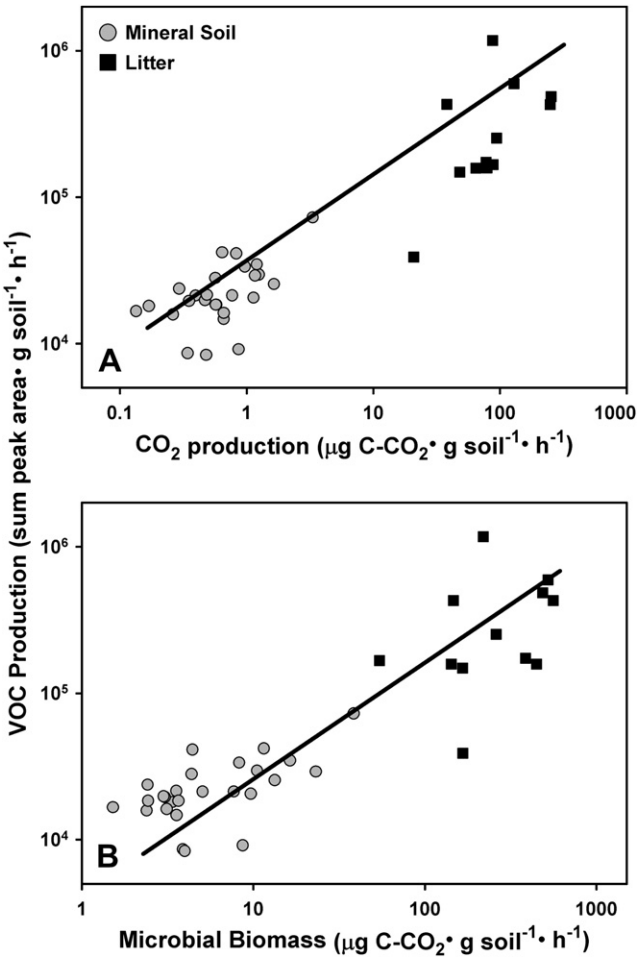

Fig. 1. Relative VOC production rates across all of the collected samples and the correlations with both CO_2 production (A) and microbial biomass (B) as measured using the substrate-induced respiration technique.

rating of less than 90 (on a scale of 0 to 100) when compared with the NIST/EPA/NIH mass spectral library. The 28 compounds with high quality matches to the library mass spectra (Table 2) can only be considered to be putatively identified as the compound identities have not been independently confirmed. Even some of the VOCs that were produced from a variety of different soil and litter samples could not be identified with a high degree of certainty; of the eight compounds that were emitted from four or more of the samples, only three had matches >90 to the mass spectral library (Table 3).

Nearly all of the soil and litter samples (>95%) emitted a compound with a retention time (RT) of 15.59 min which has been putatively identified as furfural. However, in soil samples collected from South Carolina (samples 17–28, Table 1), furfural was less prevalent with furfural contributing significantly ($P < 0.001$) less on average to their total VOC production compared to the other 28 samples (Table 3). The next most commonly observed compound (RT: 23.76 min) was only found in 63% of samples (Table 3). This compound could not be reliably identified but its closest matches in the mass spectral library were to butanoic acid and propanoic acid. Other compounds that were found to be commonly emitted from the samples are detailed in Table 3.

3.3. Variability in the types of VOCs produced

Although many of the VOCs could not be positively identified, we were able to quantitatively compare the soil and litter samples

Table 2
Selected VOCs released from the soil and litter samples with high identification quality ratings (>90) to the NIST/EPA/NIH mass spectral library

Compound	Retention time (min)	Quality rating	Sample(s) in which the compound was found
Tetrachloroethylene	4.26	97	12L
α-Pinene	4.36	91	1L, 11L
Camphene	5.26	96	1L, 11L
β-Pinene	6.37	95	1L
o-Xylene or *p*-xylene	7.4	95	12L
D-Limonene	8.05	94	1L, 6
Benzene, 1-ethyl-2-methyl-	9.82	94	12L
1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	11.44	95	16
Benzene, 1,3,5-trimethyl-	12.69	93	12L
Benzene, methoxy-	12.85	91	11L
Furfural	15.59	94	Many
5-hydroxy-methyl-furfural	17.97	91	Many
Benzene, 2-methoxy-4-methyl-1-(1-methylethyl)-	18.55	93	1L
Caryophyllene	18.7	99	1L, 16, 17
Naphthalene	21.03	99	1L
Benzene, 1,2-dimethoxy	21.07	94	11L
Ethanol, 2-(2-butoxyethoxy)-, acetate	23.34	91	5

If a compound was found in multiple samples, the highest ID quality rating was reported.

with respect to the types of VOCs emitted. As is evident in Fig. 2, there was a high degree of variability in VOC emission profiles across the collected samples. VOC emission profiles of litter samples were significantly different than those of soils ($P = 0.007$, global $R = 0.214$, Fig. 2A), and only 23 of the 100 VOCs released from the samples were emitted by both soil and litter samples. The distances between replicates of the two soil samples (Fig. 2B) and one litter sample (Fig. 2C) were relatively small and serve as useful comparisons for the relative differences in VOC emission profiles between samples. While there is variation between soils with respect to the types of VOCs emitted, many of the soils emitted similar types of VOCs and that there was more variability in VOC emission profiles between litters than between soils (Fig. 2).

3.4. Factors affecting types of VOCs produced

Despite the high degree of variability in the types of VOCs emitted from the samples (Fig. 2) and the wide range of unique soil and litter types included in this study (Table 1), some of the measured sample characteristics were found to be significant predictors of the types of VOCs emitted from the soil and litter samples (Table 4). Across the soil samples, microbial biomass, %N, %C, and CO_2 production rates were significantly correlated with the VOC emission profiles suggesting that soils with different levels of microbial activity produce distinct types of VOCs. Across the 12 litter samples, CO_2 production per unit organic C (an index of litter carbon quality) was the only significant predictor of the types of VOCs emitted from litter (Table 4). Together these results suggest that soils and litters are distinct with respect to those measured characteristics that best predict the types of VOCs emitted.

4. Discussion

4.1. Differences in net VOC production rates across soils and litters

On average, VOC production rates were 15 times higher in litter samples than the samples of mineral soil (Fig. 1). Since these rates were estimated on a per gram basis (under laboratory conditions), we do not know the relative contributions of soil versus litter to

Table 3
A comparison of the prevalence of selected VOCs among all samples

Name	Furfural	Butanoic acid or Propanoic acid	Propanoic acid	5-Hydroxy-methyl-furfural	Propanoic acid	Unknown	Unknown	Caryophyllene	Unknown	α-Pinene	D-Limonene		
Retention time (min)	15.59	23.76	24.00	17.97	23.82	11.44	11.51	18.70	20.96	4.250/4.360	8.050		
No. of samples with VOC	38	25	22	14	11	8	5	3	3	2	2		
Sample no.												No. of unique VOCs	VOC Production Rate
Soils													
1	52.3	7.92		7.48		13.7						6	5.85
2	52.0	5.72		5.78		13.3						7	7.04
3	47.8	2.67		5.07		20.2						8	10.1
4	70.5		4.69	4.82	12.5							6	6.98
5	38.3	12.7		4.36		9.80						9	8.06
6	2.98	20.0	10.7								25.4	8	13.7
7	66.7		3.51	7.55	11.7		3.75					7	8.38
8	57.2		7.77	10.5	18.2							5	2.29
9	58.3		7.80	5.79	19.6							6	5.09
10	71.8			12.1	16.1							3	2.15
11	38.1		8.57		25.0		9.73					7	5.33
12	63.0		6.73	4.14	19.0							6	4.16
13	76.6			6.50	10.7							4	4.52
14	100											1	0.69
15	55.1	21.8	14.1									4	5.32
16	41.2	8.84	5.64			8.36		20.1				7	7.14
17	32.6	9.40	8.17					45.0				5	3.67
18		52.7	31.3									4	2.63
19	34.2	39.0	23.5									4	5.57
20	14.7	59.7	25.6									3	1.73
21	16.8	53.4	25.7									4	4.63
22	13.8	54.7	27.3									4	3.70
23	41.6	38.3	20.2									3	4.39
24	5.57	62.7	31.7									3	4.51
25	15.5	55.5	29.0									3	4.86
26	16.2	49.5	26.0									6	9.06
27	14.7	46.4	23.6									7	6.33
28	11.1	51.8	27.9									4	3.92
Litters													
1L	2.70					3.24		7.58		27.4	1.38	28	289
5L					4.02		4.81		2.98			16	121
6L	91.7	8.32										2	148
7L	84.6	15.4										2	43.3
8L	21.9						13.0					6	63.2
9L	41.3			5.57	10.2		7.16					8	39.5
10L	33.3			5.90	4.01				9.81			8	107
11L	19.9			3.35					3.74	10.1		15	41.7
12L	7.49	2.06				1.86						28	108
14L	80.3	19.7										2	9.76
15L	48.3	15.0	8.42			15.8						5	37.1
16L	81.1	18.9										2	39.6

The values in the table indicate the percentage value for a particular compound's peak area divided by the sum of VOC peak areas for that sample. Darker shading indicates a more prevalent compound. The specific VOCs being compared were selected because of their presence in a large number of samples, because they were relatively common in litter samples, or because they have been discussed in previous papers. VOC production rates are in units of total VOC peak area $\times 10^3$ g dry soil^{-1}h^{-1}.

VOC emissions in the field. However, these results do suggest that the litter layer is a major source of VOCs, confirming results from studies by Hayward et al. (2001) and Hellen et al. (2006) which have also indicated that decomposing litter is likely to be a major source of VOCs (monoterpenes, in these cases) emitted from soil profiles. We found that net rates of VOC emissions were correlated with overall levels of microbial activity as those samples with higher microbial biomass and CO_2 production had higher measured VOC production rates. A similar, positive relationship between microbial activity and VOC production rates has been noted in other studies (Asensio et al., 2007; Brinton, 1998; Stahl and Parkin, 1996; Swanson et al., 2005). Although VOCs may be emitted from soil by abiotic processes (namely volatization), this study was not designed to distinguish between biotic and abiotic sources of VOCs. However, our observation of a strong correlation between VOC production and microbial CO_2 production (Fig. 1) suggests that microbial metabolism is likely to be the dominant source of VOC emissions from soil and decomposing litter. While abiotic VOC production may occur, the rates are likely to be far lower than the rates of microbial VOC production particularly considering that abiotic volatization rates would be reduced by our use of a 5-day pre-incubation of all samples prior to the start of the VOC measurements.

Gross rates of microbial VOC production may be considerably higher than the net rates of VOC production measured here as a wide range of VOCs can be readily metabolized by soil microbes

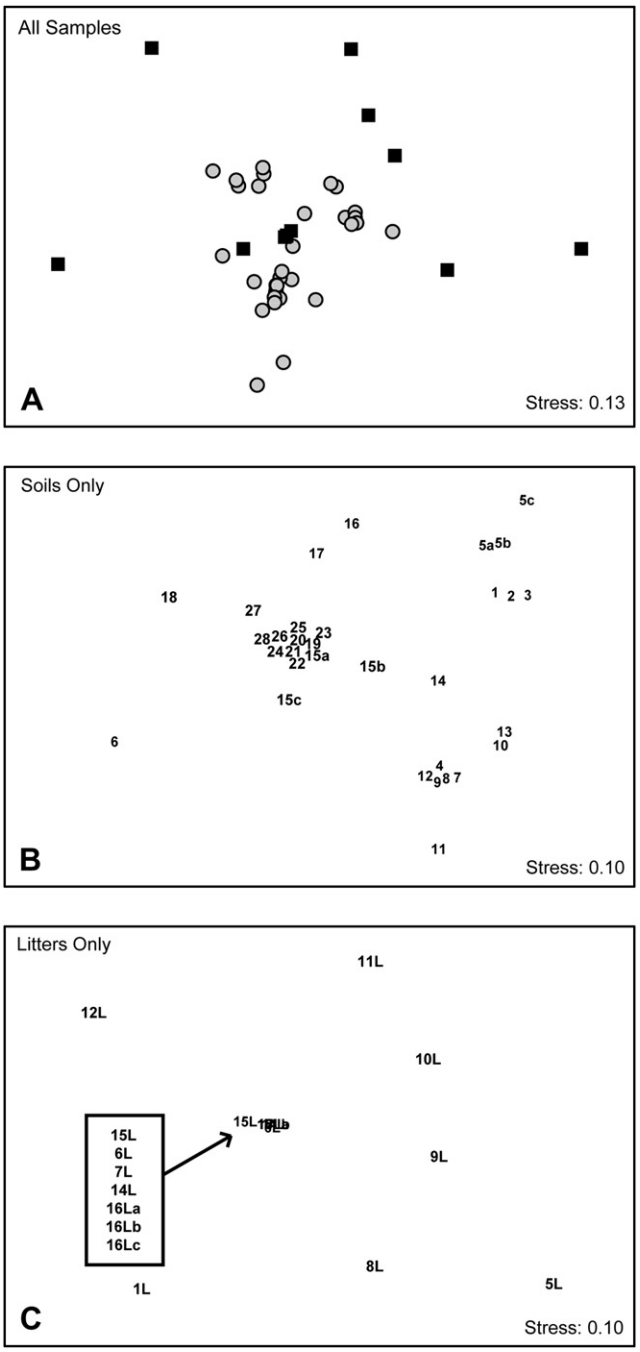

Fig. 2. A visual representation of the relation between samples with regards to their VOC emission profiles (proportional abundances of VOCs released). No axes units are used in the multi-dimensional scaling (MDS) analyses and points closer together represent samples that have more similar VOC emission profiles. Soils are represented by the symbol ●, and litters are represented by the symbol ■. Plots indicate similarities in emission profiles across all samples (A), soil samples alone (B), and litter samples alone (C).

(Cleveland and Yavitt, 1998; Owen et al., 2007; Smolander et al., 2006; Yoo and Day, 2002). At the same time, VOCs may be abiotically removed from the soil atmosphere via sorption onto clay mineral surfaces (Serrano and Gallego, 2006). This disconnection between net and gross rates of VOC production is likely to contribute to the observed variability in measured VOC production rates across samples (Fig. 1).

Table 4
Pearson's correlation coefficients for those sample characteristics that were significantly correlated ($P < 0.05$) with VOC emission profiles for soils and litters

	Among soils ($N = 27$)	Among litters ($N = 12$)
CO_2 production (μg C-CO_2 g soil^{-1}h^{-1})	0.321	NS
Microbial biomass (μg C-CO_2 g soil^{-1}h^{-1})	0.454	NS
% C	0.330	NS
% N	0.376	NS
Carbon quality (μg C-CO_2 g soil^{-1}h^{-1})	NS	0.451
pH	0.179	NS
% silt + clay	NS	NS

Correlations that were not significant ($P > 0.05$) are noted by "NS". These analyses solely examine the influence of sample characteristics on the chemical diversity of VOCs emitted, not the total concentrations of VOCs emitted. Sample number 4 (see Table 1) was not included in the statistical analyses because its carbon content (17%) was far higher than that of the other soils.

It is also important to note that the VOC production rates measured in this study do not necessarily reflect *in situ* rates. The samples were root-free, homogenized, and incubated at near-optimal temperature and moisture conditions so we could effectively compare VOC production rates across all of the samples under controlled conditions. In the field, VOC production rates are likely to be far more variable due to the effects of moisture, temperature, or plant influences. Those soils and litters with the highest observed VOC production rates (Table 3) may not necessarily have the highest rates in the field and additional research is required if we want to effectively compare soil VOC production rates *in situ*.

4.2. The chemical diversity of VOCs emitted from soil and litter

As in other studies that have used GC/MS techniques to study soil VOCs (Kai et al., 2007; Scholler et al., 2002; Stahl and Parkin, 1996), most of the VOCs could not be positively identified, suggesting that more sensitive analytical techniques are necessary if we want to survey the full extent of VOC diversity emitted from soils and litters.

Of those VOCs that could be positively identified, furfural ($C_5H_4O_2$) and 5-hydroxy-methyl-furfural ($C_6H_6O_3$), both furane compounds, were particularly noteworthy in that they were emitted in significant quantities from almost all of the litter and soil samples (but none of the control samples). Furfural has been identified as a VOC emitted by soil fungi (Stotzky and Schenck, 1976), furfural emissions from decomposing litter have been documented by Isidorov and Jdanova (2002), and furfural is one of the dominant VOCs produced during the composting of sewage sludge (Hernandez et al., 2006). Furfural may be commonly emitted from soils and litters, but due to a paucity of research on the topic and disparities in analytical techniques, this cannot be confirmed by comparison with other studies of soil and litter VOC production. However, furfural and 5-hydroxy-methyl-furfural are compounds of industrial interest as they are common byproducts of the chemical oxidation and thermal decomposition of lignocellulose and carbohydrates (Ahring et al., 1999; Chheda et al., 2007). It is also interesting to note that furfural has been documented as one of the dominant chemical compounds in soil organic carbon pools (Grandy et al., 2007) and it has been hypothesized that furfural production is a major pathway by which soil organic matter is formed and stabilized in soil (Gonzalez and Laird, 2006).

Other VOC compounds that were emitted from a range of different soils and litter samples included butanoic acid, propanoic acid, and benzene-related compounds (Tables 2 and 3). Butanoic and propanoic acids are common products of microbial fermentation and a study by Wheatley et al. (1996) also observed emission of these VOCs from soil. Likewise, soil and litter samples have been

found to produce benzene and other, similar aromatic compounds (Asensio et al., 2007; Isidorov and Jdanova, 2002; Wheatley et al., 1996). We did observe emissions of terpenoid compounds (e.g. pinene, camphene, caryophyllene, and limonene), but only from a handful of soil and litter samples (Tables 2 and 3). Although a number of studies have documented terpenoid emissions from soils, particularly forest soils (Hanson and Hoffman, 1994; Hellen et al., 2006; Smolander et al., 2006), it is not clear if these compounds were predominately produced by microbes or by plant roots. Soil microbes, particularly fungi, are capable of producing terpenoid compounds (Stahl and Parkin, 1996), but plant roots (particularly roots that have been damaged) are likely to be the dominant source of terpenoids from forest floors (Paavolainen et al., 1998; Smolander et al., 2006).

4.3. Controls on the types of VOCs emitted

As is evident in Table 3 and Fig. 2, soil and litter samples were fairly distinct with regard to the types of VOCs emitted. These differences were largely driven by the prevalence of terpenoid (e.g. α-pinene, β-pinene, D-limonene, and camphene) and benzene-related compounds emitted from the litter samples (Table 2). The general differences between soil and litter samples with respect to the types of VOCs produced (Fig. 2) could be driven by a variety of factors, including microbial community composition (Larsen and Frisvad, 1995; Scholler et al., 2002; Stahl and Parkin, 1996), the nature of the substrates metabolized by the microbes (Stotzky and Schenck, 1976; Wheatley et al., 1997), environmental conditions (Brinton, 1998), or differences between litters and soils with respect to abiotic volatilization rates.

Compared to the litter samples, there was relatively little inter-sample variability across soils in VOC composition and soils from distinct ecosystem types did not necessarily produce distinct types of VOCs (Fig. 2). For the soil samples, overall levels of microbial activity (namely microbial biomass and CO_2 production rates) seemed to be the best predictor of both net VOC emissions (see above) and the types of VOCs emitted (Table 4). The correlations between measures of microbial activity and VOC characteristics may result from a number of processes. One possibility is that higher densities of microbes lead to an increase in the production of microbial VOCs that serve as signaling molecules and/or quorum sensing molecules (Wheatley, 2002). However, a more likely explanation is that many VOCs are a direct product of microbial metabolism, and therefore, higher metabolic rates yield a greater chemical diversity of VOCs detectable by the methods employed here.

In contrast to the soil samples, organic carbon quality (as measured by rates of CO_2 production per gram organic carbon), not overall microbial activity, was the best predictor of the types of VOCs released from litter samples (Table 4). The apparent relationship between substrate quality and VOC emissions could be driven by differences in the microbial communities inhabiting distinct litter types, as distinct microbial communities are likely to produce distinct VOCs (Stahl and Parkin, 1996). Likewise, substrates that vary in lability may be metabolized in a different manner by soil microbial communities, yielding distinct VOCs. Alternatively, litters of varying quality may also differ with respect to rates of abiotic volatization. As many factors may interact to influence net rates of VOC production from both soil and litter, additional studies are required if we want to gain a mechanistic understanding of soil VOC production.

4.4. The potential influence of microbially-produced VOCs on belowground ecology

In this study, we could not quantitatively compare VOC-carbon emissions with CO_2-carbon emissions given the techniques used, and it is unlikely that VOC emissions rival CO_2 emissions as a flux of carbon from soil to the atmosphere (Hanson and Hoffman, 1994; Stahl and Parkin, 1996). However, the VOCs observed in this study are likely to have an important effect on belowground ecology by influencing biological interactions and microbial process rates.

Unfortunately, most of the VOCs released from the soil and litter samples could not be identified, so it is impossible to speculate on their ecological significance. Of those VOCs that could be identified, we know the most about the impacts of terpenoid compounds on belowground ecology. In particular, we know that terpenoids can have a variety of direct and indirect effects on the rates of microbial processes including methane oxidation, nitrification, nitrogen mineralization, and aerobic respiration (Amaral et al., 1998; Paavolainen et al., 1998; Smolander et al., 2006; White, 1994) with some studies suggesting that terpenoids may function as allelopathic compounds (Langenheim, 1994). Since terpenoids were more commonly emitted from litter samples than from soil samples, we would expect these effects to be most important in ecosystems with significant litter layers and in soils.

Other compounds identified in this study that may have important effects on belowground ecology include furfural, benzene, and the ethyl esters. Furfural is a potent inhibitor of microbial fermentation processes (Couallier et al., 2006; Zaldivar et al., 1999), and furfural derivatives can inhibit nitrification (Datta et al., 2001). Interestingly, furfural can also inhibit the activity of phytoparasitic nematodes (Rodriguez-Kabana et al., 1993). Benzene, and related compounds, can influence bacterial and fungal growth rates, as can butanoic and propanoic acids (Ezra and Strobel, 2003; Wheatley, 2002). In short, it is highly likely that soil VOCs have an important influence on belowground ecology, but the details are not well understood. In particular, we need additional research in order to determine if the concentrations of these VOCs in the soil atmosphere are sufficient to influence soil organisms and processes *in situ*.

4.5. Future research directions

This study highlights a number of questions that are ripe areas for future research on soil VOCs. In particular: Why is there an apparent predominance of furfural and furfural derivatives in soil VOC emissions? How do gross VOC production rates compare to net rates? What are the relative contributions of abiotic, plant, and microbial sources to soil VOC emissions in the field? and, what is the capacity for VOCs to mediate competitive interactions and regulate microbial processes in soil?. Addressing these questions is important given the potential influence of VOCs on belowground ecology and the possibility that soil VOCs may serve as sensitive indicators of changes in soil conditions.

Acknowledgments

We are grateful to Ken Keefover-Ring for his advice and help with the GC/MS analyses. We also wish to acknowledge Christian Lauber, Stuart Grandy, Barbara Demming-Adams, and two anonymous reviewers for valuable comments on previous drafts of the manuscript. This study was funded by the Innovative Research Program of the Cooperative Institute for Research in Environmental Sciences and the Undergraduate Research Opportunities Program at the University of Colorado.

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