THE PRODUCTION AND CONSUMPTION OF VOLATILE ORGANIC COMPOUNDS IN SOIL AND DECOMPOSING LITTER

by

CHRISTOPHER M. GRAY

B.A., University Colorado Boulder, 2008

A thesis submitted to the

Faculty of the Graduate School of the

University of Colorado in partial fulfillment

of the requirement for the degree of

Doctor of Philosophy

Department of Ecology and

Evolutionary Biology

2014

This thesis entitled:
The production and consumption of volatile organic compounds in soil and decomposing litter
written by Christopher M Gray
has been approved for the Department of Ecology and Evolutionary Biology

_____
Noah Fierer

_____
Christine Wiedinmyer

Date_____

The final copy of this thesis has been examined by the signatories, and we
Find that both the content and the form meet acceptable presentation standards
Of scholarly work in the above mentioned discipline.

Gray, Christopher M (Ph.D., Ecology and Evolutionary Biology)

The production and consumption of volatile organic compounds in soil and decomposing litter

Thesis directed by Associate Professor Noah Fierer

Non-methane biogenic volatile organic compounds (BVOCs) are reactive, low molecular weight gases that play key roles in atmospheric chemistry, and in soils, where they can alter the rates of biogeochemical cycles and impact the growth of plants and soil organisms. However, the types and quantities of BVOCs released from or taken up by soils and decomposing litter remain poorly characterized as do the biotic and abiotic controls on these fluxes. We used proton transfer reaction mass spectrometry (PTR-MS) to quantify BVOC flux rates from decomposing litter under varying biotic and abiotic conditions. Microbial production was the primary source of BVOCs emitted from decomposing litter, while the types of BVOCs emitted from the litter differed in a predictable manner among litter types. The amount of carbon (C) emitted as VOCs from the some decomposing litter types was near equivalent to the amount emitted as CO_2 from microbial respiration. Although nitrogen (N) amendments have been shown to increase CO_2 emission rates from decomposing litter, we found that N amendments reduced BVOC emissions to near zero. We also examined BVOC fluxes in soil and litter under field conditions, quantifying the contribution of tree roots to flux rates. Tree roots, directly or indirectly, contributed to half of the total C emitted from the soil as BVOCs. Methanol was the BVOC emitted at the highest net rates in all studies, while isoprene was net consumed into the intact soil at the highest rates. This finding led us to investigate the microbial community involved in the consumption of isoprene. Using amplicon sequencing and experimental amendments of incubating soil with isoprene, we found that several phyla, known to consume other

hydrocarbons, were responding positively to increasing isoprene concentrations. These microorganisms were able to consume approximately 70% of the isoprene added into the headspace of incubating soils, with consumption rates up to 770 pmol g^{-1} h^{-1}. Together these results have increased or understanding of the biotic and abiotic controls on the consumption and production of BVOCs in the soil environment and these results highlight the importance of considering these effects when modeling BVOC flux rates and C dynamics.

CONTENTS

TABLES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

FIGURES

Chapter 1

Chapter 2

Chapter 3

Chapter 4

Introduction

Non-methane biogenic volatile organic compounds (BVOCs) are light-weight, gaseous carbon (C) compounds emitted and consumed from a wide variety of life. In the atmosphere, these compounds are initially oxidized by hydroxyl radicals (OH). As OH is responsible for the breakdown of many of the most potent greenhouse gases, including methane, changes in atmospheric BVOC concentrations or flux rates could impact the residence time of those greenhouse gases in the atmosphere (Monson & Holland 2001). The products of BVOC oxidation can also contribute to tropospheric ozone and global nitrogen (N) transport in areas of high NO_X (Harley et al. 1999; Monson & Holland 2001). Furthermore, the oxidation of several BVOCs can lead to the formation of secondary organic aerosols, which can then serve as cloud condensation nuclei affecting precipitation dynamics and cloud albedo (Claeys et al. 2004; Spracklen et al. 2008).

Within the biosphere, BVOCs are part of the local and global C cycle (Guenther 2002; Kesselmeier et al. 2002). Within soils specifically, they can affect the rates the C (Amaral & Knowles 1998; Maurer et al. 2008) and N cycles (Smolander et al. 2006; Uusitalo et al. 2008). Soil ecology can also be affected as many BVOCs stimulate or inhibit the growth of species of bacteria, fungi, plants and nematodes (Bruce et al. 2004; Farag et al. 2006; Gu et al. 2007; Mackie & Wheatley 1999; Wheatley 2002). Despite our understanding on the effects of BVOCs in soil, we have relatively little information on their sources and sinks within the soil environment when compared to plants and atmosphere.

Building upon previous work by (Ramirez et al. 2010) that reported decomposing plant litter as the main contributor to soil net BVOC emissions, the following questions were asked as reported in the first chapter and Gray et al. (2010). 1) What is the relative importance of abiotic versus biotic processes to BVOC emissions from litter? 2) Does the decomposition of litter from different plant species yield different types and quantities of emitted BVOCs? 3) Can any differences in VOC emissions among litter types be predicted from either plant taxonomy and/or litter chemical characteristics?

The emission rates of several BVOCs with a large ratio of C per molecule measured in Gray et al. (2010) and the finding that BVOC emissions were microbial in nature prompted the following questions which were addressed in the second chapter and Gray & Fierer (2012). 1) How does the total C lost as BVOCs from decomposing litter compare to the total C lost as respiration? 2) How do N amendments affect BVOC emissions from decomposing litter?

As the previous two studies were conducted in a controlled laboratory setting, the following questions were investigated in a field setting and described in the third chapter and Gray et al. (2014). (1) What are the types and amounts of BVOCs emitted or consumed (soil uptake) from undisturbed soils in situ from a subalpine forest floor during the growing season? (2) How much does the presence of active roots and root rhizodeposition contribute to BVOC fluxes from soil? (3) How do temperature and soil moisture relate to the temporal variability in soil BVOC flux rates? It should be noted that the moisture differences between ambient air and chamber air in this experiment could have led to the measured consumption of formaldehyde in this experiment. However, comparing the differences in moisture detected here to those reported by [Warneke] suggest that this is not the case.

Isoprene, which has several key roles in atmospheric chemistry, was identified as having the largest net consumption rates of all BVOCs measured in Gray et al. (2014). This led to the further investigation of its consumption in bulk soil by asking the following questions as addressed here in the fourth chapter. 1) Do microbial isoprene consumption rates scale linearly with the isoprene concentration provided to the soil microorganisms with significant consumption even at very low isoprene concentrations? 2) Is isoprene consumption associated with increases in the relative abundances of specific bacterial taxa (primarily those in the Actinobacterial and Alphaproteobacterial phyla) and specific fungal taxa (within the Sordariomycete and Eurotiomycete group).

Chapter 1

Introduction

Biogenic volatile organic compound (VOC) emissions from terrestrial ecosystems can have important effects on atmospheric chemistry and ecosystem-level processes. In the atmosphere, biogenic VOCs are initially degraded by hydroxyl radicals, a photochemically produced compound in the atmosphere that is responsible for the oxidation of several types of greenhouse gases, including methane. Because biogenic VOCs, along with greenhouse gases, can be competitive reactants for available hydroxyl radicals, many regional and global atmospheric chemistry models explicitly consider biogenic VOC emissions when predicting the tropospheric lifetime of greenhouse gases (Hauglustaine et al. 1998; Lelieveld et al. 1998; Poisson et al. 2000). Additionally, the products of biogenic VOC oxidation can serve as cloud condensation nuclei affecting local cloud albedo and precipitation dynamics (Spracklen et al. 2008) or they can react with tropospheric NO_x, affecting nitrogen transport (Harley et al. 1999; Monson & Holland 2001). As all VOCs are comprised of a carbon skeleton, their emissions from the biosphere are also a component of local and global carbon cycling (Guenther 2002; Kesselmeier et al. 2002). There is also evidence that VOCs can influence biogeochemical processes within soils, altering rates of carbon cycling by serving as substrates for microbial metabolism (Shennan 2006), inhibiting microbial processes associated with the nitrogen cycle (Amaral et al. 1998; Bending & Lincoln 2000; Smolander et al. 2006), and either stimulating or inhibiting the growth of specific microbial taxa (Bruce et al. 2004; Wheatley 2002).

The emissions of VOCs from living plants have been studied for decades and, although key knowledge gaps remain, we have a reasonably good understanding of the biotic and abiotic controls over plant VOC emissions (Kreuzwieser et al. 1999). In contrast, VOC emissions from soil and litter have received far less attention even though recent studies suggest that they may represent important sources of VOCs in terrestrial systems (Asensio et al. 2007; Leff & Fierer 2008). There have been some recent efforts to incorporate soil and litter sources into VOC models (Jacob et al. 2005), but such models are often constrained by the paucity of relevant data. For example, Warneke et al. (1999) used abiotic emissions from leaf litter of a single plant species (*Fagus sp.*), to estimate global VOC emissions from soil, assuming that biotic sources of VOCs from the litter are insignificant and that litter from different species emit similar types and quantities of VOCs.

Recent research on VOC emissions from soil and decomposing litter suggest that microbes may be important sources of VOCs and that such emissions are highly variable across litter types. Isidorov & Jdanova (2002) found differences between essential oils extracted from leaf litter and the VOCs that were emitted, suggesting that microbial enzymes were breaking down these oils into volatiles. Likewise, Leff & Fierer (2008) sampled VOC emissions from litter and soil and found a high correlation between respiration, microbial biomass and VOC emission levels, suggesting that microbial decomposition processes are the dominant source of VOC emissions. They also found that the litter produced a greater diversity and quantity of VOCs compared to soil. However, we still do not know the relative importance of biotic versus abiotic sources of VOC emissions during litter decomposition and how VOC emissions vary among types of decomposing litter from different plant species. We would expect the types and quantities of VOCs emitted to vary across litter types due to differences in litter chemistry and/or

differences in decomposer communities. Both microbial community composition (Bunge et al. 2008; Lechner et al. 2005) and substrate-type (Van Lancker et al. 2008) have been shown to influence VOC production by microorganisms.

We measured VOC emissions from both sterile and non-sterile litter from 12 plant species over a 20-d laboratory incubation, using proton transfer reaction mass spectrometry (PTR-MS) to quantify the concentration of specific VOCs above the litter and facilitate calculation of compound-specific emission rates. The study was designed to address the following questions that currently represent gaps in our understanding of terrestrial VOC emissions. First, what is the relative importance of abiotic versus biotic processes to VOC emissions from litter? If biotic controls on global emission models are important, current models that assume strictly abiotic emissions will have to be revised. Second, does the decomposition of litter from different plant species yield different types and quantities of emitted VOCs? And if so, can these differences in VOC emissions among litter types be predicted from either plant taxonomy and/or litter chemical characteristics? Addressing these questions will help improve our estimates of VOC emissions from decomposing litter in the field and improve our predictions of how VOC emissions from terrestrial ecosystems may be altered over time and space with shifts in vegetation type.

Methods

Sample collection

Leaf litter was collected from 12 plant species in September and October of 2008 from California, Colorado, Montana and North Carolina (Table 1).

Table 1 – Litter characteristics of the litter from 12 plant species included in this study

					Litter C fractions (%)			
Species	Family	Litter location	N (%)	C:N	Cell soluble	Hemi-cellulose	Cellulose	Lignin
Centaurea maculosa	Asteraceae	Missoula, MT	1.15	39.14	32.33	20.42	37.83	9.42
Rhododendron maximum	Ericaceae	Otto, NC	0.41	122.46	59.23	7.45	18.02	15.30
Quercus macrocarpa	Fagaceae	CU Boulder, CO	0.77	64.78	58.32	12.96	14.85	13.87
Quercus rubra	Fagaceae	CU Boulder, CO	1.27	37.09	60.02	15.63	11.45	12.90
Eucalyptus sp.	Myrtaceae	Arroyo Grande, CA	0.70	75.47	63.60	8.28	13.32	14.80
Fraxinus pennsylvanica	Oleaceae	CU Boulder, CO	1.21	36.11	66.43	11.95	12.25	9.36
Pinus contorta	Pinaceae	Niwot Ridge, CO	0.69	76.53	36.25	13.89	19.73	30.13
Pinus ponderosa	Pinaceae	Boulder Canyon, CO	0.64	86.31	48.86	13.62	18.94	18.58
Miscanthus sp.	Poaceae	Superior, CO	0.72	61.33	39.19	27.52	24.61	8.68
Thinopyrum intermedium	Poaceae	Boulder Canyon, CO	0.58	77.66	28.87	32.39	34.18	4.56
Populus deltoides	Salicaceae	CU Boulder, CO	0.46	97.57	71.50	11.49	12.23	4.78
Populus tremuloides	Salicaceae	Niwot Ridge, CO	0.52	95.75	74.58	8.06	6.99	10.37

Litter samples from deciduous species were collected within 5 days of leaf fall. For evergreen species, litter was collected from the ground, selecting litter that appeared newly dropped. Dead grass leaves were clipped from tillers that had gone dormant at the end of the growing season. All samples were oven dried at 60°C then stored at 4°C prior to the start of the experiment.

Sample characterization

Subsamples of each litter type were ground to a fine and coarse powder with a Wiley mill (60 and 20 mesh respectively). The percentage of carbon (%C) and nitrogen (%N) in the litter was measured using the fine powder and a CHN 4010 Elemental Combustion System (Costech Analytical Technologies, Valencia, CA) (Table 1). The coarse powder was analyzed using a plant fiber analyzer (Ankom Technology, Macedon, New York, USA) to determine litter solubility and quantities of various C fractions (Hobbie & Gough 2004). Briefly, the coarse powder was sealed in filter bags and digested at 100°C in a dilute neutral detergent to determine the cell soluble fraction lost. What remained was then digested in a weak acid detergent to quantify the hemicelluloses fraction lost. Finally, the remaining filter bags were digested using

sulfuric acid and the mass lost was contributed to the cellulose in the leaf. The mass remaining was considered an estimate of the lignin fraction of the litter.

Lab incubation and VOC measurements

Each litter sample was cut into pieces of approximately 40 mm^2 and thoroughly homogenized. The samples were then divided into eight 125 mL glass jars, each with 1.6 g dry weight (±0.06 g) of cut homogenized litter. Six jars without litter were added for experimental "blanks" and were used as background measurements of VOC concentrations in the ambient air, for a total of 102 jars (8 jars per litter type and 6 "blanks"). Each 125 mL glass jar was placed into a 500 mL glass jar, which was then closed with a gas-tight Teflon sealed cap. Two holes were drilled into each cap and fitted with brass Swagelok connectors and Whatman 0.2 μm polyVENT filters to exclude contamination from airborne microbes. All jars were sterilized by autoclaving at 121°C for 45 minutes. Autoclaving most likely released VOCs from the litter samples and may have altered litter chemistry to some degree. Thus, autoclaving may have either increased measured VOC emissions (by accelerating litter breakdown) or decreased measured emissions (by driving off VOCs prior to the start of the incubation). However, our goal was to compare VOC emissions between litter types and treatments, not necessarily to quantify VOC emissions as they may occur from decomposing litter in the field. Autoclaving was determined to be the most effective means of sterilization and, regardless of our choice of sterilization method, VOC emissions would have likely been altered to some degree. Four jars of each litter type were kept sterile as abiotic controls for a total of 48 abiotic samples (4 per litter type). All jars were stored in the dark at room temperature (~22°C) for the duration of the experiment. Autoclaved deionized water was added through the filters to bring the sterilized litter sample to 80% of water holding capacity (WHC). Water was also added through the filters into the outer 500 mL glass

jar to keep humidity levels constant during the incubation period. The other four autoclaved jars of each litter type were inoculated with non-sterile soil and used as biotic controls. Homogenized soil slurry was created by mixing 20 grams of soil (a mixture of 3 local soil types) in 800 mL of autoclaved deionized water. To inoculate each litter type, 1 mL of the soil slurry was added directly to the litter. Autoclaved deionized water was then added to bring the litter to 80% WHC. As with the sterile samples, water was added to the outer 500 mL jar to keep air humidity levels constant. Blank jars received autoclaved deionized water in both the 125 mL jar and 500 mL jar but no litter or inoculum was added.

All jars were incubated in the dark at room temperature (~22°C) throughout the 20-d incubation period. The filters were left unsealed in order to allow for diffusive gas exchange in between VOC measurement periods. Sterility of the litter in the abiotic jars was confirmed throughout the experiment both visually and by monitoring CO_2 fluxes (or lack thereof) using a LI-COR 6400 (LI-COR Biosciences, Lincoln, Nebraska, USA). Measurements of headspace VOC concentrations started three days after setup and continued on regular intervals for 20 days. VOC concentration measurements were taken using PTR-MS (Ionicon GmbH, Innsbruck, Austria) as described by Lindinger et al. (1998). Briefly, headspace air containing VOCs is drawn through a drift tube containing hydronium (H_3O^+) ions. The H_3O^+ reacts with the VOC (R), transferring a proton, which increases the mass by 1 amu and gives the VOC a positive charge (Eq. 1).

$$H_3O^+ + R \rightarrow RH^+ + H_2O \quad (1)$$

A quadrupole detector in the PTR-MS selects for compounds with characteristic masses at a resolution of 1 amu. A secondary electron multiplier quantifies the amount of each selected mass. The PTR-MS was operated at 125 Townsends (Td; 1 Td = 10^{-17} V cm^2 molecule^{-1}) to keep

fragmentation of the target compounds low and the clustering of water and H_3O^+ ions low. The concentration of the measured VOC were calculated with the following equation

$$[RH^+] = [H_3O^+]_0(1 - e^{-k[R]t}) \approx [H_3O^+]_0 k[R]t, \quad (2)$$

where t is the time of flight of H_3O^+ through the drift tube and k is the rate coefficient for the proton-transfer reaction. Ambient air concentrations were concurrently determined using VOC measurements taken from the blank jars. These were then averaged at each time point and subtracted from the experimental jar measurements. Emission rates (E) were calculated using the equation:

$$E = ([C]F) / aW \ . \quad (3)$$

In equation 3, [C] is the concentration of the measured VOC in nanomole per mole, F is the flow rate though the headspace in liters per hour, W is the dry weight of the sample in grams and a is the standard molar volume of 22,414 $L \cdot mol^{-1}$. Emission rates are reported as nanomoles of VOC per gram dry litter per hour. Identification of VOCs was based solely on molecular mass and comparison to VOCs described in other studies. Thus, the identity of individual compounds has not been confirmed and any identifications are considered to be putative.

Data Analysis

All analyses were run using the R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Total VOC emissions (for the entire incubation period) were calculated by integrating compound emission rates over time, and the resulting total emissions were compared between treatments using Welch's two-sample t-test (Figure 1).

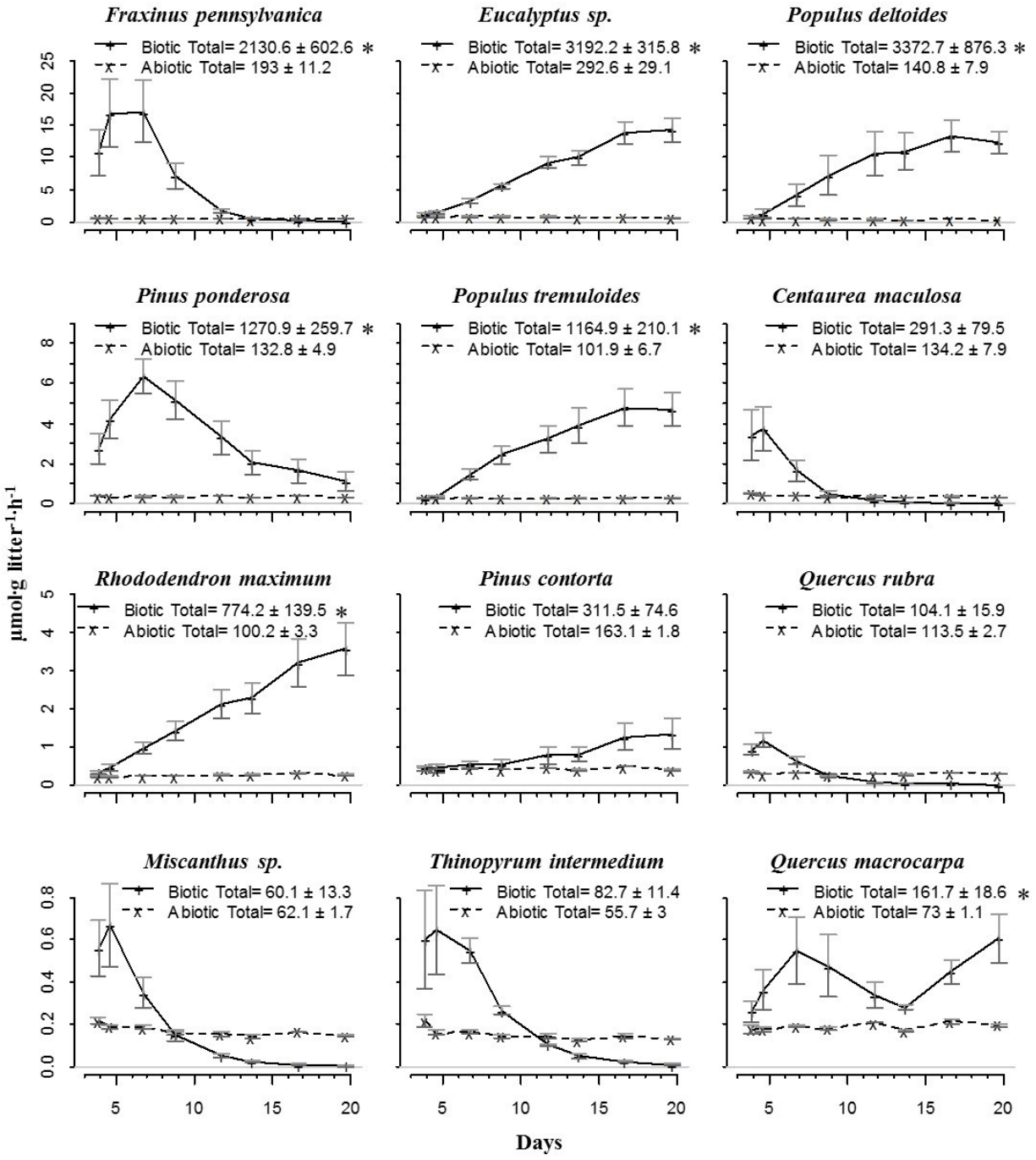

Figure 1 – Net emissions from 21 measured VOC masses over a 20 day period. Biotic emissions (solid line) peak at levels higher than the emissions from non-sterile samples (dashed line) in all litter types. Y axis values are in nmol of total VOCs emitted per gram of dry litter per hour (nmol·g litter^{-1}·h^{-1}). Inset values are total VOCs emitted over the measure time with standard error. (*) indicates a statistical difference ($P < 0.05$) between the total VOCs emitted over the measured time.

Total emissions among litter types were compared with analysis of variance (ANOVA). An analysis of similarity (ANOSIM) was performed on the individual percentages of emitted VOCs to determine the similarity in VOC emission profiles between the abiotic and biotic treatments and between litter types. Finally, the percentages of VOCs emitted from each litter type (VOC profiles) were compared to the measured litter characteristics using Mantel tests (Mantel 1967).

Results

Abiotic vs. Biotic emissions

For the non-sterile ('biotic') litters, net VOC emissions over the 20-d experiment ranged from 29 to 4816 nmol · g-litter^{-1} while the net emissions from the sterile ('abiotic') litters ranged from 50 to 344 nmol · g-litter^{-1} (Figure 1 and Table 2).

Table 2 – Net VOC emissions from the decomposing litter of 12 plant species[a]

Mass (m/z)	Suspected Compound(s)	*Centaurea maculosa* Biotic	*Centaurea maculosa* Abiotic	*Rhododendron maximum* Biotic	*Rhododendron maximum* Abiotic	*Quercus macrocarpa* Biotic	*Quercus macrocarpa* Abiotic	*Quercus rubra* Biotic	*Quercus rubra* Abiotic	*Eucalyptus sp.* Biotic	*Eucalyptus sp.* Abiotic	*Fraxinus pennsylvanica* Biotic	*Fraxinus pennsylvanica* Abiotic
33+51	methanol	285.654 (79.071)	70.058 (5.616)	763.284 (139.307)	76.709 (3.209)	150.059 (18.877)	47.328 (1.457)	94.591 (15.604)	48.910 (3.372)	2817.840 (355.878)	66.195 (2.311)	2113.636 (601.559)	140.538 (12.054)
43	n-propanol / acetic acid	0.943 (0.134)	3.143 (0.283)	1.385 (0.066)	2.055 (0.090)	1.511 (0.185)	3.227 (0.586)	1.426 (0.092)	6.431 (0.411)	12.051 (1.948)	5.248 (0.325)	1.799 (0.121)	4.192 (0.408)
45	acetaldehyde	0.110 (0.081)	44.637 (1.315)	0.056 (0.018)	13.051 (0.329)	0.101 (0.014)	14.856 (0.721)	0.265 (0.022)	41.388 (0.692)	0.538 (0.056)	15.594 (0.986)	0.608 (0.299)	34.556 (1.482)
47	formic acid ethanol	0.369 (0.201)	0.163 (0.025)	0.301 (0.072)	0.184 (0.037)	0.154 (0.069)	0.190 (0.047)	0.389 (0.033)	0.423 (0.118)	1.160 (0.183)	0.492 (0.046)	1.506 (0.270)	0.239 (0.021)
57	butanol	0.370 (0.091)	0.845 (0.078)	0.348 (0.058)	0.098 (0.019)	1.106 (0.098)	0.162 (0.040)	1.448 (0.086)	0.225 (0.015)	3.473 (0.531)	1.196 (0.079)	1.864 (0.224)	0.647 (0.035)
59	propanal / acetone	2.345 (0.640)	6.667 (0.934)	7.041 (0.403)	4.163 (0.118)	6.080 (1.225)	2.699 (0.064)	2.620 (0.151)	5.698 (0.158)	120.279 (38.289)	10.812 (0.159)	5.132 (0.697)	3.938 (0.391)
61	acetic acid	0.518 (0.144)	1.735 (0.229)	0.436 (0.099)	1.572 (0.068)	0.404 (0.063)	2.864 (0.640)	0.434 (0.076)	5.387 (0.437)	0.962 (0.144)	3.656 (0.319)	0.678 (0.062)	3.138 (0.404)
69	isoprene	0.076 (0.017)	2.682 (0.244)	0.197 (0.031)	0.177 (0.015)	0.206 (0.027)	0.270 (0.015)	0.263 (0.006)	0.535 (0.027)	3.052 (0.202)	1.433 (0.090)	0.687 (0.086)	1.908 (0.061)
73	methyl ethyl ketone (MEK)	0.405 (0.167)	2.763 (0.111)	0.474 (0.212)	0.317 (0.013)	0.949 (0.299)	0.608 (0.031)	1.324 (0.133)	3.299 (0.195)	7.941 (4.261)	2.182 (0.114)	1.804 (0.598)	2.591 (0.151)
93	toluene	0.013 (0.004)	0.016 (0.000)	0.022 (0.006)	0.016 (0.009)	0.019 (0.005)	0.014 (0.002)	0.023 (0.008)	0.022 (0.006)	4.256 (0.846)	2.627 (0.300)	0.007 (0.003)	0.017 (0.005)
135	unknown	0.002 (0.001)	0.007 (0.004)	0.005 (0.002)	0.007 (0.002)	0.017 (0.004)	0.004 (0.002)	0.021 (0.005)	0.006 (0.002)	8.602 (2.066)	6.422 (0.808)	0.006 (0.002)	0.006 (0.002)
81+137	monoterpenes	0.006 (0.004)	0.107 (0.004)	0.053 (0.015)	0.014 (0.011)	0.123 (0.025)	0.167 (0.027)	0.074 (0.011)	0.275 (0.029)	205.058 (40.930)	174.871 (28.777)	0.067 (0.020)	0.158 (0.024)

Mass (m/z)	Suspected Compound(s)	*Pinus contorta* Biotic	*Pinus contorta* Abiotic	*Pinus ponderosa* Biotic	*Pinus ponderosa* Abiotic	*Miscanthus sp.* Biotic	*Miscanthus sp.* Abiotic	*Thinopyrum intermedia* Biotic	*Thinopyrum intermedia* Abiotic	*Populus deltoides* Biotic	*Populus deltoides* Abiotic	*Populus tremuloides* Biotic	*Populus tremuloides* Abiotic
33+51	methanol	293.634 (74.937)	86.136 (2.017)	1217.715 (256.632)	66.278 (5.633)	54.603 (12.353)	26.288 (1.372)	77.131 (11.635)	30.294 (1.485)	3340.120 (867.458)	94.567 (5.718)	1150.668 (208.908)	69.851 (7.371)
43	n-propanol / acetic acid	2.390 (0.698)	7.148 (0.471)	3.422 (0.255)	6.503 (1.462)	0.937 (0.218)	3.940 (0.641)	0.901 (0.072)	2.034 (0.198)	3.102 (0.520)	3.683 (0.261)	2.036 (0.081)	2.617 (0.338)
45	acetaldehyde	0.076 (0.007)	38.310 (2.055)	1.070 (0.164)	18.492 (0.761)	0.040 (0.013)	20.135 (0.675)	0.041 (0.017)	15.682 (0.782)	3.679 (2.062)	31.354 (2.467)	0.710 (0.313)	22.961 (1.640)
47	formic acid or ethanol	0.268 (0.095)	1.208 (0.114)	1.337 (0.188)	0.423 (0.112)	0.173 (0.047)	0.128 (0.027)	0.126 (0.025)	0.150 (0.026)	6.148 (3.173)	0.244 (0.047)	0.818 (0.288)	0.178 (0.021)
57	butanol	0.533 (0.082)	0.614 (0.038)	2.242 (0.225)	0.377 (0.047)	0.360 (0.080)	0.432 (0.041)	0.285 (0.060)	0.489 (0.095)	2.619 (1.044)	0.520 (0.020)	0.858 (0.183)	0.272 (0.018)
59	propanal / acetone	3.814 (0.516)	9.542 (0.100)	21.989 (2.361)	14.155 (2.328)	1.922 (0.572)	5.804 (0.385)	2.956 (0.661)	3.487 (0.249)	11.248 (1.381)	5.316 (0.840)	6.404 (0.336)	2.364 (0.162)
61	acetic acid	1.416 (0.957)	6.016 (0.444)	0.690 (0.101)	5.423 (1.478)	0.461 (0.182)	3.239 (0.727)	0.442 (0.037)	1.400 (0.162)	0.804 (0.131)	2.756 (0.322)	0.674 (0.169)	2.088 (0.326)
69	isoprene	0.240 (0.038)	1.517 (0.114)	1.442 (0.082)	1.091 (0.055)	0.182 (0.014)	0.408 (0.010)	0.082 (0.016)	0.287 (0.031)	0.631 (0.106)	0.487 (0.022)	0.492 (0.046)	0.354 (0.019)
73	methyl ethyl ketone (MEK)	0.038 (0.017)	2.436 (0.184)	2.448 (0.454)	1.794 (0.155)	0.729 (0.196)	0.695 (0.048)	0.081 (0.046)	0.601 (0.065)	2.378 (1.212)	0.691 (0.045)	1.193 (0.521)	0.507 (0.038)
93	toluene	0.506 (0.047)	0.481 (0.038)	1.012 (0.122)	0.635 (0.115)	0.007 (0.001)	0.010 (0.002)	0.009 (0.002)	0.015 (0.002)	0.033 (0.007)	0.009 (0.004)	0.030 (0.005)	0.012 (0.003)
135	unknown	0.216 (0.029)	0.173 (0.022)	1.180 (0.097)	0.559 (0.123)	0.006 (0.003)	0.001 (0.000)	0.008 (0.003)	0.001 (0.000)	0.042 (0.018)	0.019 (0.010)	0.004 (0.001)	0.015 (0.008)
81+137	monoterpenes	7.848 (1.348)	8.085 (0.715)	14.388 (2.358)	15.681 (2.266)	0.006 (0.006)	0.060 (0.008)	0.004 (0.003)	0.093 (0.054)	0.311 (0.076)	0.148 (0.031)	0.068 (0.015)	0.103 (0.035)

[a] The top 12 compounds from the measured 21 masses are shown. Values are in nmol of VOC emitted per gram of dry litter ($nmol \cdot g^{-1}$) totaled over 20 days. Parentheses contain standard error. The identities of the suspected compounds have not been confirmed and are considered putative.

On average, net biotic emissions were 946 nmol · g-litter^{-1} (611%) greater than abiotic emissions.

Fraxinus pennsylvanica, the *Eucalyptus sp.*, and *Populus deltoides* had the largest disparity

between biotic and abiotic emissions, with biotic emissions exceeding abiotic emissions by an average of 1042%, 1044% and 2229% respectively. Seven of the 12 litter types showed significantly ($P < 0.05$) higher net VOCs emissions from the biotic treatments. However, those litter types that were not significantly higher during the 20-d experiment still had a larger variation of individual emission rates when compared to the abiotic emission rates (mean standard deviation of 0.70 among biotic treatments and 0.04 among abiotic treatments of these five litter types). ANOSIM statistics comparing the proportional representation of the types of VOCs emitted from biotic and abiotic treatments showed that the VOC profiles were also significantly different (Global $R = 0.83$, $P < 0.001$) (Figure 2, Figure 3A).

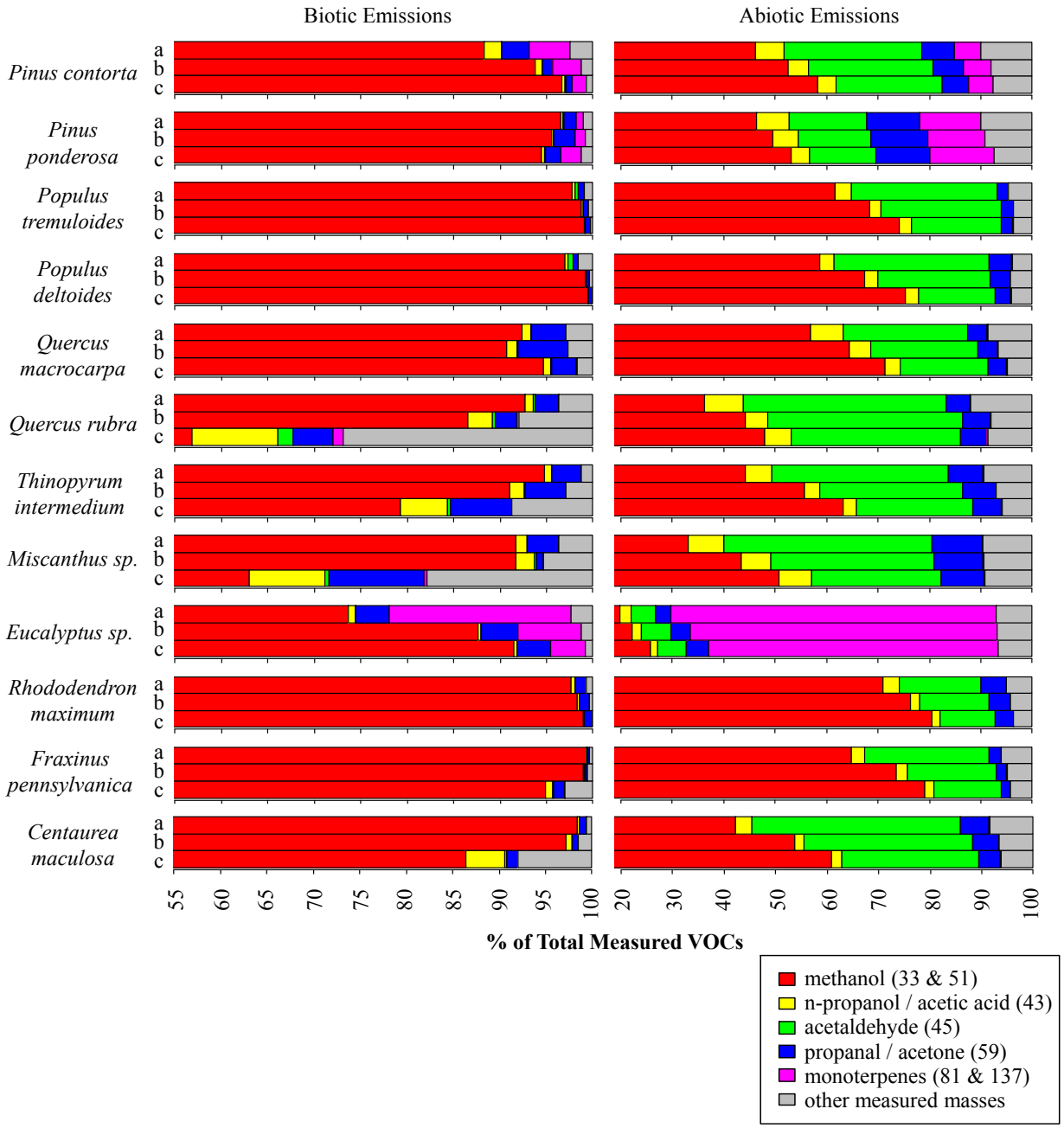

Figure 2 – The proportional contribution of measured VOCs to the net emissions from the decomposing litter of 12 plant species during a 20 days incubation. The top bar (a) is the average emissions from hour 93 to hour 212. The middle bar (b) is the average emissions from hour 212 to hour 330. The bottom bar (c) is the average emissions from hour 330 to hour 473. Legend shows possible compound(s) with the measured protonated mass(es) in parentheses.

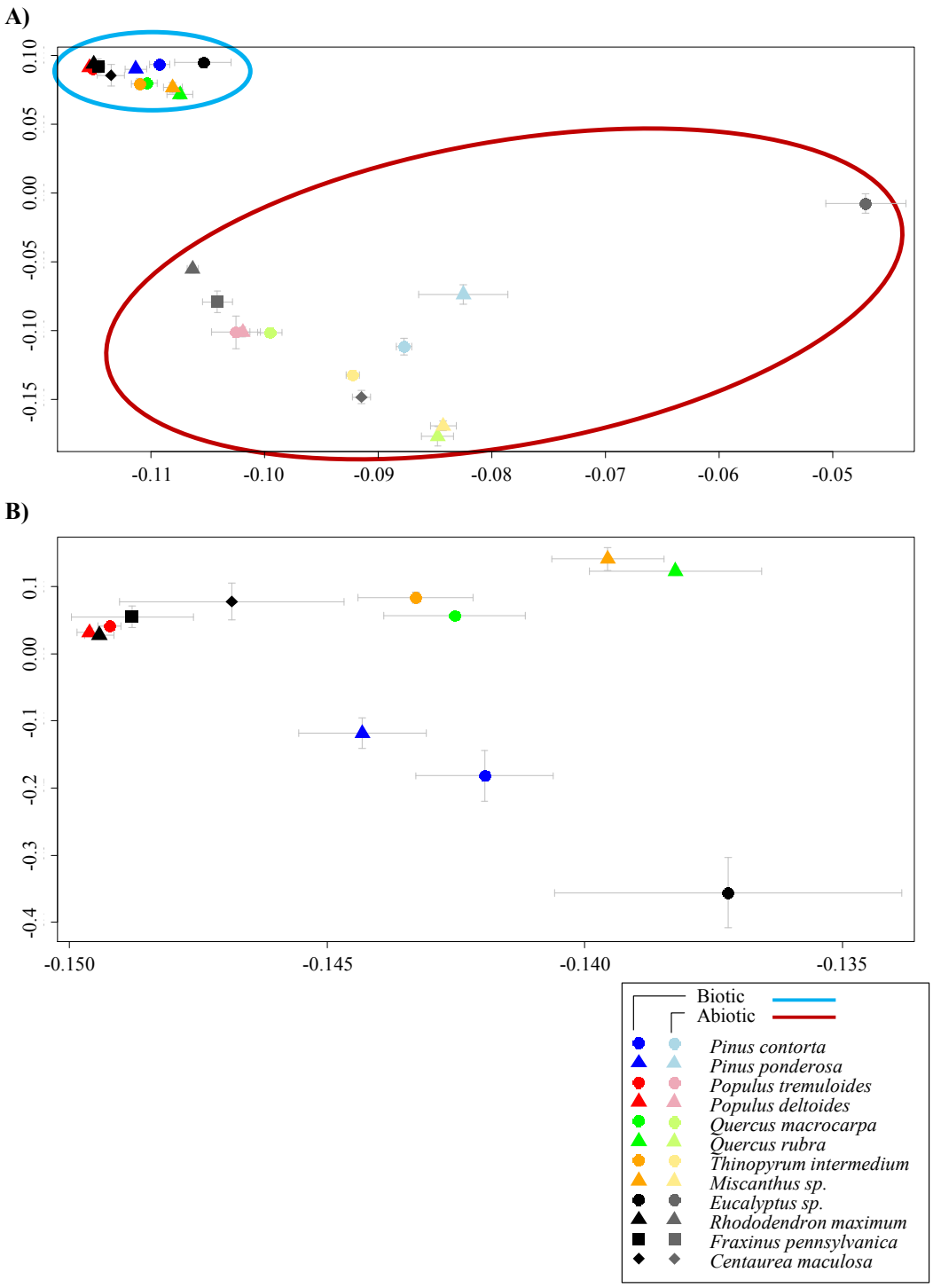

Figure 3 – PCA plots transforming the differences between measured plant species litter VOC emissions into two dimensions. Plant species detritus with similar VOC emissions during decomposition are closer in the PCA plot. (A) depicts biotic and abiotic emissions, while (B) shows only the biotic emissions. The blue circle indicates the grouping of the non-sterile samples and the red circle indicates sterile samples.

VOC emissions from the abiotic litters were dominated by protonated masses 33, 45 and 59 (suspected to be methanol, acetaldehyde, and a combination of propanal and acetone respectively) with average percentages of total VOCs emitted of 56%, 23%, and 5%, respectively (Figure 2). In contrast, VOC emissions from the biotic litters were dominated by protonated masses 33, 59 and 43 with average percentages of 95%, 2%, and 1% respectively (Figure 2).

Differences in biogenic VOC emissions between litter types

Total VOC emissions were significantly affected by litter type ($P < 0.001$) with emission rates highest in litter from *Fraxinus pennsylvanica*, the *Eucalyptus* sp., and *Populus deltoides* (Figure 1). The types of VOCs emitted were also significantly different between litter type (Global $R = 0.64$, $P < 0.001$)(Figure 3B). Although protonated mass 33 (methanol) was the dominant VOC in all cases (representing 78 – 99% of total VOC emitted), the relative proportions of various VOCs varied across litter types with far more variability between plant species than between replicate litter samples from the same species (Figure 3B). In particular, decomposing *Eucalyptus* sp. emitted relatively high levels of protonated mass 137 (suspected to be monoterpene) throughout the 20-d incubation with emission levels reaching 20% of total VOC emissions within the first 8 days (Figure 2). *Quercus rubra* and *Miscanthus* sp. emitted less of protonated mass 33 during the last 6 days of the incubation, reaching 57% and 63% of the total VOC emission respectively.

Predictability of biogenic VOC emissions

There was a significant relationship between the types of VOCs emitted and plant family (Global $R = 0.097$, $P = 0.006$), a pattern also evident in Figure 3B which shows that VOC profiles from litter of related plant species were often similar. However, VOC emissions were not highly predictable from the measured litter chemical characteristics. There were some weak

but statistically significant correlations between total net emissions and soluble cell mass, hemi-cellulose and to a lesser degree cellulose ($r = 0.57$, -0.49 and -0.44 respectively with all $P < 0.002$). The remaining litter characteristics (and combinations thereof) had no significant relationships with the total VOCs emitted from the non-sterile samples ($r < 0.05$ and $P > 0.13$ in all cases). Likewise, Mantel tests showed no significant correlation between these litter chemical characteristics, or combinations thereof, and the percentages of VOCs emitted (VOC profiles) from the biotic treatments (Mantel $r < 0.593$, $P > 0.01$ in all cases).

Discussion

In nearly all cases, biotic VOC emission rates exceeded those from the abiotic controls throughout most of the incubation period (Figure 1). However, in only seven of the 12 litter types did the total biotic VOC emissions statistically differ from abiotic emissions during the 20-d experiment. The lack of statistical difference was either due to slow increases in biotic emissions (*Pinus contorta*) or an initial increase in emissions above the control followed by a marked decrease in emissions (to below the control) over the course of the incubation (*Centaurea maculosa*, *Quercus rubra*, *Miscanthus sp.* and *Thinopyrum intermedium*). From this experimental design, we were unable to ascertain whether the decrease in VOC emissions to values below the controls is caused by a decrease in VOC production or an increase in VOC consumption (see Shennan 2006). Nevertheless, the dynamic differences in net biotic emissions over 20 days of decomposition suggest that field emissions should be measured in order to include the biotic component in regional and global VOC emission models. Using abiotic emissions alone for models (e.g., Warneke et al. (1999) not only underestimates the net VOC flux but also leads to incorrect assumptions about the specific types of VOCs emitted during litter decomposition (Figure 2 & 3A).

Litter from all of the species included here differed with respect to the quantities and types of VOCs emitted as they were being actively decomposed. This finding was similar to studies by Leff & Fierer (2008) and Isidorov & Jdanova (2002) that also found differences in VOCs emissions between different litter types. Although litter chemistry itself was not a great predictor of VOC emissions, the decomposition of litter from closely related species in the same family (e.g. Pinaceae & Salicaceae) often yielded similar VOC emission profiles (Figure 3B). The types and quantities of VOCs emitted during litter decomposition are likely to be regulated by the characteristics of the decomposer communities, the specific litter components being consumed, or some combination thereof. Many microorganisms have been found to produce different VOCs depending on the substrates catabolized. For example, indoor fungal molds will produce different VOCs depending on the nature of the substrate being consumed (Van Lancker et al. 2008). Likewise, each litter type likely harbored different microbial decomposer communities (Moorhead & Sinsabaugh 2006; Strickland et al. 2009) and this could contribute to the observed differences in VOC profiles. Lechner et al. (2005) and Bunge et al. (2008) found that different bacteria produced different VOC profiles when grown on the same media. Future work determining the specific factors regulating the types and quantities of VOCs emitted from microorganisms during litter decomposition is necessary in order to gain a more predictive understanding of VOC emissions from litter.

In all biotic cases, protonated mass 33 (methanol) was the dominant VOC emitted accounting for 78 to 99% of the emitted VOCs (Figure 2). Methanol emissions have been shown to be emitted from flowering plants (Macdonald & Fall 1993) with rates related to pectin levels within the primary cell walls of the plant tissue (Galbally & Kirstine 2002). However, since we did not measure the pectin levels of the plant litter, we do not know if the same patterns exist for

litter decomposition. A comparable study utilizing PTR-MS (Asensio et al. 2008) also found that methanol was the primary VOC emitted from the combined litter and soil in a Mediterranean shrubland. However, studies using GC-MS to examine soil or litter VOCs (Isidorov & Jdanova 2002; Leff & Fierer 2008) have not reported methanol emissions because the GC-MS analyses did not permit the detection of such low molecular weight compounds. The contributions from other compounds varied across litter types and families, with the largest average from protonated masses 59 and 43. However, as incubations continued over the 20-d experiment, the VOC profiles often changed (Figure 2), demonstrating that the types of VOCs emitted can be influenced by the progression of decomposition. Studies using longer-term incubations will allow for a greater understanding of how VOC emission profiles change throughout the decomposition process, particularly if such studies include GC-MS, proton transfer reaction time of flight mass spectrometry (PTR-TOF-MS) or other such techniques to better confirm the identity of the compounds emitted.

Although the results from this lab-based study, with litter incubated under nearly optimal temperature and moisture conditions, cannot necessarily be used to predict the specific rates of VOC emissions in the field, these results do point at the potential importance of VOC emissions from decomposing litter. In particular, this work highlights the relatively high emissions of methanol from all litter types, an important observation given that methanol is an important component in the OH and ozone global budgets and is also a significant atmospheric source of formaldehyde and carbon monoxide (Jacob et al. 2005). Recent attempts to model global methanol do not take soil emissions from biotic decomposition into consideration (Galbally & Kirstine 2002; Jacob et al. 2005). This study suggests that including methanol emissions from the biotic decomposition of plant litter might improve the accuracy of global methanol modeling

efforts. However, we note that net methanol emissions will not necessarily be positive in all cases as under certain scenarios we might expect methanol consumption to exceed methanol production rates. Also, the differences in VOC emissions from the different plant species' litter suggests that changes in vegetation type can have an important influence on microbially-derived VOC emissions when considered at local, regional, and global scales.

Chapter 2

Introduction

Biogenic, non-methane, volatile organic compounds (VOCs) are reactive, low molecular weight gases produced by the activity of plants, animals, and microorganisms in a wide range of natural systems. The importance of VOCs to atmospheric chemistry, including their influence on the formation of greenhouse gases, tropospheric ozone and secondary organic aerosols, has been well documented (reviewed in Atkinson 2000; Kansal 2009; Monson & Holland 2001). However, research on VOC emissions has historically focused primarily on plant sources (e.g. isoprene, monoterpenes) even though emissions from decomposing litter can be substantial (Asensio et al. 2008; Gray et al. 2010; Isidorov & Jdanova 2002; Leff & Fierer 2008; Ramirez et al. 2010). The majority of the VOCs released during litter decomposition appear to be derived from microbial activities, not abiotic sources (Gray et al. 2010), and many of these VOCs are reactive, with potential effects on atmospheric chemistry (e.g. methanol, acetaldehyde, acetone, and monoterpenes). We do not currently know how net VOC emissions from decomposing litter directly compare to CO_2 emission rates, but both types of emissions are relevant to terrestrial carbon (C) dynamics given that they represent gaseous losses of C from terrestrial systems. Also, as VOCs often contain more C per molecule than CO_2, VOC emissions could potentially account for a significant fraction of gaseous C loss during decomposition, yet VOCs are rarely, if ever, included in estimates of C emissions from decomposing litter.

There are likely a number of factors that independently control the emissions of microbially derived VOCs from decomposing litter including moisture, temperature, substrate

(litter type), and the types and activity of microbial decomposers. In addition, we hypothesize that changes in nitrogen (N) availability could influence the types and quantities of VOCs emitted from decomposing litter just as N availability influences litter mass loss and CO_2 emission rates (Fog 1988; Knorr et al. 2005). With anthropogenic activities increasing ecosystem N availability worldwide and N deposition rates expected to increase 2.5 fold by the year 2100 (Lamarque et al. 2005), understanding how increases in N may affect litter decomposition rates is critical for predicting ecosystem C dynamics. However, nearly all studies examining N effects on litter decomposition have focused on either litter mass loss rates or changes in CO_2 emissions; to our knowledge, it has not yet been experimentally determined how the magnitude and types of VOCs emitted from decomposing litter are affected by N additions. If N additions have important effects on litter VOC emissions, the results may not only be relevant to model predictions of biogenic VOC fluxes from terrestrial ecosystems to the atmosphere, the results could also have implications for understanding how terrestrial C dynamics are impacted by N additions if VOC emissions represent a significant portion of total gaseous C emissions from decomposing litter.

The objectives of this study were to compare the gaseous C lost as VOCs from decomposing litter to the C lost as CO_2 and to determine the effect of N additions on VOC emissions from decomposing litter. We measured CO_2 and VOC emissions concurrently from the decomposing litter of 12 plant species in order to compare the relative importance of these two C sources to net C emissions. We hypothesized that for litter types that emit relatively large amounts of VOCs during decomposition, the amount of C emitted in the form of VOCs could be comparable to that emitted as CO_2. Furthermore, we hypothesized that the N effects on VOC emissions will mirror the N effects on CO_2 emissions because VOCs, like CO_2, are largely

produced via microbial catabolism (Bunge et al. 2008; Schulz & Dickschat 2007; Wheatley et al. 1996). Alternatively, N additions could lead to opposing VOC and CO_2 responses if N additions shift the quantities and types of VOCs produced by altering the decomposer community (Campbell et al. 2010) and/or altering the metabolic pathways (such as fermentation reactions; Schulz & Dickschat 2007) used by the decomposer community.

Materials and Methods

Litter Collection

The methods employed were similar to those used in a previous study (Gray et al. 2010). Recently senesced litter was collected from 12 species of plants representing a taxonomically diverse set of species, with a broad range of litter chemistries (Table 1).

Table 1 – Characteristics of the 12 litter types used in this study (adapted from Gray et al. 2010). Definitions of the individual litter C fractions can be found in Hobbie & Gough 2004).

						Litter C fractions (%)			
Species	Family	Collection Location	N (%)	C:N	Lignin:N	Cell soluble	Hemi-cellulose	Cellulose	Lignin
Centaurea maculosa	Asteraceae	Missoula, MT	1.15	39.14	8.16	32.33	20.42	37.83	9.42
Rhododendron maximum	Ericaceae	Otto, NC	0.41	122.46	36.97	59.23	7.45	18.02	15.30
Quercus macrocarpa	Fagaceae	CU Boulder, CO	0.77	64.78	17.97	58.32	12.96	14.85	13.87
Quercus rubra	Fagaceae	CU Boulder, CO	1.27	37.09	10.17	60.02	15.63	11.45	12.90
Eucalyptus sp.	Myrtaceae	Arroyo Grande, CA	0.70	75.47	20.99	63.60	8.28	13.32	14.80
Fraxinus pennsylvanica	Oleaceae	CU Boulder, CO	1.21	36.11	7.71	66.43	11.95	12.25	9.36
Pinus contorta	Pinaceae	Niwot Ridge, CO	0.69	76.53	43.39	36.25	13.89	19.73	30.13
Pinus ponderosa	Pinaceae	Boulder Canyon, CO	0.64	86.31	29.06	48.86	13.62	18.94	18.58
Miscanthus sp.	Poaceae	Superior, CO	0.72	61.33	12.08	39.19	27.52	24.61	8.68
Thinopyrum intermedium	Poaceae	Boulder Canyon, CO	0.58	77.66	7.80	28.87	32.39	34.18	4.56
Populus deltoides	Salicaceae	CU Boulder, CO	0.46	97.57	10.32	71.50	11.49	12.23	4.78
Populus tremuloides	Salicaceae	Niwot Ridge, CO	0.52	95.75	19.82	74.58	8.06	6.99	10.37

Litter samples were oven dried at 60 °C then stored at 4 °C prior to the start of the experiment. Total litter C and N contents were determined using a CHN 4010 Elemental Combustion System (Costech Analytical Technologies, Valencia, CA) and concentrations of various C fractions were measuring using a plant fiber analyzer (Ankom Technology, Macedon, New York, USA).

Each litter type was cut into pieces of approximately 40 mm^2 in size and homogenized before being divided into eight 125 mL glass jars. Six jars without litter were used to measure background VOC concentrations in the ambient air, for a total of 102 jars (8 jars per litter type and 6 "blanks"). Control jars were brought up to 90% of water holding capacity (WHC) with deionized water (DI) and 0.8 mL of a homogenized soil slurry to reinoculate the litter while the jars with N additions were brought to 90% of WHC using DI mixed with 10 mg N • g $litter^{-1}$ (as NH_4NO_3) and 0.8 mL of the homogenized soil slurry. This N amendment concentration was chosen to simulate a N fertilization rate of 100 kg N • ha^{-1} • y^{-1} (assuming 1 kg litter • m^{-2}), a rate similar to that used in comparable studies of N effects on litter decomposition (Agren et al. 2001; Carreiro et al. 2000; Hobbie 2005). However, since the fertilizer was added as a single dose, we do not know if the N effects observed in this study would necessarily parallel those effects observed in field sites receiving chronic amendments of N. Blank jars received DI but no litter or N was added. Each 125 mL jar was placed into a 500 mL glass jar containing 10 mL of water to keep the internal humidity constant and to maintain the litter near 90% of WHC. When VOC and CO_2 emissions were not being measured, all jars were stored in the dark at room temperature (21-23 °C) and kept unsealed in order to allow for the free exchange of air.

Emission Measurements

Measurements of VOC emissions were made using a proton transfer reaction mass spectrometer (PTR-MS; Ionicon GmbH, Innsbruck, Austria) at increasing intervals (Figure 1) for 125 days following the protocol described in Gray et al. 2010).

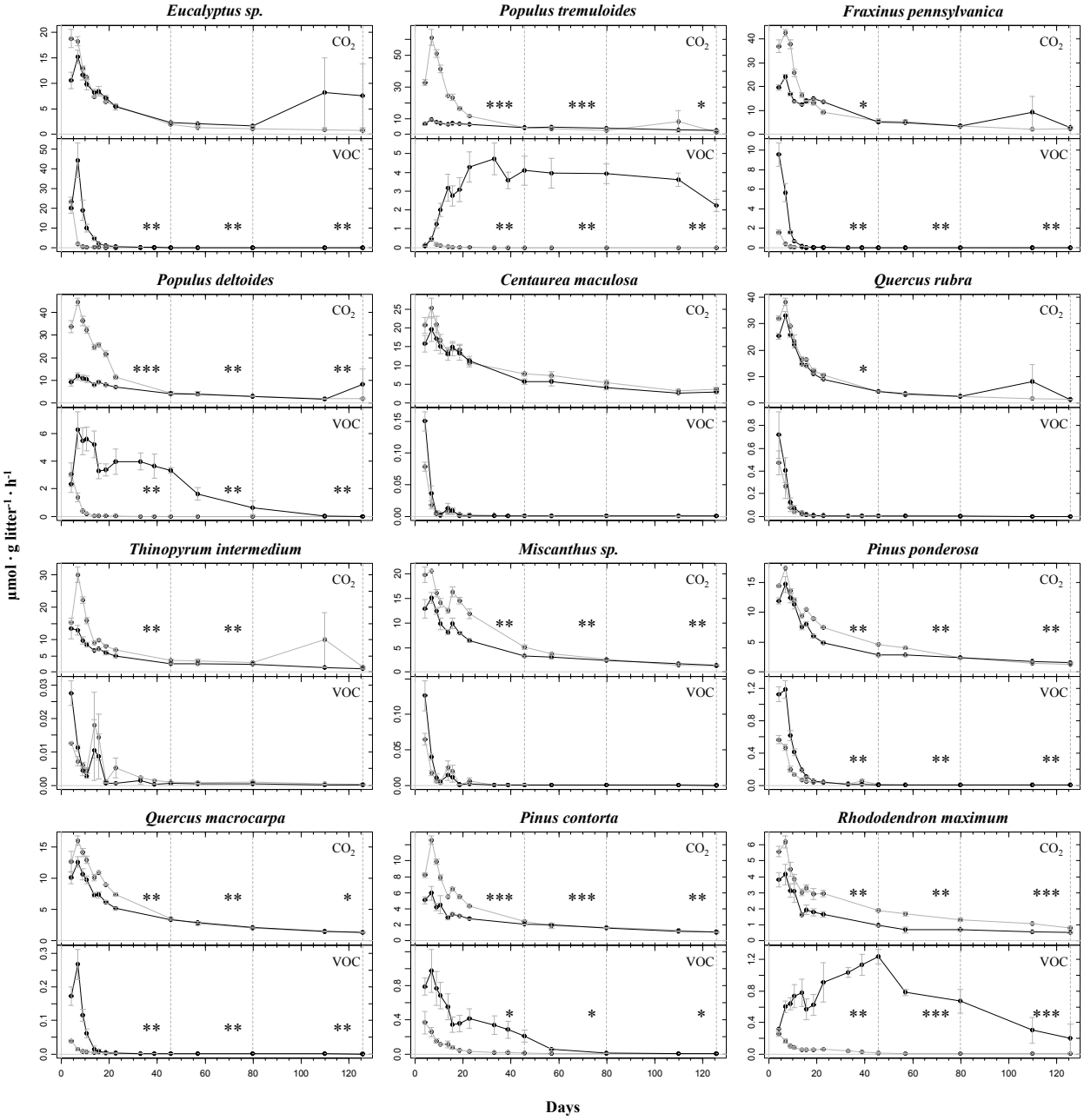

Figure 1 – Measured CO_2 and total measured VOC emissions during the 125 d incubation period. Emissions from the N amended samples are shown in gray. Significant differences ($p<0.05$= *, $p<0.01$= **, $p<0.001$= ***) between control and N amended samples were determined at three time intervals from the start of the experiment (end points indicated with vertical dashed lines). Vertical bars indicate ± 1 S.E.M.

The PTR-MS only measures the molecular mass of compounds to a resolution of 1 atomic mass unit, so the identity of individual compounds can only be considered putative (Lindinger et al. 1998). Directly following the measurement of VOC emissions, static CO_2 emission measurements were completed. For the CO_2 measurements, the jars were sealed and 3 mL of air were drawn from the headspace to measure the initial CO_2 concentration using an infrared gas analyzer (IRGA; CA-10a, Sable Systems, Inc., Las Vegas, NV, USA). The jars remained sealed for 1 to 11 hours after which CO_2 measurements were repeated (the longer times were used when CO_2 production rates decreased towards the end of the incubation period). CO_2 concentrations were never allowed to exceed 2%, to prevent CO_2 toxicity. Net CO_2 emissions were calculated by subtracting the initial CO_2 concentration from final CO_2 concentration and dividing by the length of time between CO_2 measurements (μmol CO_2 • g dry litter^{-1} • h^{-1}).

We compared CO_2 emissions to VOC emissions using the same metric, μg C • g dry litter^{-1} • h^{-1} (either total VOC-C or CO_2-C). The totaled molar emissions of CO_2 and VOCs over the 125 day experiment were converted into C emission rates using the following equation

$$E_g = E_M \bullet r \bullet M_C, \tag{1}$$

where E_g is the totaled emissions in μg C • g dry litter^{-1} • h^{-1}, E_M is the totaled molar emissions in μmol CO_2 or VOC • g dry litter^{-1} • h^{-1}, r is the molar ratio of C in each measured compound and M_C is the molar mass of C. The r values used were estimated based on compounds corresponding to the detected masses. If a mass detected by the PTR-MS had multiple possible compounds associated with it, the compound with the lowest r value was used (Table 2).

Table 2 – Molar ratios used for converting VOC molar emissions into emissions of carbon (C) for the dominant VOCs emitted (those VOCs representing >1.2% of total VOCs emitted). Compounds used represent the compounds with lowest molar ratio of C of those that are possibly emitted from the samples studied here.

Measured Protonated Masses	Compound used for molar ratio of C	Molar Ratio (*R*)
33 & 51	methanol	1
43	acetic acid	2
45	acetaldehyde	2
47	formic acid	1
59	acetone	3
69	furan	4
81 & 137	monoterpenes	10
all other measured masses	N/A	1

For example, as detected by the PTR-MS, mass 47 is most often associated with formic acid (1 C per molecule) and ethanol (2 C per molecule). In this case, an *r* value of 1 was used, as we were unable to determine if we were detecting formic acid, ethanol or some combination of the two. Therefore, the *r* values used for the VOC calculations and the resulting C emissions are assumed underestimates. Also, all measured VOC masses contributing less than an average of 1% to the total measured VOCs were assumed to have an *r* of 1.

Statistical Analyses

All analyses were run using the R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Total CO_2 and VOC emissions (for the noted periods) were calculated by summing the area between each consecutive measurement over time, and the resulting total emissions were compared between treatments using Welch's two-sample t-test (Figure 1). For both CO_2 and VOC emissions, the N response for each litter type was calculated as:

$$N_R = (R_N - R_C) / R_C, \quad (2)$$

where N_R is the N response or the percentage change of emission rates with additions of N, R_N is the emission rate of the samples with N amendments and R_C is the emission rate of the unamended control samples. An analysis of similarity (ANOSIM) was performed on the relative percentages of emitted VOCs to determine the similarity in VOC emission profiles between the N amended and unamended treatments for each litter type. Linear and logarithmic regressions were used to identify correlations between the litter characteristics and both the CO_2 and VOC emissions, respectively (Table 3).

Table 3 – Results from regressions of totaled VOC and CO_2 emissions against litter characteristics. Nitrogen response was calculated using equation 2. Significance indicated to the right of the R value (- = p < 0.10, * = p < 0.05, ** = p < 0.01, *** = p < 0.001).

Control

	CO_2			log of VOC		
	slope	R value		slope	R value	
N (%)	16046.15	0.66	**	-3.57	0.14	
C (%)	-755.72	0.27	-	0.28	0.16	
Lignin:N	-357.95	0.56	**	0.09	0.15	
Cell soluble	90.29	0.06		0.14	0.64	**
Hemi-cellulose	14.82	0		-0.3	0.74	***
Cellulose	-69.86	0.01		-0.22	0.59	**
Lignin	-359.24	0.18		0.08	0.04	

Nitrogen Response

	CO_2			log of VOC		
	slope	R value		slope	R value	
N (%)	-1	0.45	*	2.45	0.19	
C (%)	-0.01	0.01		-0.09	0.05	
Lignin:N	0	0.02		-0.04	0.1	
Cell soluble	0	0.01		-0.08	0.58	**
Hemi-cellulose	0.01	0.04		0.16	0.59	**
Cellulose	0.01	0.02		0.12	0.46	*
Lignin	-0.02	0.06		-0.01	0	

Results

CO_2 emissions

In 10 of the 12 litter types, the addition of N led to a significant increase in CO_2 emissions. CO_2 emissions ranged from 13% to 203% higher in those litters receiving N compared to the unamended controls across the first 42 days of the incubation period (Figure 1). In 7 of the 12 litter types, CO_2 emissions continued to be significantly higher with N additions through to the end of the 125 day incubation (Figure 1). *Populus tremuloides* had the highest response to added N with total CO_2 emissions doubling over the length of the experiment (Table 4).

Table 4 – VOC and CO_2 emissions (in units of µmol • g litter^{-1}) totaled over the duration of the 125 d incubation of unamended litter (U) and nitrogen amended litter (N). VOCs accounting for <1.2% of total VOCs on average are grouped under "Other".

Measured Protonated Masses		*Centaurea maculosa*	*Rhododendron maximum*	*Quercus macrocarpa*	*Quercus rubra*	*Eucalyptus sp.*	*Fraxinus pennsylvanica*	*Pinus contorta*	*Pinus ponderosa*	*Miscanthus sp.*	*Thinopyrum intermedium*	*Populus deltoides*	*Populus tremuloides*
33&51	U	7.2	1973.9	28.2	41.1	1929.9	767.4	422.6	146.6	6.8	1.7	5500.5	10337.3
	N	4.6	62.0	3.1	28.4	160.3	80.4	23.8	48.6	3.8	2.2	205.6	44.2
43	U	0.3	3.4	0.5	3.5	50.3	1.8	5.2	1.0	0.4	0.2	20.7	11.7
	N	0.3	1.0	0.2	1.5	13.9	0.6	3.2	1.3	0.5	0.2	1.2	1.5
45	U	0.0	0.7	0.0	1.0	0.9	0.4	0.6	0.3	0.2	0.1	9.0	2.6
	N	0.0	0.2	0.0	0.9	0.2	0.1	0.4	0.0	0.4	0.4	1.5	0.2
47	U	0.7	1.7	0.1	1.1	1.6	0.5	0.7	0.3	1.0	0.6	3.1	2.7
	N	0.2	0.1	0.3	1.4	0.5	0.5	1.0	0.1	2.0	1.3	4.8	0.9
59	U	0.1	13.7	2.9	3.6	3091.0	5.5	13.7	3.7	0.1	0.5	26.0	41.2
	N	0.1	2.2	0.6	2.8	709.4	0.5	5.0	2.8	0.1	0.2	1.0	2.0
69	U	0.2	0.6	0.2	0.3	4.9	1.0	6.4	7.3	0.1	0.3	2.6	7.6
	N	0.0	0.4	0.1	0.6	3.3	0.3	5.2	5.9	0.1	0.3	0.5	2.3
81&137	U	0.2	0.6	0.2	0.1	134.8	0.1	36.5	30.3	0.1	0.1	3.1	1.0
	N	0.3	0.6	0.1	0.5	21.4	0.1	26.2	31.0	0.1	0.3	0.8	0.2
Other	U	0.9	4.2	1.4	12.9	93.6	4.5	11.2	7.0	1.2	0.7	52.3	20.3
	N	0.9	1.9	1.1	6.8	30.5	1.6	8.3	9.9	1.2	1.5	3.9	2.2
VOC Total	U	9.5 ±0.5	1998.9 ±127.5	33.6 ±4.9	63.5 ±15.2	5307.0 ±735.4	781.3 ±93.3	497.0 ±123.5	196.4 ±13.6	9.9 ±1.5	4.2 ±0.7	5617.3 ±721.9	10424.4 ±1412.7
	N	6.7 ±1.5	68.4 ±9.5	5.5 ±0.8	42.8 ±13.1	939.4 ±77.4	84.1 ±10.7	73.2 ±11.2	99.6 ±8	8.2 ±1.6	6.3 ±1.3	219.2 ±46.4	53.6 ±7.2
CO_2 Total	U	19113 ±2407	3128 ±253	10228 ±329	20585 ±3601	14447 ±3557	22815 ±3170	6072 ±277	10644 ±406	11536 ±464	9737 ±585	14065 ±531	13121 ±682
	N	22537 ±2083	5593 ±294	12050 ±485	18421 ±711	8976 ±459	21397 ±1336	8275 ±239	13370 ±94	16471 ±701	18907 ±5192	22742 ±1312	26935 ±4213

CO_2 emissions totaled over the duration of the experiment from the unamended litters correlated negatively with lignin:N and positively with litter N concentrations ($p<0.01$ in both cases, Table 3). The magnitude of the CO_2 response to N additions significantly decreased ($p<0.05$) with

increasing litter N concentrations, i.e. CO_2 emissions from those litters with higher initial N concentrations increased less after N amendments than CO_2 emissions from those litters with lower initial N concentrations (Table 3).

VOC emissions

In contrast to the observed increases in CO_2 emissions with N additions, total measured VOC emissions significantly decreased in response to the N additions in 8 of the 12 litter types (Figure 1). The magnitude of this decrease in totaled emissions ranged from over 99% in *Populus tremuloides* to 49% in *Pinus ponderosa* litter (Table 4). The four litter types that were not significantly affected by additions of N; *Quercus rubra, Centaurea maculosa*, and both of the grass species (*Thinopyrum intermedium* and *Miscanthus sp.*), were among those litter types with the lowest VOC emission rates. Unlike the CO_2 responses, total VOC emissions from the unamended litters exponentially increased as the litter became more labile (higher cell soluble content and lower hemi-cellulose and cellulose content) ($p<0.01$ in all cases, Table 3). The totaled VOC emission response to N decreased with increasing percentages of labile cell soluble compounds in the litter ($p<0.01$) and decreasing percentages of the less labile hemi-cellulose and cellulose ($p<0.01$ and $p<0.05$ respectively) suggesting that N additions led to a larger depression of VOC emissions as the lability of litter increased.

Methanol was the largest contributor to the measured VOC emissions, contributing an average of 72% and 55% of total VOCs emitted (on a molar basis) from the unamended and N amended samples, respectively (Figure 2).

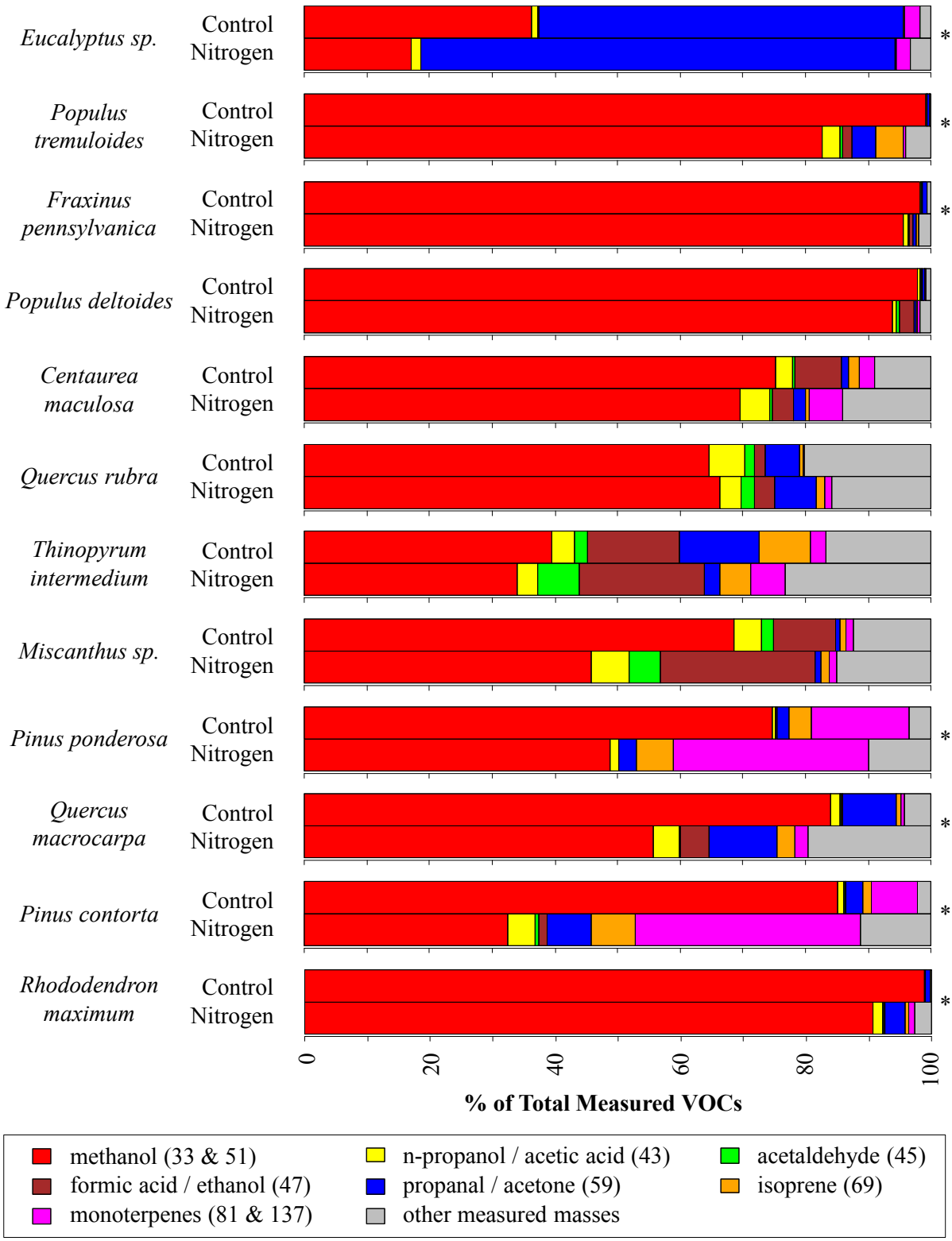

Figure 2 – The relative proportions of measured VOCs emitted during the 125 d incubation. Asterisks (*) indicates a statistical difference ($p \leq 0.05$) between control and after nitrogen additions. Legend shows the putative identity of the compound(s) with the measured protonated mass(es) in parentheses.

The identity of other major VOCs emitted depended on the litter type. Mass 59 (likely a combination of propanal and acetone) was a large proportion of emissions from *Eucalyptus sp.* (67%) while only contributing to an average of 4% of the emissions from other litter types. Across the grass species and *Pinus sp.*, mass 47 and monoterpenes (mass 81 + 137) contributed the second largest proportion to the total VOC emissions respectively. N additions caused a significant change in the relative amounts of VOCs emitted in 7 of the 12 litter types sampled (Figure 2). The decrease in total VOC emissions in response to N additions was mainly related to a corresponding decrease in methanol emissions. For example, methanol emissions from *Pinus contorta* decreased by 94% with N additions and similar decreases in the relative emissions of methanol were observed for other litter types (Table 4).

Total C emissions

From the unamended litters, the amount of C emitted as VOCs was not correlated with the amount of C emitted as CO_2 (Figure 3).

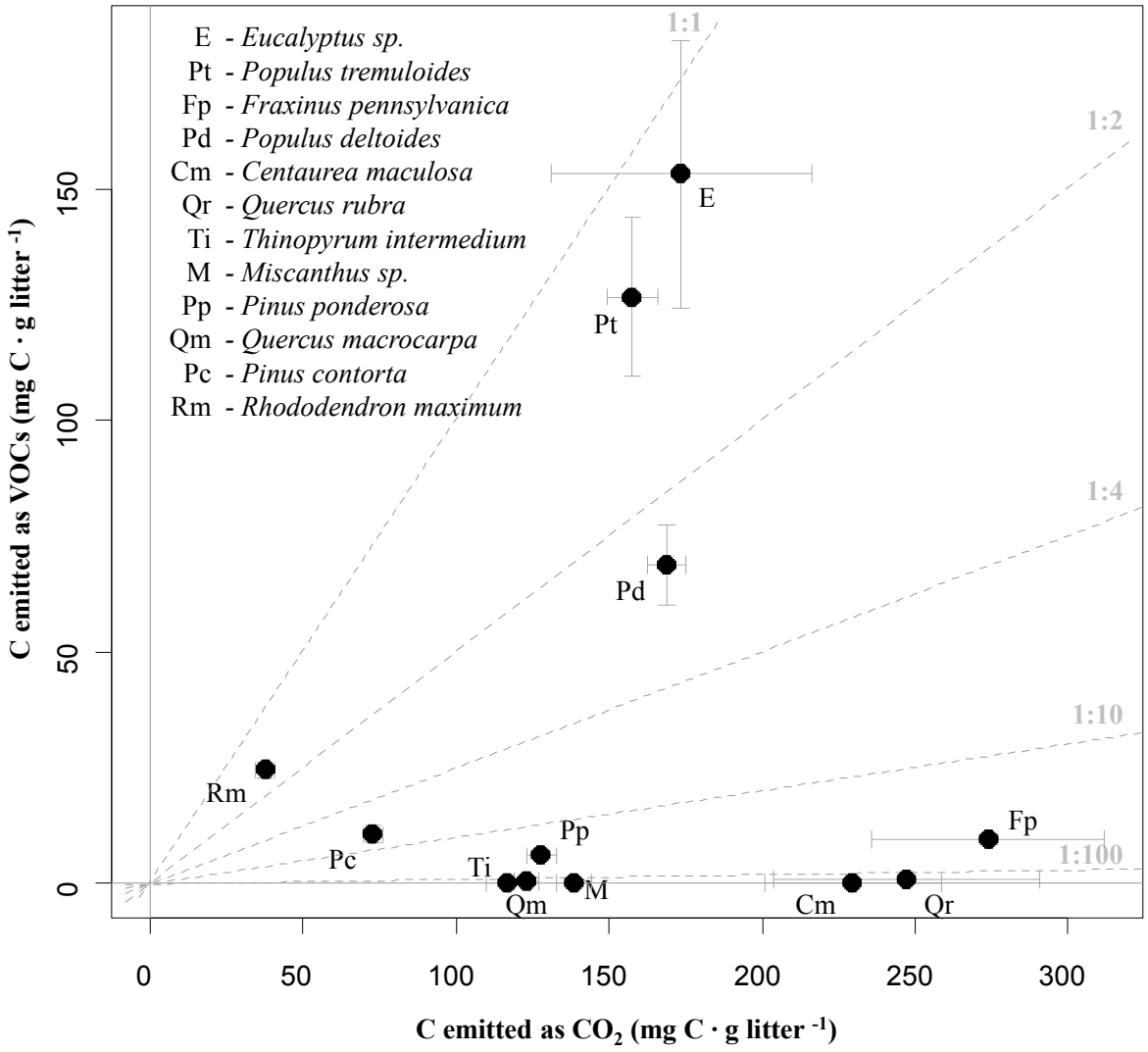

Figure 3 – Total C emitted as CO_2 plotted against total measured C emitted as VOCs during the 125 d incubation. There was no statistical relationship between the amount of C emitted as CO_2 and the total C emitted as VOCs ($p>0.75$). One S.E.M. is indicated by the vertical and horizontal bars for both C emitted as CO_2 and as VOCs.

C emitted over the 125 day incubation as VOCs ranged from 0.075 mg C • g litter^{-1} to 194 mg C • g litter^{-1} with a mean of 33 mg C • g litter^{-1} and a median of 6 mg C • g litter^{-1}. C emitted as CO_2 ranged from 31 to 388 mg C • g litter^{-1} with a mean of 156 mg C • g litter^{-1} and a median of 137 mg C • g litter^{-1}. VOC emissions represented a large portion of overall C emissions in several litter types. *Eucalyptus sp.* and *Populus tremuloides* emitted the largest amounts of C in the form of VOCs with means of 127 and 153 mg C • g litter^{-1}, which represented 88% and 80%

respectively, of the C emitted as CO_2 from these two litter types. Figure 3 shows that other litter types, besides *Eucalyptus sp.* and *Populus tremuloides*, also emitted amounts of C as VOCs that were within an order of magnitude of the amounts emitted as CO_2. *Centaurea maculosa* and *Thinopyrum intermedium* emitted the least amount of C as VOCs relative to CO_2 (0.07% and 0.08% respectively). For some of litter types, the effects of N amendments on gaseous C emissions were significant when only CO_2 emissions were considered, but not significant when C emissions as VOCs were included (Figure 4).

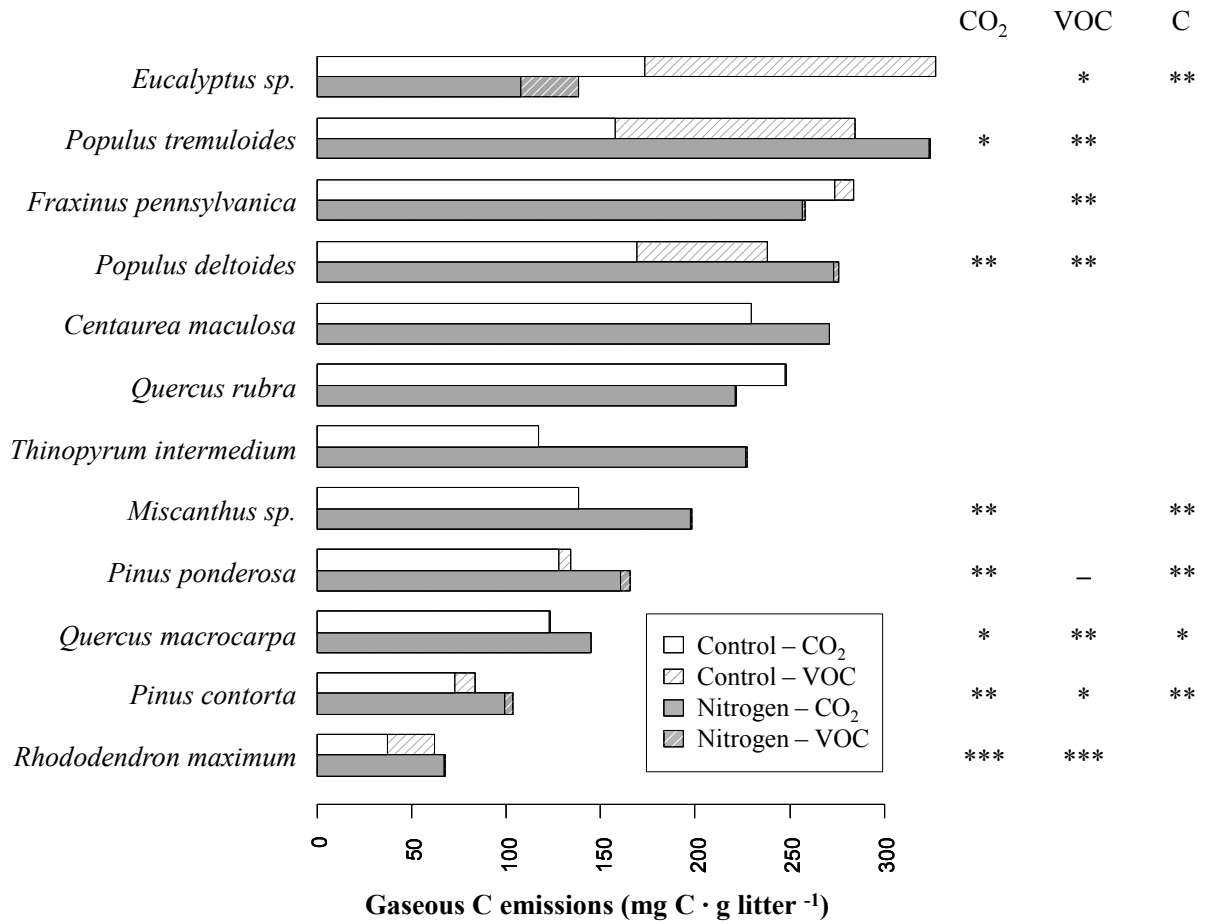

Figure 4 – Total measured C emitted during the 125 d incubation without (white) and with addition of N (gray). Total C split into emissions as CO_2 (solid) and as VOCs (hatched). Statistical significance of N amendments on the three measurements of emitted C are indicated with the symbols ($p<0.1$= – , $p<0.05$= * , $p<0.01$ = ** , $p<0.001$= ***).

In other words, since CO_2 emissions often increased with N amendments, but VOC emissions typically decreased, the net effect of N on total gaseous C emissions was negligible for some of the litter types once both VOC and CO_2 emissions were summed.

Discussion

C emissions

We found that, for certain litter types, the amount of C lost from decomposing litter in the form of VOCs was comparable to the amount lost as CO_2. Estimated C emissions from VOCs ranged from 0% (*Centaurea maculosa*) to 88% (*Eucalyptus sp.*) of that emitted as CO_2 (Figure 3). In general, litter from non-woody species emitted a lower percentage of C as VOCs than woody species, but additional research is needed to confirm this pattern. However, our finding that C emissions from VOCs and CO_2 can be within the same order of magnitude accentuates the importance of including VOC emissions when examining C losses from decomposing litter, as solely measuring CO_2 production could lead to a significant underestimation of gaseous C losses from decomposing litter.

Across all litter types, the amounts of C emitted as VOCs did not correlate with the amounts emitted as CO_2, suggesting that the controls on microbial VOC and CO_2 emissions are distinct. CO_2 emissions were negatively correlated with the lignin:N ratio of the litter, which agrees with many (but not all) previously-observed patterns of leaf litter decomposition (Hobbie 2008; Knorr et al. 2005; Melillo et al. 1982; Taylor et al. 1989). In contrast, total VOC emission rates were not correlated with the lignin:N ratio but were correlated with individual litter C fractions, which were poor predictors of CO_2 emissions (Table 3). The fact that the controls over VOC and CO_2 emissions are distinct suggests that predicting VOC emissions from litter in terrestrial systems is not simply a matter of modeling VOC emissions as a fixed proportion of

CO_2 emissions or by applying standard chemical indices (like lignin:N ratios) that are commonly used to infer litter decomposability.

VOC emissions after N amendments

As expected in the initial stages of decomposition, CO_2 emissions generally increased after N additions (Craine et al. 2007; Knorr et al. 2005). However, with most litter types we observed a significant decrease in VOC emissions to near zero by day 46 of the experiment with added N (Figure 1). The strong effect of N additions on VOC emissions suggests that VOC production is primarily a biological process, as we know of no mechanism by which the added N would abiotically inhibit VOC production. This is supported by our previous work (Gray et al. 2010), which also demonstrated that microbial activities are responsible for the majority of VOC emissions. However, the biotic mechanisms responsible for the decrease in net VOC emissions with additions of N are unknown. As we only measured net emissions, we were unable to determine whether gross VOC production decreased or gross consumption increased. Increased N availability might favor increased consumption of VOCs (Dalmonech et al. 2010) with VOCs catabolized to CO_2 by methylotrophic taxa (for example). Alternatively, additional N could reduce VOC production by either altering the physiologies of the microbial decomposers or altering the types of taxa present. Bunge et al. (2008) found that distinct microbial taxa emit different types and amounts of VOCs, thus, it is possible that the commonly observed impacts of N additions on microbial community composition (Campbell et al. 2010; Feng et al. 2010; Jangid et al. 2008), could, in part, account for changes in VOC emission rates. Not only were the total amounts of VOCs emitted affected by the N amendments used in this study, but the relative contribution of different VOCs to the totaled VOCs emissions (VOC profile) was also affected (Figure 2). A shift in the microbial community composition or microbial physiologies brought on

by increased N availability (Dalmonech et al. 2010; Papanikolaou et al. 2010; Treseder 2008; van Diepen et al. 2010) could have altered the production and/or consumption of certain VOCs over others. Although additional research is required to determine the mechanisms involved, our results clearly indicate that high levels of N fertilization inhibit VOC emissions and alter the relative contribution of individual VOCs.

The decrease in C from VOC emissions after N amendments was enough to account for the increase in C emissions from CO_2 in 3 of the 7 litter types that had a positive CO_2 response to additional N (Figure 4). Thus, changes in CO2 emissions with added N are not necessarily equivalent to changes in litter decomposition rates (total gaseous C emissions from litter), as VOC emissions typically decreased with N amendments leading to no significant effect (or less of an effect) of N on total gaseous C losses from decomposing litter.

Conclusions

VOC emissions from decomposing litter could be decreasing globally as terrestrial ecosystems are receiving elevated inputs of N from anthropogenic activities. These changes in VOC emissions could affect terrestrial C dynamics, and perhaps atmospheric chemistry, given that litter decomposition is likely to represent an important source of certain VOCs to the atmosphere. However, additional research is required to determine how litter VOC emissions directly compare to emissions from other known sources of biogenic VOCs (e.g. plants). Likewise, additional research is needed to determine why N amendments have such strong effects on litter VOC fluxes and whether these effects are related to shifts in gross VOC production or consumption and microbial community changes. Our finding that the amount of C lost as VOCs from decomposing litter can potentially be in the same magnitude as the amount of C lost as CO_2, highlights that research into the C dynamics of decomposing litter should include

both CO_2 emissions as well as VOC emissions. Including only CO_2 emissions will likely underestimate gaseous losses from litter, overestimate the effects of N on litter decomposition rates and, perhaps, lead to an overestimation of C inputs in terrestrial systems from decomposing litter.

Chapter 3

Introduction

Non-methane biogenic volatile organic compounds (BVOCs) are low molecular weight carbon (C) compounds that are produced primarily by plants and microbes in terrestrial systems. These compounds can have wide-ranging impacts on atmospheric chemistry, terrestrial nutrient cycles, and soil ecology (Atkinson & Arey 2003; Insam & Seewald 2010). In the atmosphere, the oxidation of BVOCs results in the formation of tropospheric ozone and the formation of secondary organic aerosol particles, which lead to increased cloud albedo and altered precipitation dynamics (Atkinson 2000; Kesselmeier & Staudt 1999). Within terrestrial systems, BVOCs can alter the rates of specific microbial processes associated with the C and nitrogen (N) cycles. For example, monoterpenes, a well-studied class of BVOCs, inhibit the oxidation of methane in soils (Amaral & Knowles 1998; Maurer et al. 2008), and inhibit several N cycling processes, including nitrification and N mineralization (Paavolainen et al. 1998; Smolander et al. 2006; Uusitalo et al. 2008; White 1994). In soils, various BVOCs have been shown to alter the growth and activity of plants (Farag et al. 2006), fungi (Bruce et al. 2004), nematodes (Gu et al. 2007), and bacteria (Wheatley 2002). Several interspecies interactions within the soil also appear to be mediated by BVOCs, including the formation of nodules in legumes (Horiuchi et al. 2005) and the antagonistic interactions between bacteria and fungi (Bruce et al. 2004; Mackie & Wheatley 1999).

BVOCs clearly have the potential to alter the structure and functioning of terrestrial systems in a myriad of ways (Insam & Seewald 2010), but research into BVOC fluxes has

historically concentrated on plant foliar emissions, with soil sources and sinks of BVOCs largely ignored. For example, a widely-used model to calculate BVOC flux rates from a system (MEGAN: Model of Emissions of Gases and Aerosols from Nature) primarily considers the dynamics of foliar emissions and uses a single variable to account for any uptake by the canopy or soil (Guenther et al. 2012). However, several studies comparing canopy-level fluxes to forest floor fluxes suggest that the forest floor (ground vegetation and soil) can be an important source and sink of certain BVOCs to the atmosphere (Aaltonen et al. 2011; Cleveland & Yavitt 1997; Hellen et al. 2006), yet the rates and controls on soil BVOC fluxes remain poorly characterized. The work that has been done suggests that BVOC fluxes can vary considerably across soil and litter types. For example, previous work on BVOC emissions from decomposing litter has shown that the types and quantities BVOCs will vary depending on the plant litter type in question with most of these BVOCs produced by microbial processes (Gray et al. 2010). Under laboratory conditions these BVOC fluxes can reach as high as 63 μmol g-litter^{-1} h^{-1} and the amount of carbon (C) emitted as BVOCs can be equivalent to the amount of C emitted from decomposing litter as CO_2 (Gray & Fierer 2012). There is also evidence that biotic processes within mineral soil can lead to the net consumption of specific BVOCs (Asensio et al. 2007; Ramirez et al. 2010; Scheutz et al. 2004) and that the presence of active roots in soil can increase uptake of certain BVOCs and increase net emission of others (Asensio et al. 2007; Back et al. 2010; Chen et al. 2004; Steeghs et al. 2004). However, only a few studies have examined BVOC fluxes in the field and the biotic or abiotic controls on these fluxes (Asensio et al. 2007; Asensio et al. 2008; Greenberg et al. 2012). In particular, consumption (i.e. uptake) of BVOCs into soil is poorly studied as most previous studies have used air free of BVOCs, rather than ambient air, to flush soil chambers before quantification of flux rates. This method cannot capture consumption

rates and alters the natural concentration gradients between soil and the sampled air, artificially increasing diffusion into the sampled air and thus leading to overestimation of net emission rates. Also, much of the previous work on soil or litter emissions of BVOCs have used analytical techniques that do not measure methanol, one of the dominant BVOCs emitted from soils and decomposing litter (Asensio et al. 2008; Gray & Fierer 2012; Greenberg et al. 2012).

To address some of these gaps in our current understanding of BVOC fluxes from soils and the controls on these fluxes, we designed a study to answer the following questions. (1) What are the types and amounts of BVOCs emitted or consumed (soil uptake) from undisturbed soils *in situ* from a subalpine forest floor during the growing season? (2) How much does the presence of active roots and root rhizodeposition contribute to BVOC fluxes from soil? (3) How do temperature and soil moisture relate to the temporal variability in soil BVOC flux rates? To answer these questions we utilized a high sensitivity proton transfer reaction mass spectrometer (PTR-MS) to measure BVOC fluxes in soil chambers receiving ambient air from intact plots and from plots on which trees had been girdled, removing the potential for shoot-to-root rhizodeposition, in a subalpine forest in Colorado, USA.

Methods

Site description

Our study site was located near the Niwot Ridge AmeriFlux tower in northern Colorado, USA (40°1'58" N, 105°32'47" W; elevation 3050m). This subalpine system is dominated by *Abies lasiocarpa* (subalpine fir), *Picea engelmannii* (Engelmann spruce), and *Pinus contorta* (lodgepole pine) with interspersed groves of *Populus tremuloides* (quaking aspen). The understory is sparse, containing tree seedlings and patches of *Vaccinium myrtillus* (whortleberry). Soils are sandy and derived from granitic moraine with a distinct, thin (<6 cm)

organic horizon. Additional site details can be found in Scott-Denton et al. (2006) and Monson et al. (2010).

The trees in three plots (~50m^2) dominated by *P. contorta* were girdled and the soil around the plots was trenched in the spring of 2009, three months before we began soil BVOC measurements. The timing of the girdling and trenching was chosen to reduce the unintended effects of the disturbances on soils within the plots (Scott-Denton et al. 2006; Weintraub et al. 2007). Trees were girdled by scraping away a 15-20 cm swath of bark and phloem at breast height. Thus, only the outer layer of xylem (wood) was exposed in the girdled area. The girdling of trees severs the phloem connection between shoots and roots and effectively blocks photosynthate from reaching the roots or rhizosphere. Girdling, combined with trenching to 20 cm depth around the perimeter of the plots to remove invading shallow roots, removed active roots and shoot-to-root rhizodeposition (both herein referred to as an 'active root system'), and has been shown previously to effectively eliminate approximately 50% of the soil respiration rate in non-girdled plots (Scott-Denton et al. 2006; Weintraub et al. 2007). Three similar plots were selected as control plots where neither girdling nor trenching was implemented. The number of trees in each plot ranged from 3 to 7. Throughout the experiment, above ground cover was clipped to ground level at weekly intervals in the girdled and trenched plots (herein referred to as the 'girdled plots') and control plots to exclude BVOC emissions that might originate from understory vegetation. Chambers were placed near the center of the plots in undisturbed areas and located so they did not cover the clipped, but sparse herbaceous ground cover.

Environmental data were taken from sensors at the Niwot Ridge AmeriFlux tower site, which was located within 300 m of the study plots. Measurements used in this study included air temperature at 2 m (Vaisala HMP-35D), barometric pressure at 12m (Vaisala PTB-101B),

volumetric soil moisture 3 cm below the surface (Campbell Scientific Instruments CS615) and a precipitation gauge (Met One Model 385). Data are recorded from these instruments every 30 minutes and made publicly available as part of the AmeriFlux Network (http://public.ornl.gov/ameriflux/index.html). Values for soil moisture are not meant to represent the actual moisture at our plots, but rather represent the relative changes throughout the experiment in response to precipitation events. Figure 1 provides the precipitation, soil moisture and air temperature data during the experiment and information on when BVOC flux rates were measured from the plots.

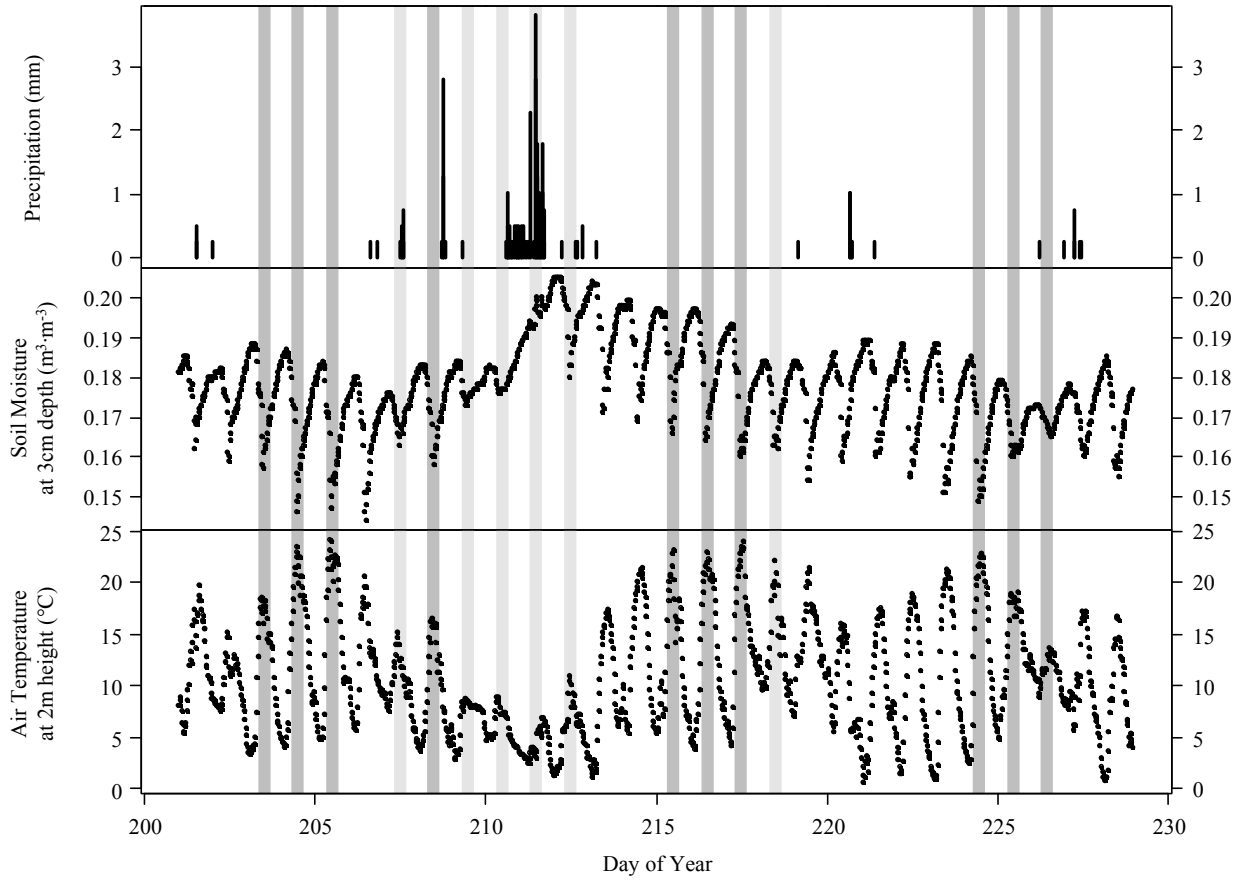

Figure 1 – Recorded precipitation, soil moisture (at 3cm) and air temperature (at 2m) taken from the Niwot Ridge Ameriflux site, Colorado. Dark gray bars indicate measurement time periods without a visibly wet litter layer or rain and light gray bars indicate measurement time periods with visibly wet soil or rainfall.

BVOC flux measurements

A stainless steel collar with an area of 0.132 m^2 was placed in each of 3 girdled and 3 control plots one month before BVOC measurements began (Figure 2).

Figure 2 – (Top) Trenched experimental plot with girdled trees and stainless steel collar placed into the soil. (Bottom) Soil chamber during the measurement of BVOCs with an ambient line to quantify ambient BVOCs and a chamber line to quantify soil BVOCs.

Each collar was inserted 2 - 5 cm into the soil with the exact depth dependent on the presence or absence of rocks beneath the surface and an approximate 10 L headspace volume. Two equal lengths of Dekoron tubing (3/8" O.D. Type 1300; effects determined minimal relative to chamber emissions) were positioned between each plot and the centrally-located proton transfer reaction mass spectrometer (PTR-MS). One Dekoron line was connected to a stainless steel lid that was placed on top of the collars while sampling, and the other was placed at the inlet of the stainless steel lid to capture BVOC concentrations in ambient air (Figure 2). We sampled on 16 days within a four week period during the 2009 growing season. Days not included were either due to limited access to the site or when methodological issues made it impossible to take measurements. On each day of sampling (Figure 1), one plot from each of the control and girdled replicate plots was selected at random for sampling. Chamber lids were placed on top of the collars and ambient air was pulled through chambers and lines for 1 hour prior to and during sampling with a diaphragm pump at ~400 mL min^{-1} with ~100 mL min^{-1} of the flow diverted to the PTR-MS for analysis. Temperature and humidity within the chambers were not measured but are assumed to have changed little as all chambers were shaded by the canopy. Description and operation of the PTR-MS has been previously described in detail (Lindinger et al. 1998). The specific PTR-MS techniques and settings used for this study follow those described previously (Gray & Fierer 2012; Gray et al. 2010). Since the PTR-MS only characterizes compounds, or fractions of compounds, by their molecular weight, the identities of the BVOCs measured are considered putative. On each day of sampling, selected BVOC masses (Table 1) were measured 4 times: once every 50 minutes over a 3.5 hour period.

Table 1. BVOC flux rates from an alpine soil[a]

Protonated Mass (m/z)	putative ID	conservative molar ratio	BVOC flux (nmol $m^{-2}h^{-1}$)		C flux (ng-C $m^{-2}h^{-1}$)	
			control	girdled	control	girdled
Protonated masses with highest average emission rates						
33+51*	methanol	1	53.35 ± 31.84	11.22 ± 11.73	640.8 ± 382.4	134.7 ± 140.8
43	propanol/ acetic acid	2	5.12 ± 4.32	3.06 ± 3.16	123.1 ± 103.7	73.4 ± 76.0
45*	ethanal/ acetaldehyde	2	9.12 ± 5.55	1.92 ± 2.77	219.1 ± 133.3	46.2 ± 66.5
47*	formic acid/ ethanol	1	6.92 ± 6.38	-1.07 ± 2.59	83.1 ± 76.6	-12.9 ± 31.2
59	propanal/ acetone	3	6.03 ± 7.14	-0.09 ± 2.84	217.4 ± 257.3	-3.1 ± 102.2
61	acetic acid	2	4.35 ± 6.04	0.72 ± 4.99	104.5 ± 145.1	17.2 ± 119.9
73	methyl ethyl ketone	4	3.64 ± 8.60	4.60 ± 13.82	174.9 ± 413.1	221.2 ± 664.1
81+137	monoterpene	10	5.38 ± 4.22	5.34 ± 3.25	646.5 ± 507.4	640.8 ± 389.8
Protonated masses with highest average uptake rates						
31	formaldehyde	1	-3.09 ± 1.01	-3.39 ± 1.11	-37.1 ± 12.1	-40.7 ± 13.3
69	isoprene/ furan	4	-2.03 ± 1.32	-1.86 ± 0.76	-97.7 ± 63.3	-89.2 ± 36.5
75	methyl acetate/ propionic acid	3	-2.46 ± 2.75	-2.09 ± 1.57	-88.5 ± 99.0	-75.2 ± 56.7
91		1	-1.12 ± 0.40	-0.87 ± 0.41	-13.4 ± 4.8	-10.5 ± 4.9
Sum flux rate of all other measured masses						
All others[b]		1	6.21 ± 13.88	4.41 ± 8.34	74.6 ± 166.7	53.0 ± 100.2

[a] The 9 protonated masses with highest average emissions (soil emissions), the 4 masses that exhibited the highest rates of soil uptake, and the sum of other measured masses along with putative compound identifications, a conservative carbon molar ratio for the given protonated mass(es), average fluxes (molar BVOC flux and grams of carbon flux) and standard error from control and girdled/trenched plots. "*" indicate significant differences between treatments after Bonferroni correction ($\alpha < 0.001$).

[b] Other protonated masses measured included: 41, 42, 57, 63*, 65*, 71, 79, 83, 85, 87, 89, 93, 95, 97, 99, 101, 103, 107, 109*, 111, 113, 115, 117, 121, 123, 125, 127, 129, 133, 135, 139, 141, 143, 145.

Each line measurement contained the average of three PTR-MS cycles taken over a span of 3 minutes. All measurements were taken between 10:30 and 15:30 local time to capture mid-day fluxes. Masses 49, 53, 67, 77, 105, 119, 131, 147 and 149 were measured but excluded from all calculations as they were determined to be indistinguishable from the background levels of the PTR-MS system.

Data from the PTR-MS (in ppbv) were converted to soil BVOC flux rates using the following equation:

$$F_{BVOC} = ((C_{Ch} - C_{Am}) \bullet Q \bullet P) / (R \bullet A \bullet T)$$

where F_{BVOC} is the flux rate in nmol m^{-2} h^{-1}, C_{Ch} is the measured chamber BVOC concentration converted to mole fraction (nmol mol^{-1}), C_{Am} is the measured ambient BVOC concentration in mole fraction (nmol mol^{-1}), Q is the flow rate through the chamber in L h^{-1}, P is the barometric pressure in kPa, R is the gas law constant of 8.3145 L kPa mol^{-1} K^{-1}, A is the footprint area of the soil chambers in m^2, and T is ambient air temperature in K. Because many BVOCs vary in their molar C concentrations and to facilitate comparisons to fluxes measured in other studies, molar BVOC fluxes were also converted to an estimated C mass flux using the equation:

$$F_C = F_{BVOC} \bullet r \bullet G_C$$

where F_C is the C flux rate in ng-C m^{-2} h^{-1}, F_{BVOC} is the flux rate as nmol m^{-2} h^{-1}, r is the conservatively estimated molar ratio of C to the measured protonated mass (Table 1), and G_C is the molar mass of C in ng-C $nmol^{-1}$.

Data analysis

All analyses were run using R statistical software (R Foundation for Statistical Computing, Vienna, Austria). Two sampling days from the girdled plots (days 205 and 217) were removed from all analyses due to debris that had infiltrated the Dekoron lines. Only data from sampling days without visibly wet litter were used to compare flux rates of individual protonated masses, summed BVOC flux rates (flux rates for each individual protonated mass summed for each measurement point) and the effects of active roots on BVOC emissions (Figure 2). We excluded days with wet litter from these analyses to get a baseline estimate of BVOC

fluxes under conditions that are more typical for this site. For each individual protonated mass measured, Welch's T-Tests were used to compare flux rates from the control plots to the rates from the girdled plots. Due to the large number of individual tests (48 masses and the summed rate), a Bonferroni adjusted α of 0.001 was used for the determination of significance.

To measure the effects of temperature and soil moisture on BVOC emissions, all sampling days, including those with visibly wet and dry litter, were included in the analyses to maximize the range in temperature and soil moisture conditions across which BVOC emissions were measured. Using multiple linear regressions, air temperature and soil moisture were fit to individual protonated mass flux rates as well as the summed BVOC flux rates.

Results and Discussion

General characteristics of BVOC fluxes

From the control plots, individual BVOC fluxes summed at each measurement point averaged 2.0 µg-C m^{-2} h^{-1} and ranged from -1.8 to 7.2 µg-C m^{-2} h^{-}1 with Figure 3 showing the mean flux of each protonated mass along with the sum of all positive and negative fluxes (net emission and soil uptake, respectively) from control and girdled plots.

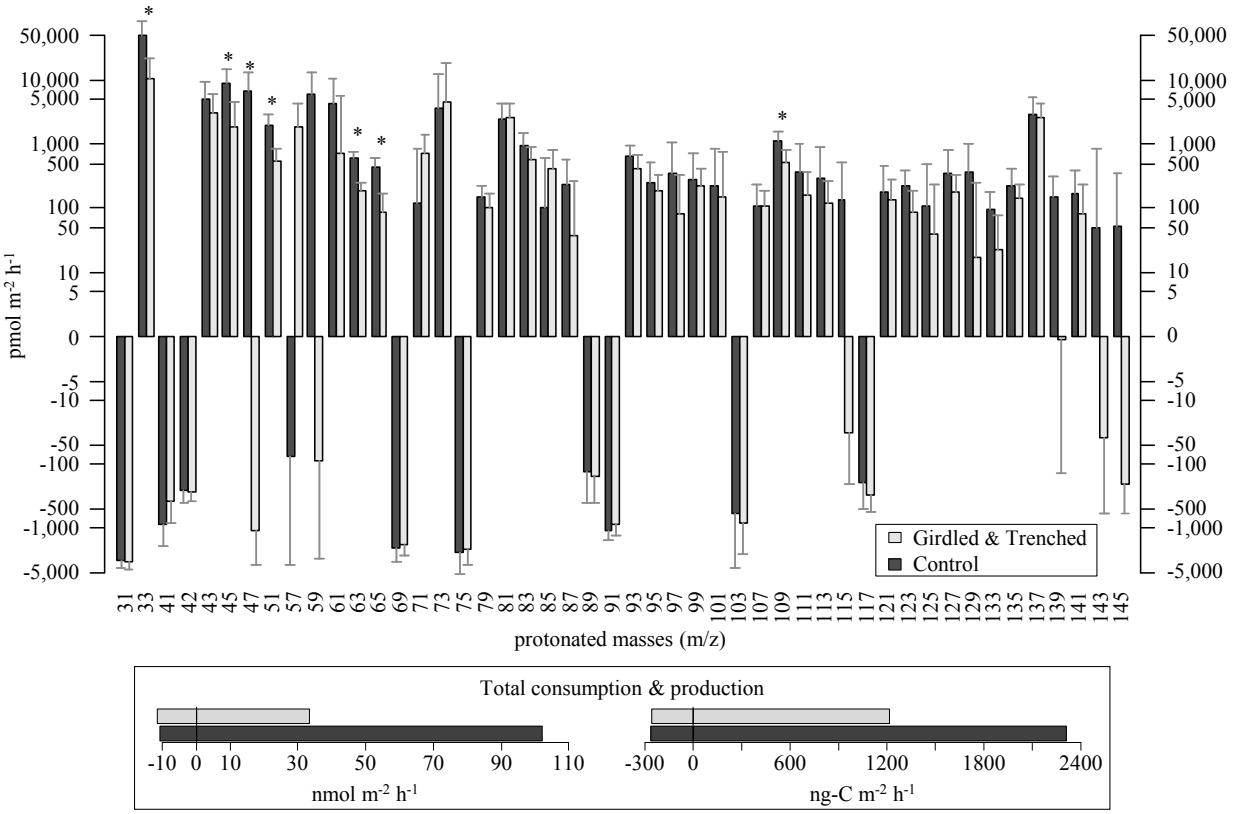

Figure 3 – Soil fluxes of measured BVOCs from a field site primarily consisting of lodgepole pines (*Pinus contorta*) at Niwot Ridge, Colorado. Fluxes of protonated masses are from undisturbed control plots (dark) and from recently trenched plots with surrounding trees girdled (light). One S.E.M. is indicated by vertical error bars. Significance after Bonferroni correction (α^*=0.001) for multiple T-tests is indicated with "*". Inset figure gives the summed fluxes for all measured masses that were produced on average (right of 0) and for all those that were consumed on average (left of 0). Molar fluxes and C fluxes from control and girdled and trenched plots are given.

The summed BVOC fluxes from this study were comparable to C fluxes from BVOCs measured from a boreal forest floor where BVOC fluxes varied between 0.6 and 9.8 μg-C m^{-2} h^{-1} (Aaltonen et al. 2011). Our measured BVOC emissions are lower than what would be estimated from the results of a laboratory study (~6 mg-C m^{-2} h^{-1}) that measured emissions during the decomposition of fresh *P. contorta* litter (Gray & Fierer 2012). The discrepancies between this study and the laboratory study could be due to BVOC uptake within the mineral soil (Ramirez et al. 2010) that should decrease net rates measured in the field or the discrepancies could be related to the laboratory study having incubated fresh litter under nearly optimal moisture and

temperature conditions, thus maximizing net BVOC fluxes. In addition, we found that the estimated net C emissions as BVOCs (i.e. the summed BVOC C flux) was 5 orders of magnitude lower than C fluxes in the form of CO_2 previously measured at this site (Scott-Denton et al. 2006). Compared to CO_2 emissions, BVOC emissions do not represent a major pathway by which belowground C is transported to the atmosphere. However, this may not necessarily be true in other systems (Gray & Fierer 2012) or during other times of the year (Aaltonen et al. 2013). Furthermore, gross BVOC flux rates within the soil could be much higher than net rates would indicate and even low concentrations of BVOCs within soils could have important effects on belowground processes and community dynamics (Insam & Seewald 2010).

The range of summed BVOC fluxes observed here, with individual BVOCs showing either net positive efflux out of soil or net uptake into soil, are likely the outcome of many independent biotic and abiotic processes. Although we cannot separate abiotic from biotic sources with these results, previous work has suggested that the abiotic contribution is likely minimal (Gray et al. 2010). Table 1 provides detailed mean flux rates for the 9 masses with the highest mean emission rates and the 4 masses with the highest mean uptake rates. Methanol (33^+ and 51^+) had the largest mean molar emission rate of 53.35 nmol m^{-2} h^{-1} and a maximum measured rate of 189.08 nmol m^{-2} h^{-1}. This is in agreement with other studies showing that methanol was the dominant BVOC emitted from soils (Asensio et al. 2008; Greenberg et al. 2012). According to above-canopy measurements of methanol fluxes at this site, which are estimated to be ~30 μmol m^{-2} h^{-1}, our emissions represent roughly 0.2% of the above-canopy flux (Baker et al. 2001; Karl et al. 2002). This is in agreement with Greenberg et al. (2012) who found that methanol emissions from soil comprised only 0.4% of the above-canopy flux from a *Pinus ponderosa* forest. Although the contribution of soil and litter to total ecosystem methanol

emissions is low, other systems, including deciduous forests, are likely to have far higher fluxes of methanol given that the decomposition of deciduous litter types can represent large sources of methanol (Gray & Fierer 2012). Nevertheless, methanol emissions at this site could still be important to soil processes (e.g. C dynamics within the soil) as methanol is readily consumed by the broad diversity of C1-oxidizing bacteria and fungi found in soil (Kolb 2009).

Monoterpenes (137^+ and 81^+), a class of BVOCs with a C number of 10, had the largest mean estimated C emission rate (as opposed to molar emission rate) of 646.5 ng-C m^{-2} h^{-1} (Table 1) and a maximum rate of 3,827 ng-C m^{-2} h^{-1}. These rates are similar to those reported previously from coniferous forests (Aaltonen et al. 2011; Greenberg et al. 2012; Hayward et al. 2001; Hellen et al. 2006). At our study site, Rinne et al. (2000) found that the above-canopy flux of α-pinene (a major monoterpene emitted from this ecosystem) was roughly 15,800 ng-C m^{-2} h^{-1}. This puts an estimated soil contribution to the above-canopy fluxes at 4% with the maximum contribution reaching 24%. Our estimated contribution falls in the range of forest floor contribution estimates by Aaltonen et al. (2011) and Hellen et al. (2006) at ~10% and ~60% respectfully, but was larger than the 0.3% found by Greenberg et al. (2012). We could be overestimating the contribution of soil to above-canopy monoterpene emissions because monoterpenes, as measured by the PTR-MS, comprise many different compounds, only one of which is α-pinene. However, it does suggest that forest floor monoterpene emissions could reach levels high enough to be important for local BVOC inventories and models of local atmospheric chemistry. Alternatively, if α-pinene is only a small fraction of the soil emissions, then the soil emissions during this time of the year would likely more closely resemble the results from Greenberg et al. (2012). Further studies are required to determine under what circumstances monoterpenes from the forest floor might be contributing significantly to canopy-level

emissions. Beyond their potential effects on atmospheric chemistry, we note that the monoterpene fluxes observed here could have important effects on belowground processes given that even low concentrations of monoterpenes are capable of inhibiting N mineralization (Smolander et al. 2006), net nitrification (Uusitalo et al. 2008), denitrification and methane oxidation rates (Amaral et al. 1998).

Although methanol and monoterpenes were typically observed to have net positive emission rates from these soils, all compounds displayed net soil uptake at some point during the experiment. Unlike other studies that flush their chambers with air scrubbed of all BVOCs and thus are unable to detect net uptake rates, our measurement method allowed us to quantify net consumption of ambient atmospheric VOCs in soil. Formaldehyde (31^+) had the largest mean molar uptake rate of 3.09 nmol m^{-2} hr^{-1}, while isoprene/furan (69^+) had the largest estimated C uptake rate of 97.7 ng-C m^{-2} h^{-1}. A portion of the measured uptake into the soil could be due to abiotic mechanisms within the soil, such as adsorption onto soil particles or dissolution into soil water. However, several past studies have suggested that microorganisms living in mineral soil can catabolize BVOCs emitted from the litter layer or the surrounding canopy (Asensio et al. 2007; Asensio et al. 2008; Ramirez et al. 2010). Likewise, Cleveland & Yavitt (1997) observed microbial consumption of isoprene in the soil and suggested that the rates could be relevant to ecosystem flux rates and the global isoprene budget. As isoprene consumption is likely enzymatically driven (Cleveland & Yavitt 1998), increases in ambient concentrations of isoprene would be expected to increase uptake rates. If this is the case, further studies should be done to determine the significance of soil uptake rates at different ambient concentrations, including uptake rates in forested systems where ambient levels of isoprene have been measured at 35

ppbv (Wiedinmyer et al. 2005), over ten times higher than levels measured during our experiment.

Effect of root presence on BVOC fluxes

The presence of an active root system increased the summed molar BVOC flux by 76% and the BVOC C flux by 53%, on average (Figure 3). In terms of the fractional contribution of an active root system to net soil fluxes, an active root system contributed to the C flux from BVOCs at the same ratio as that for CO_2. At this site, using similar girdling and trenching techniques, the root system was found to be responsible for 44% of the CO_2 emitted from the soil (Scott-Denton et al. 2006) and a review of 37 studies from forested sites found that the mean root system contribution was 48.6% (Hanson et al. 2000). These results suggest that the effect of an active root system on net C emissions from soil is similar regardless of whether C emissions are measured as emissions of CO_2 or BVOCs. In other words, the contribution of roots to belowground BVOC and CO_2 emissions appears to be similar at around 50%. We do not know if this similarity is merely coincidental or if there are shared mechanisms (i.e. a direct links between respiration and the processes leading to BVOC emissions) that drive this apparent similarity in root contributions to C emission from soil.

The effect of root presence on BVOC emissions was not equivalent across all measured masses. Several individual protonated masses showed significant changes in flux rates between the control and girdled plots (Figure 3). For example, methanol (33^+ and 51^+) fluxes from girdled plots were on average 21% of those from control plots, a finding in agreement with research suggesting that methanol is a product of root metabolism in some tree species (Folkers et al. 2008). However, as we were unable to separate root from rhizosphere flux and given that BVOC emissions have been detected from roots, rhizosphere and associated fungi (Back et al. 2010;

Chen et al. 2004; Lin et al. 2007), we do not know if the methanol is coming directly from the roots themselves. In addition, emissions of mass 47^+ (likely formic acid and/or ethanol) significantly changed from net positive emissions (6.9 nmol m^{-2} h^{-1}) in control plots to net uptake (average rate of -1.1 nmol m^{-2} h^{-1}) in plots where active roots were removed. This pattern highlights the likely role of the root system as a source of mass 47^+ and the ability of soil processes (likely microbial catabolism) to consume this BVOC. Monoterpenes, likely the most frequently studied of the non-methane BVOCs emitted from soils, showed no change in flux rates between control and girdled plots. This suggests that monoterpene fluxes originated from either the needle litter or the mineral soil itself, a finding in agreement with results reported previously (Hayward et al. 2001; Hellen et al. 2006).

Effects of temperature and moisture

At this site, air temperature and soil moisture were strongly correlated (Figure 4 – $p<<0.001$, $R^2=0.59$); the cooler days generally coincided with higher soil moisture levels (Figure 2).

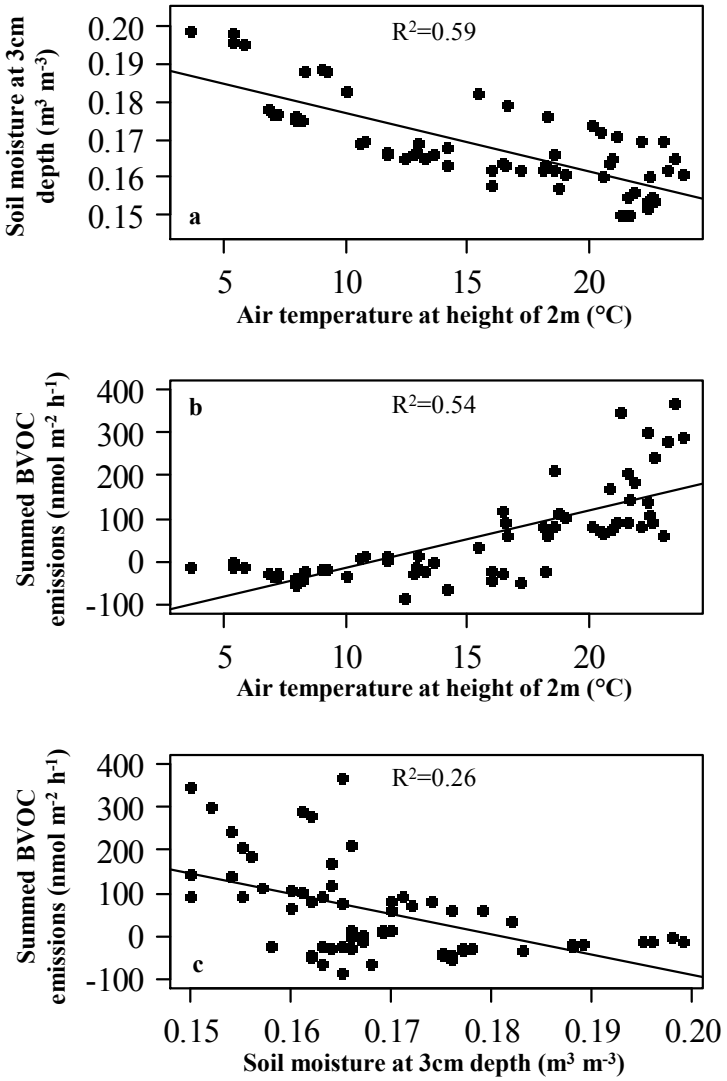

Figure 4 – (**a**) Soil moisture was correlated with air temperature at the study site. (**b,c**) Summed BVOC flux from all measured masses correlated with air temperature and soil moisture.

For this reason, we were unable to quantify the independent effects of temperature and moisture variability on BVOC fluxes. However, multiple linear regressions testing the effects of air temperature and soil moisture on the summed BVOC flux from all measured masses showed that air temperature is the only independent variable significantly correlated with BVOC flux ($p<<0.001$, $R^2=0.54$) and including soil moisture in the statistical model led to only marginal increases in the predictive strength ($p<<0.001$, $R^2=0.65$). Our finding that BVOC emissions increased with increasing temperatures could be a result of both biotic (e.g. plant and microbial

metabolisms) and abiotic processes (e.g. increased evaporation of soluble compounds and physical degradation of labile carbon). Other studies have also found that BVOC flux rates from soil generally increase with increasing temperature (Aaltonen et al. 2011; Asensio et al. 2008; Greenberg et al. 2012). Neither air temperature nor soil moisture correlated with the summed BVOC flux from the girdled plots suggesting that these variables are more strongly linked to BVOC fluxes from the roots or associated rhizosphere rather than to fluxes from litter or mineral soil alone.

This correlation between net BVOC emissions and temperature was largely driven by the dominant compounds described in Table 1, with the emissions of individual compounds, including methanol, acetaldehyde, and acetone/propionaldehyde exhibiting significant, positive correlations with air temperature (Figure 5).

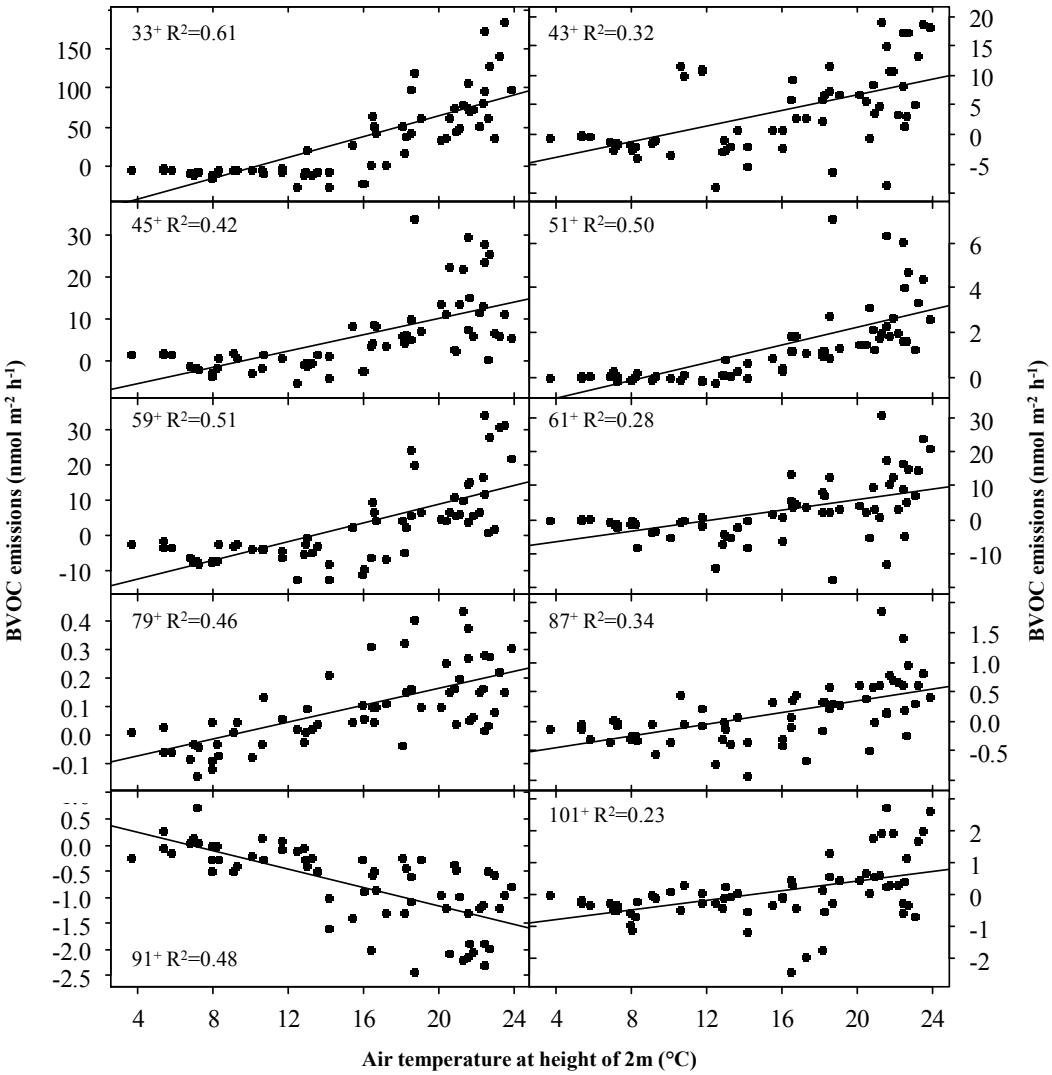

Figure 5 – Individual BVOC fluxes that correlated with air temperature. Measured protonated mass and R^2 values are inset.

Alternatively, compounds that were detected at 91^+ (several possibilities including diethyl sulfide 2,3-butanediol and thioacetic acid methyl ester) showed increases in net uptake rates with increasing temperature (Figure 5). Although the flux rates of these BVOCs are assumed to be primarily biotic in origin, the relationships with temperature were not exponential, as would be expected of an enzymatically-driven process. This could be due to the interactions between temperature and moisture effects, the limited range of temperatures observed at the study site, or because we measured net flux rates instead of gross rates. More controlled, experimental work is

needed to isolate the effects of temperature and moisture on BVOC emissions from soil and to identify how these environmental factors directly influence the gross consumption and production of these compounds.

Conclusion

There was appreciable net production and consumption of many BVOCs during the growing season in the subalpine soils examined here. The dominant compounds emitted from the soils were methanol and monoterpenes, with monoterpene emission rates approaching estimated above-canopy flux rates. Formaldehyde and isoprene were the dominant compounds taken up by the soil. Future research on soil flux rates should utilize techniques that permit the quantification of consumption rates as we clearly show that consumption of BVOCs does occur *in situ*. The activity from roots and associated rhizosphere in this system contributed to over 50% of the C emitted from the system as BVOCs. Although we observed a correlation between air temperature at the site and BVOC flux rates, more experimental work needs to be conducted under controlled conditions to better understand how temperature and soil moisture independently affect flux rates. Also, methods should be developed to independently measure gross production and consumption within intact soils as the specific controls on these processes are likely distinct.

Chapter 4

Introduction

Isoprene (2-methyl-1,3-butadiene [C_5H_8]), is the second-most abundant volatile organic compound in the atmosphere (after methane) with global emissions of isoprene predicted to be > 500 TG yr^{-1} (Guenther et al. 2012). The atmosphere acts as the primary sink for isoprene through oxidation with hydroxyl radicals (OH). In areas with high levels of nitrogen oxides, the oxidation of isoprene leads to the formation of tropospheric ozone, a major pollutant and greenhouse gas. Other effects of atmospheric isoprene oxidation include the formation of tropospheric carbon monoxide, global transport of nitrogenous compounds, extended residence times for atmospheric trace gases and the formation of secondary organic aerosols (Claeys et al. 2004; Granier et al. 2000; Monson & Holland 2001). Isoprene is clearly a key atmospheric trace gas given its myriad of effects on chemical reactions in the atmosphere.

The sources of atmospheric isoprene have been relatively well-studied, with terrestrial plants accounting for 90% of isoprene emissions to the atmosphere (Pacifico et al. 2009; Sharkey et al. 2008). In contrast, the terrestrial sinks of isoprene remain poorly understood, even though it has been estimated that soil may serve as an important sink of atmospheric isoprene (Cleveland & Yavitt 1997). Cleveland & Yavitt (1997) estimated the global soil isoprene sink at 20.4 Tg yr^{-1}, approximately 4% of global emissions. They went on to demonstrate that the isoprene sink was microbially-driven and followed a microbial temperature curve and a maximum consumption rate at 30 °C (Cleveland & Yavitt 1998). Likewise, two later studies combined the dynamics of isoprene plant emissions with soil consumption rates in enclosed ultraviolet light-depleted

mesocosms that consequently have high levels of isoprene (Pegoraro et al. 2005; Pegoraro et al. 2006). They also concluded that soil acts as a significant atmospheric sink of isoprene (3% of global emissions). However, all studies thus far have relied on closed static chambers that measure consumption of initially high levels of isoprene over the span of hours until the isoprene is exhausted within the closed chamber. This technique utilizes initial isoprene concentrations that are much higher than typically measured in ambient air and does not allow the soil microbial population to adapt to varied concentrations of isoprene, therefore leading to a likely underestimation of the potential rates of isoprene consumption.

Several soil microorganisms in pure cultures have been shown to consume isoprene including members of the *Arthrobacter* (Cleveland & Yavitt 1998), *Nocardia* (Van Ginkel et al. 1987) and *Rhodococcus* genera (Vlieg et al. 2000). However, since this previous research on isoprene-degrading soil microorganisms has focused exclusively on those bacteria that could be readily cultured, there are likely far more taxa associated with isoprene degradation in soil that could be identified using culture-independent molecular methods. Moreover, although bacteria, primarily in the Actinobacteria and Alphaproteobacteria, are the only confirmed isoprene-degrading microorganisms, we suspect that fungi may also be capable of degrading isoprene as several Sordariomycete and Eurotiomycete fungal isolates are able to consume similar hydrocarbons, specifically short chain *n*-alkanes (Shennan 2006).

Here we use a dynamic flow through system to determine isoprene consumption rates at atmospherically-relevant concentrations over the course of 45 days combined with high-throughput sequencing to identify taxa associated with the consumption of isoprene in two distinct soils. We hypothesized that (1) microbial isoprene consumption rates would scale linearly with the isoprene concentration provided to the soil microorganisms with significant

consumption even at very low isoprene concentrations, (2) in both soils, isoprene consumption will be associated with increases in the relative abundances of specific bacterial taxa (primarily those in the Actinobacterial and Alphaproteobacterial phyla) and specific fungal taxa (within the Sordariomycete and Eurotiomycete group).

Methods

Soil collection

Two soil types were collected in October, 2013 near the Mountain Research Station in Nederland, CO, USA (40°01'52.0"N, 105°32'06.6"W). Additional site details can be found at http://niwot.colorado.edu/site_info/climate/climate.html. Several samples, each 5 cm deep, were collected and pooled together separately from beneath adjoining *Populus tremuloides* and *Pinus contorta* stands. Soils were collected after the leaves had dropped from the deciduous *P. tremuloides*. Decomposing litter from *P. tremuloides* and *P. contorta* both emit isoprene at similar rates (Gray & Fierer 2012). However, since *P. tremuloides* had recently dropped its leaves (and *P. contorta* had not), microorganisms able to consume isoprene were expected to be more abundant, or at least more active, in the soil beneath the decomposing *P. tremuloides* leaves. All soil samples collected were located within 100 m of each other and were stored at 4 °C within 2 hours of collection. The following day, all soils were sieved at 2 mm and homogenized. The water holding capacity (WHC) was determined for each soil. Soil subsamples were sent to the Soil, Water and Plant Testing Laboratory at Colorado State University, Fort Collins, CO, USA for analysis of soil edaphic characteristics (Table 1).

Table 1. Soil properties

Soil collected beneath:	pH	%OM	NO_3 (ppm N)	%N	%C	C:N	Texture Estimate
Populus tremuloides	5.6	17.2	1.5	0.4800	9.092	18.94	Sandy Loam
Pinus contorta	5.0	10.5	5.0	0.1938	6.242	32.21	Sandy Loam
	P (ppm)	K (ppm)	Zn (ppm)	Fe (ppm)	Mn (ppm)	Cu (ppm)	EC (mMhos/cm)
Populus tremuloides	9	256	17.4	457	9.3	11.45	0.2
Pinus contorta	5	174	11.2	216	24.4	7.30	0.2

Soil incubations

Approximately 20 g of each soil was added to 125 ml glass jars in replicates of 6 for each soil and each experimental concentration of isoprene (0, 2, 20, 200 ppb) for a total of 48 jars with soil. For each isoprene concentration, two 125 ml glass jars were included without soil as no soil controls. De-ionized (DI) water was added to each jar to bring the soil up to 60% WHC. Blank jars received 10 ml of DI water. Each 125 ml jar was placed inside of a 500 ml glass chamber. To keep relative humidity near 100% during the experiment, 50 ml of DI water was added to the larger 500 ml glass jar. A Teflon lined cap, with Swagelok brass bulkhead fittings for an inlet and outlet, was used to seal each 500 ml chamber. Inlet tubing passed into the 500 ml chamber but was kept outside of the 125 ml jar to ensure the incoming air was humidified before reaching the soil (Figure 1). Teflon tubing, 1 m long, was attached to the outlet to minimize diffusion of ambient air into the chamber.

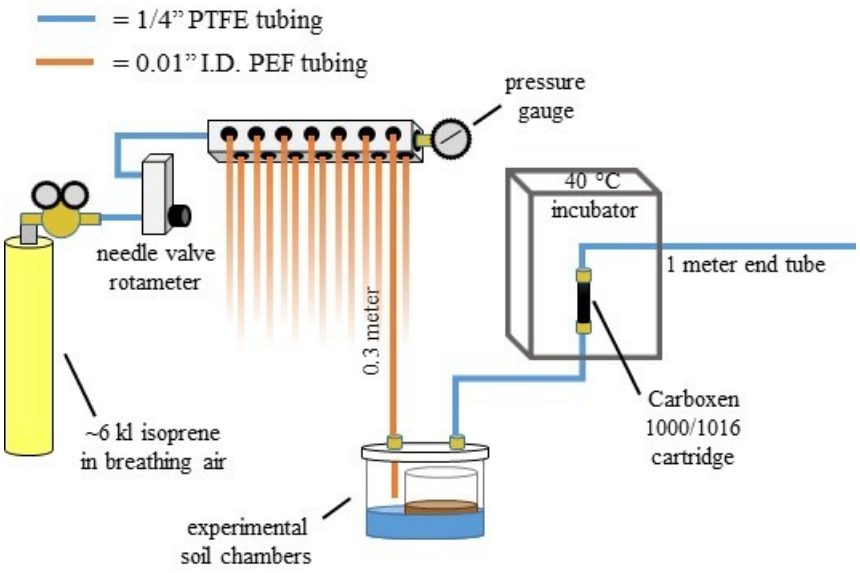

Figure 1 – Diagram illustrating the simplified experimental design.

Breathing air tanks with known concentrations of isoprene (Table 2) were used to continuously flow air to the designated chambers at approximately 30 ml min^{-1} for a total of 45 days.

Table 2. Measured source isoprene concentrations

	Experimental Chambers			
Sampling Period (every ~9 days)	0 ppb	2 ppb	20 ppb	200 ppb
	Actual Source Tank Concentrations			
1	< 0.2 ppb	0.9 ppb	21 ppb	187 ppb
2	< 0.2 ppb	1.9 ppb	23 ppb	191 ppb
3	< 0.2 ppb	2.2 ppb	23 ppb	197 ppb
4	< 0.2 ppb	2.4 ppb	26 ppb	203 ppb
5	< 0.2 ppb	2.5 ppb	27 ppb	205 ppb
Autoclaved	< 0.2 ppb	2.3 ppb	23 ppb	202 ppb

Breathing air tanks were replaced after sampling for headspace isoprene concentrations and before the tanks were empty (approximately 9 days). The sieving of the soils did not remove all of the seeds in the soils and seedlings appeared in several chambers after two weeks of

incubation. All seedlings were removed after the 3rd sampling period, on the 27th day of the experiment. No further seedlings grew after that point.

To check for abiotic consumption of isoprene in soils, the above process was repeated with the following adjustments. To exclude airborne microorganisms, 0.2 µm PTFE membrane filters (Whatman) were added to the inlet and outlet of the 500 ml chambers. All samples were then autoclaved at 121 °C for 45 min. The necessary DI water to wet the soil and the 500 ml chamber was added through the 0.2 µm filters after autoclaving. The autoclaved chambers were incubated for 8 days under the 4 isoprene concentrations.

Isoprene quantification

Isoprene concentrations in the outlet flow of each chamber was measured 5 times during the 45 day experiment (approximately every 9 days) and once at the end of the 8 day autoclave control experiment. Isoprene was collected onto adsorbent cartridges made in house (9 cm long, 0.64 cm o.d. glass or stainless steel tubes, respectively packed with 0.14 g or 0.22 g each of Carboxen 1000 and Carboxen 1016 solid adsorbent (Sigma-Aldrich). Chamber outlet flow was routed to the adsorbent cartridges, which were temperature controlled in an incubator set at 40 °C to keep water in the outlet air from adsorbing onto the cartridges. This temperature was determined not to affect the efficiency of isoprene adsorption onto the cartridge. Sampling times and volumes ranged from 8 min and ~240 ml for the 200 ppb samples to 60 min and ~1800 ml for the 2 ppb and 0 ppb samples. Sample cartridges were kept at 4 °C until they could be analyzed (within 14 days) by thermal desorption (Perkin-Elmer ATD400) onto a gas chromatography – flame ionization detector (GC-FID) instrument (Hewlet-Packard 5890). An Agilent PLOT Al/KCl capillary column (50 m length, 0.53 µm ID, 15 µm film thickness) was used with the FID set to 240 °C. The GC oven temperature was programmed for 60 °C to 200°C

with an initial time of 2 min, a heating rate of 15 °C min^{-1} and a final time of 20 min. Hydrogen was used as the carrier gas at a constant flow of 12 ml min^{-1}. Chromatograms of isoprene were analyzed by manual integration using PeakSimple (SRI Instruments, Torrance, CA) and authenticated isoprene standards. Isoprene concentration results from the soils incubated under 0 ppb are not included in the analyses as they were determined to be below the conservative estimated detection limit of the setup (0.2 ppb). Resulting isoprene concentrations higher than the detection limit were then used to calculate the percentage of isoprene consumed compared to the chambers with no soil for each experimental chamber and sample period. The isoprene consumption rate for each soil chamber was calculated using the following equation:

$$J = ((x_a - x_c) \bullet Q) / (a \bullet W)$$

where J is the isoprene flux from the soil in nmol of isoprene per gram of dry weight soil (gdw) per min, x_a is the average measured isoprene concentration in the blank chamber in ppb, x_c is the measured isoprene concentration in the soil chamber in ppb, Q is the flow rate through the chamber in L min^{-1}, a is the molar volume of air adjusted to the temperature and pressure in the laboratory in L mol^{-1}, and W is the total dry weight of the soil in g.

Microbial analyses

To examine the microbial community shifts associated with exposure to the different isoprene concentrations, we analyzed each of the 48 soil samples by sequencing a portion of the 16S rRNA gene (for bacteria or archaea) or the first internal transcribed spacer (ITS1) region of the rRNA operon (for fungi). The day following the final isoprene sampling, each of the 48 soil samples were swabbed using sterile swabs in triplicate for a total of 144 swabbed samples. Genomic DNA was extracted from the soil using the PowerSoil DNA isolation kit (MoBio, Carlsbad, CA) following the manufacturer's directions with an additional incubation for 10 min

at 65 °C before bead beating to assist in the breakdown of cell walls. Target bacterial, archaeal and fungal DNA was amplified and sequenced using the high-throughput sequencing approach similar to that described in Crowther et al. (2014). Briefly, PCR reactions were conducted in triplicate for each of the 144 genomic DNA samples using primers targeted to the V4 region of the 16S rRNA gene for bacteria and archaea. To analyze the fungal communities, we PCR-amplified soil DNA using primers targeting the first internal transcribed spacer (ITS1) region of the rRNA operon. The PCR primers contained 12-bp barcodes unique to each DNA sample which allowed for the multiplexing (pooling) of samples. Samples were pooled together in equimolar concentrations and sequenced on an Illumina MiSeq platform located at the University of Colorado Next Generation Sequencing Facility.

The resulting MiSeq sequences were demultiplexed using a custom Python script. Quality filtering and operational taxonomic unit (OTU) clustering at 97% similarity was conducted using the USEARCH/UPARSE pipeline (Edgar 2010; Edgar 2013). Singleton sequences were removed before OTU determination. 16S and ITS OTU sequences were aligned, respectively, to the Greengenes August 2013 database (DeSantis et al. 2006) and the UNITE December, 19 2013 database (Koljalg et al. 2013). All 16S sequences were rarefied to 15,700 randomly selected reads per sample and ITS sequences were rarefied to 33,700 reads per sample to correct for differences in sequencing depth. Taxonomic identities were assigned using the RDP classifier (Wang et al. 2007) and the above-mentioned databases with a confidence threshold of 0.5.

Data analysis

To determine whether increasing isoprene concentrations resulted in differences to bacterial and fungal community composition, bacterial and fungal OTU relative abundance data was square-root transformed to minimize the influence of rare taxa and then used to generate

Bray-Curtis distance matrices. The distance matrices were analyzed by Analysis of Similarity (ANOSIM) using Primer-version 6 software (Primer-E, Plymouth, UK). Principal coordinate analyses in the R statistical software (R core team 2014) were used to visualize the community data. To identify which groups at multiple taxonomic ranks responded to increases in isoprene concentrations, multiple regression analyses were conducted in R. To determine whether there was abiotic flux of isoprene from soils, t-tests were used to compare the concentrations of isoprene in the flow of the autoclaved chambers to that of the autoclaved blanks.

Results and Discussion

Isoprene consumption

By the end of the 45 day incubation, microorganisms in the soil were consuming an average of 68% of the isoprene provided to the soils (Figure 2) with consumption rates reaching 770 pmol g^{-1} h^{-1}.

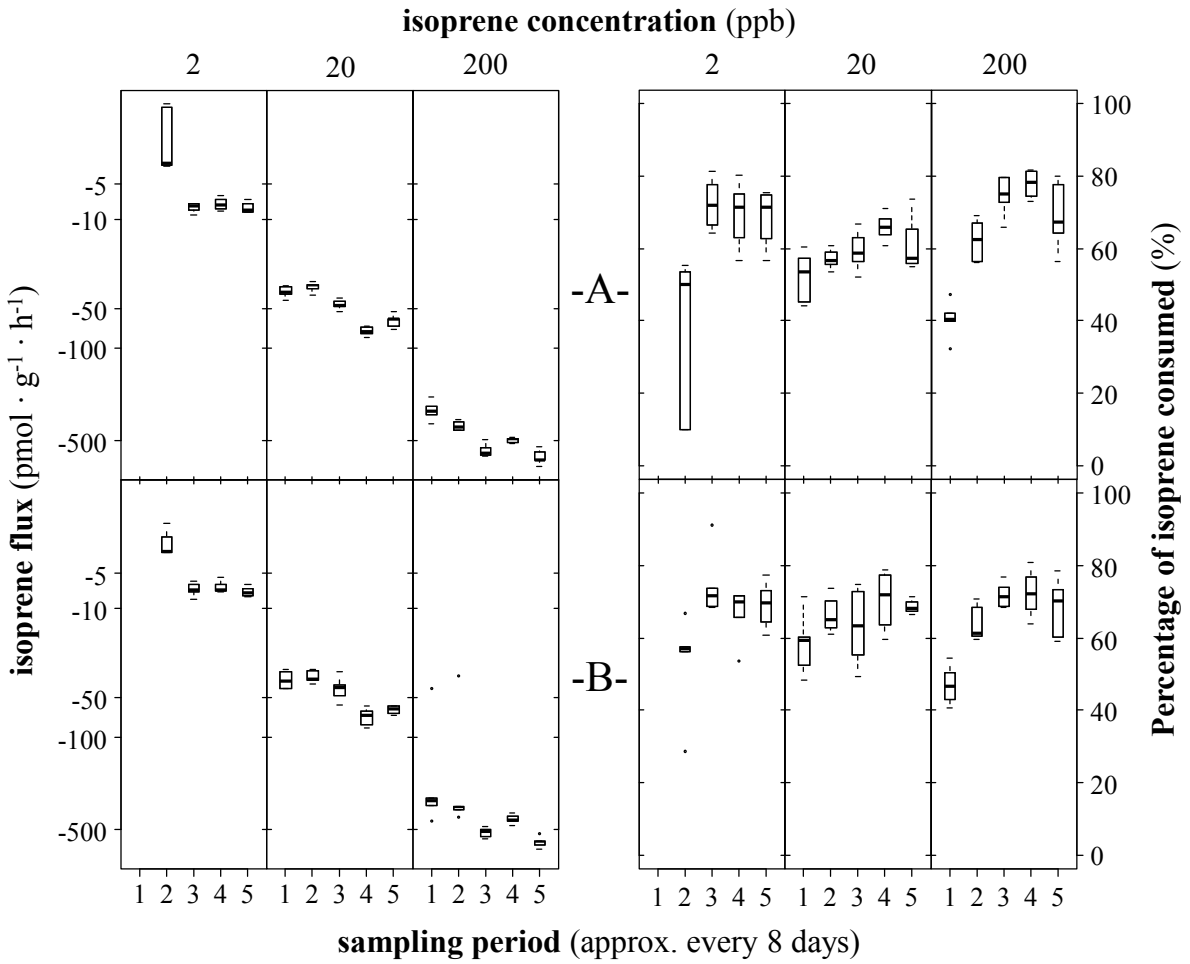

Figure 2 – Isoprene flux rates (negative value represents consumption) and the percentage of the provided isoprene consumed from decomposing soils incubated under varying concentrations of isoprene. Soil was collected from beneath the deciduous *Populus tremuloides* (A) and the evergreen *Pinus contorta* (B).

The isoprene concentrations provided to the soils (2 and 20 ppb) spanned the range of isoprene concentrations we might expect to see in the atmosphere as well as a higher concentration (200ppb). Wiedinmyer et al. (2005) measured ground level isoprene concentrations averaging 10.7 ppbv and a maximum 35.8 ppbv across 5 sites in Illinois and Missouri. In rural Texas, ground level isoprene concentrations averaged 2.6 with a range of 0.3 to 10.2 ppbv (Wiedinmyer et al. 2001). Even at the lowest isoprene concentration used in this study (2 ppb), consumption rates at the time of the final sampling event were measurable at 7.66 pmol g^{-1} h^{-1}, representing 69% of the isoprene consumed.

The percent consumed ranged from 55% to 99% across all isoprene concentrations at the last sampling event. Previous research measured consumption rates as high as 7.4 nmol m^{-2} h^{-1} in a field setting near this study's soil collection site, which was 67% of ambient isoprene concentration and within the range of consumption rates measured in this study (Gray et al. 2014). The average isoprene consumption rate in the field study was 2.0 nmol m^{-2} h^{-1} or 33% of the measured ambient concentration in that study. The cause of the lower consumption rates in the field study could be due to the drier soil and colder temperatures, which have been shown to decrease isoprene consumption in soils (Cleveland & Yavitt 1998; Pegoraro et al. 2005). Calculated consumption rates were comparable to those measured by Cleveland & Yavitt (1998). In that study, isoprene consumption was measured at 24 pmol g^{-1} h^{-1} in a soil with stated ambient isoprene concentrations of approximately 10 ppb, while we measured average rates of 7.7 and 62 pmol g^{-1} h^{-1} at 2 and 20 ppb, respectively. Together these results suggest that soil microorganisms are able to consume a substantial percentage of the isoprene in the ambient air above the soils.

Isoprene consumption in soils is primarily a microbially-mediated process as evidenced by a complete lack of consumption seen in autoclaved controls (Table 3).

Table 3. Mean isoprene concentrations from autoclaved controls

	Experimental chambers		
Autoclaved Soil	2 ppb	20 ppb	200 ppb
	mean isoprene concentration in ppb (p-value from t-test between soil and control)		
Populus tremuloides	2.3 (0.83)	23.7 (0.64)	210.9 (0.69)
Pinus contorta	2.4 (0.24)	23.5 (0.73)	210.0 (0.60)
No soil control	2.3	22.8	213.4

Our results agree with other studies that saw little to no isoprene consumption in autoclaved soils (Cleveland & Yavitt 1998). Also, the consumption of isoprene in soils has been shown to follow first order reaction kinetics suggesting that the concentrations of isoprene tested here and other studies (ranging from 0 ppb to 1500 ppb) is below the Michaelis constant (K_m) for Michaelis-Menten kinetics (Cleveland & Yavitt 1998; Pegoraro et al. 2005). As this was a laboratory-based study, we are unable to extrapolate these results to larger scales due to various environmental controls on soil microorganisms (e.g. moisture and temperature), however this does add further support to the argument that it may be important to consider microbial metabolism of isoprene in soils when trying to describe and predict isoprene dynamics in the atmosphere (Cleveland & Yavitt 1997; Pegoraro et al. 2005).

Microbial community

Not surprisingly, the two soils harbored distinct bacterial and fungal communities even though they were collected within 100 m of each other (Figure 3).

	Populus tremuloides				Pinus contorta			
Bacteria	**0 ppb**	**2 ppb**	**20 ppb**	**200 ppb**	**0 ppb**	**2 ppb**	**20 ppb**	**200 ppb**
Proteobacteria [A]	**34.00**	**33.41**	**37.57**	**38.28**	**43.60**	**41.75**	**47.14**	**45.76**
Alphaproteobacteria [A]	13.45	13.48	15.74	16.08	25.23	23.62	27.70	27.05
Gammaproteobacteria	5.70	5.30	5.86	5.83	9.35	8.94	9.32	8.84
Deltaproteobacteria [A]	7.08	7.34	7.32	7.75	4.89	4.80	5.78	5.41
Betaproteobacteria [A]	7.20	6.72	8.12	8.11	2.93	3.19	3.29	3.34
Acidobacteria	**17.07**	**17.08**	**17.09**	**16.82**	**18.38**	**18.71**	**17.43**	**17.50**
Bacteroidetes [A, B]	**14.57**	**14.84**	**12.18**	**11.74**	**14.20**	**14.69**	**11.84**	**12.36**
Verrucomicrobia [A]	**14.30**	**14.84**	**12.87**	**12.49**	**10.56**	**11.28**	**9.79**	**10.22**
Planctomycetes [A]	**7.28**	**6.77**	**6.95**	**6.64**	**4.84**	**4.78**	**4.73**	**4.77**
Actinobacteria [A, B]	**2.46**	**2.49**	**3.63**	**4.41**	**1.99**	**2.14**	**2.77**	**2.74**
Gemmatimonadetes [B]	**2.36**	**2.64**	**2.64**	**2.70**	**1.22**	**1.24**	**1.39**	**1.40**
Chloroflexi	**1.75**	**1.68**	**1.59**	**1.64**	**0.26**	**0.27**	**0.30**	**0.28**
Fungi								
Ascomycota	**39.96**	**42.14**	**38.52**	**39.77**	**51.95**	**48.72**	**50.59**	**49.22**
(undetermined) [A]	12.39	15.94	12.69	13.72	8.26	9.03	10.30	9.29
Archaeorhizomycetes	0.49	0.66	0.54	0.44	26.33	22.38	19.56	19.46
Dothideomycetes	11.93	11.39	9.53	9.39	4.08	4.17	4.69	4.38
Leotiomycetes [A]	2.97	2.84	3.51	3.81	7.50	7.10	8.78	7.84
Eurotiomycetes	1.27	0.95	1.28	1.32	3.90	4.03	4.55	6.23
Basidiomycota	**48.76**	**44.72**	**49.31**	**46.42**	**35.90**	**38.74**	**36.64**	**36.13**
Agaricomycetes	34.27	32.56	36.12	33.86	28.76	31.01	27.76	26.80
Tremellomycetes [A]	10.75	8.80	8.82	7.85	0.82	0.88	0.90	0.93
Wallemiomycetes [B]	0.04	0.01	0.03	0.03	3.92	3.92	4.46	4.78
(undetermined)	1.47	1.36	1.85	2.07	1.17	1.67	1.70	1.76
Microbotryomycetes [B]	2.07	1.79	2.17	2.36	0.66	0.61	0.86	1.02
(undetermined)	**6.60**	**8.39**	**6.82**	**8.42**	**5.10**	**5.98**	**5.68**	**6.08**
Zygomycota [B]	**4.42**	**4.53**	**5.13**	**5.15**	**7.02**	**6.53**	**7.05**	**8.54**
Glomeromycota	**0.24**	**0.22**	**0.19**	**0.22**	**0.00**	**0.00**	**0.00**	**0.00**
Chytridiomycota	**0.02**	**0.00**	**0.03**	**0.02**	**0.00**	**0.00**	**0.00**	**0.00**

Figure 3 – Relative abundances of the 8 most abundant bacterial phyla, the 4 most abundant classes within Proteobacteria, the fungal phyla and the 5 most abundant classes within Ascomycota and Basidiomycota incubated under varying concentrations of isoprene (0, 2, 20, 200 ppb). Colors range from white (lowest value) to red (highest value) within each phylum, class and soil. Superscripts (A: *Populus tremuloides*; B: *Pinus contorta)* indicate a significant correlation between relative abundance and isoprene concentrations.

Proteobacteria were more abundant in the *P. contorta* soil, while the bacterial phyla Verrucomicrobia, Planctomycetes, Actinobacteria, Gemmatimonadetes and Chloroflexi had higher relative abundances in the *P. tremuloides* soil. The *P. tremuloides* soil was dominated by Basidiomycete fungi, while the *P. contorta* soil was dominated by Ascomycetes. This could be explained by the differences in associated plant species or soil edaphic factors which differed

dramatically between the two soil types (Table 1). Although these two soil types harbored distinct bacterial and fungal communities, the microbial communities in these soils were each significantly different along the isoprene concentration gradient (Figure 4) and many of the bacterial and fungal taxa that responded to the isoprene additions were similar across the two soil types.

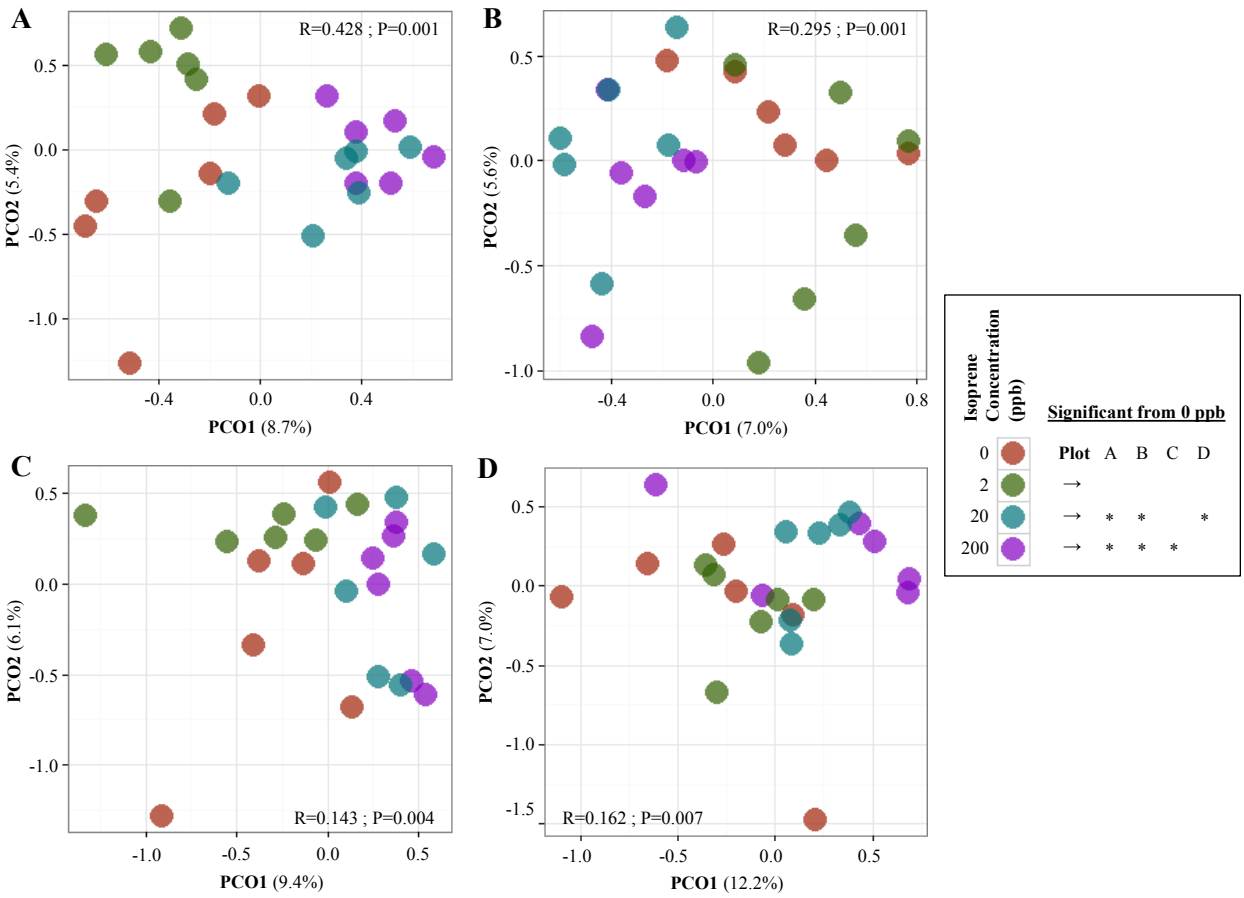

Figure 4 – Principle coordinate analyses (PCoA) showing the similarity of bacterial (A,B) and fungal (C,D) OTUs. Soil samples were collected beneath a high isoprene emitting evergreen tree species (*Populus* tremuloides; A,C) and non-isoprene emitting conifer tree species (*Pinus contorta*; B,D). Results from ANOSIM are set in figures and the legend.

Significant differences in relative abundances associated with the increasing isoprene concentrations were detected for various bacterial and fungal taxa, however, there were no significant differences seen for archaea. Those taxa that increased in relative abundance across

the isoprene gradient may not necessarily be actively consuming isoprene. Presumably, the changes measured here are associated with an increased growth of specific isoprene-degrading microbial taxa. However, the measured community shifts could also be related to isoprene-induced mortality (e.g. the decrease in the relative abundance of Bacteriodetes; Figure 3). Likewise, we are unable to distinguish whether the taxa increasing in abundance with increasing isoprene concentrations were directly consuming isoprene or if they were indirectly stimulated my isoprene amendments (e.g. bacteria or fungi that consume those bacteria that directly metabolize isoprene). In short, we are unable to directly identify whether the isoprene-induced shifts in microbial communities were a product of direct or indirect responses to isoprene metabolism, a problem that plagues even stable isotope-based approaches (Abraham 2014).

ANOSIM results suggest that the bacterial communities responded more to the increased isoprene concentrations than the fungal communities (Figure 4). The relative abundances of Proteobacteria and Actinobacteria significantly increased in the *P. tremuloides* soil in response to the isoprene amendments, while Actinobacteria and Gemmatimonadetes increased in relative abundance in the *P. contorta* soil (Figure 3). Many of the bacterial taxa that increased in relative abundance are related to those taxa that have previously been associated with the consumption of isoprene and other hydrocarbons. For example, most of the known bacteria able to grow on isoprene (i.e. *Mycobacterium sp.*, *Nocardia sp.* and *Rhodococcus sp.*) are Actinobacteria, a phylum which was highly correlated with increasing isoprene concentrations (Cleveland & Yavitt 1998; Shennan 2006). Likewise, taxa within the Alpha-, Beta- and Gammaproteobacteria sub-phyla have been demonstrated to metabolize several alkanes, alkenes and alkadienes (Shennan 2006). In our study, several rare phyla including Fibrobacteres and candidates OP3 and

WS3 increased in relative abundance with increasing isoprene concentrations, suggesting that the ability to degrade isoprene might be more widely distributed that previously thought.

Of the 17,811 unique bacterial OTUs identified between the two soils, 935 significantly increased in relative abundance with increasing isoprene concentrations (uncorrected p-values). Figure 5 highlights the subset of those bacterial taxa with the strongest correlations between relative abundance and isoprene concentration.

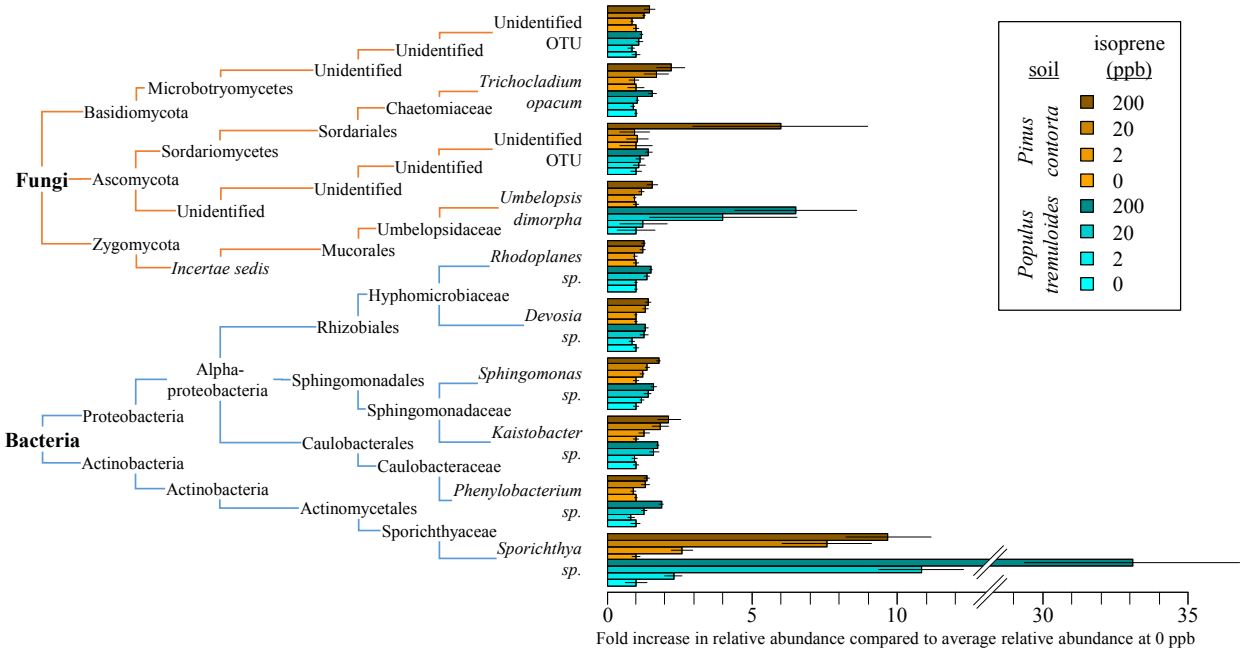

Figure 5 – Fold increases in relative abundances compared to the average relative abundance with no added isoprene (0 ppb) of 10 distinct OTUs and their associated taxonomy.

Of those, *Sporichthya sp.* had the strongest response (33 fold increase) to isoprene with the average relative abundance of 0.01% in the *P. tremuloides* soil with no added isoprene increasing to 0.36% under 200 ppb of isoprene (Figure 5). Many of these taxa are related to taxa associated with the metabolism of hydrocarbons. For example, Miqueletto et al. (2011) sequenced a close relative of *Sporichthya polymorpha* and an uncultured taxa in the Hyphomicrobiaceae family in petroliferous soil with elevated levels of petroleum based hydrocarbons. Likewise, *Kaistobacter sp.* has been detected in methane enrichments (Kravchenko et al. 2010) and in diesel-

contaminated arctic soils (Ferrera-Rodriguez et al. 2013), while its family, Sphingomonadaceae is known for the ability to degrade several aromatic hydrocarbons (Timmis et al. 2010). Using a [^{15}N]DNA-based stable isotope probing technique, Bell et al. (2011) found Sphingomonadaceae and Caulobacteraceae to exhibit the highest percentage of enrichment (indicating increased cell replication relative to other taxonomic groups) in petroleum-contaminated arctic soils. The wide diversity of taxa identified here that responded positively to increasing isoprene concentrations along with the previous research on related taxa consuming related hydrocarbons suggests that the taxa increasing in abundance are directly involved in the consumption of isoprene.

Previous work has focused on isoprene catabolism by bacteria with the assumption that soil bacteria are likely the most important degraders of isoprene (Acuna Alvarez et al. 2009; Shennan 2006). However, several fungal groups did increase in relative abundance in this study (Figure 3). The only fungal phylum with a significant increase in relative abundance was Zygomycota in the *P. contorta* soil. Although none of the fungal phyla or classes that increased in abundance are known to have taxa able to consume isoprene or alkanes, there were several fungal taxa that responded positively to isoprene and were closely related to fungi known to grow on short-chain alkanes (e.g. ethane, propane and butane). Several taxa in the Eurotiomycetes responded positively to increasing isoprene concentrations and were closely related to known consumers of alkanes and other hydrocarbons (Shennan 2006). Certain members of the Trichocomaceae family within the Eurotiomycetes are known to grow on hydrocarbons, including *Aspergillus versicolor* (Cerniglia & J. 1973), *Paecilomyces variotii* (Lowery et al. 1968), *Penicillium janczewskii* (McLee et al. 1972) and *Penicillium ochrochloron* (Cerniglia & J. 1973). There were 5 taxa between the two soils in the Trichocomaceae family that increased in relative abundance with increased isoprene, three of which were identified as

Paecilomyces carneus, *Penicillium glabrum* and *Eupenicillium pinetorum*. Other taxa outside of the Eurotiomycetes included *Exophiala equine*, which was identified in our study as responding positively to increasing isoprene. (Davies et al. 1973) isolated the closely related *Exophiala jeanselmei* var *lecanii-corni*, which was able to utilize ethane, propane and n-butane. Together, these results suggest that fungal taxa have the ability to consume not only alkanes, but also isoprene.

Conclusion

We identified that soil microbial communities are capable of consuming a consistently large proportion of the available isoprene across atmospherically relevant concentrations. Soils have the potential to uptake an estimated 3-4% of global atmospheric isoprene. However, additional research is needed to understand how these results translate to a field setting and how soil and soil edaphic factors influence isoprene consumption rates before we can incorporate microbial isoprene metabolism into global and local models of isoprene emissions. Furthermore, we were able to identify a wide range of microbial taxa that increased in relative abundance in response to the isoprene amendments, including a number of fungal taxa that have not previously been considered to be important isoprene degraders. Further research is necessary to determine if the increase in relative abundance in the taxa identified here were actively consuming isoprene. Also, additional soils should be investigated as differences in soil edaphic factors lead to different taxa increasing in relative abundance.

References

Aaltonen H, Aalto J, Kolari P, Pihlatie M, Pumpanen J, Kulmala M, Nikinmaa E, Vesala T, Back J (2013) Continuous VOC flux measurements on boreal forest floor. Plant and Soil 369(1-2): 241-256

Aaltonen H, Pumpanen J, Pihlatie M, Hakola H, Hellen H, Kulmala L, Vesala T, Back J (2011) Boreal pine forest floor biogenic volatile organic compound emissions peak in early summer and autumn. Agricultural and Forest Meteorology 151(6): 682-691

Abraham WR (2014) Applications and impacts of stable isotope probing for analysis of microbial interactions. Applied Microbiology and Biotechnology 98(11): 4817-4828

Acuna Alvarez L, Exton DA, Timmis KN, Suggett DJ, McGenity TJ (2009) Characterization of marine isoprene-degrading communities. Environmental Microbiology 11(12): 3280-3291

Agren GI, Bosatta E, Magill AH (2001) Combining theory and experiment to understand effects of inorganic nitrogen on litter decomposition. Oecologia 128(1): 94-98

Amaral JA, Ekins A, Richards SR, Knowles R (1998) Effect of selected monoterpenes on methane oxidation, denitrification, and aerobic metabolism by bacteria in pure culture. Applied and Environmental Microbiology 64(2): 520-525

Amaral JA, Knowles R (1998) Inhibition of methane consumption in forest soils by monoterpenes. Journal of Chemical Ecology 24(4): 723-734

Asensio D, Penuelas J, Filella I, Llusia J (2007) On-line screening of soil VOCs exchange responses to moisture, temperature and root presence. Plant and Soil 291(1-2): 249-261

Asensio D, Penuelas J, Prieto P, Estiarte M, Filella I, Llusia J (2008) Interannual and seasonal changes in the soil exchange rates of monoterpenes and other VOCs in a Mediterranean shrubland. European Journal of Soil Science 59(5): 878-891

Atkinson R (2000) Atmospheric chemistry of VOCs and NO_x. Atmospheric Environment 34(12-14): 2063-2101

Atkinson R, Arey J (2003) Gas-phase tropospheric chemistry of biogenic volatile organic compounds: a review. Atmospheric Environment 37:

Back J, Aaltonen H, Hellen H, Kajos MK, Patokoski J, Taipale R, Pumpanen J, Heinonsalo J (2010) Variable emissions of microbial volatile organic compounds (MVOCs) from root-associated fungi isolated from Scots pine. Atmospheric Environment 44(30): 3651-3659

Baker B, Guenther A, Greenberg J, Fall R (2001) Canopy level fluxes of 2-methyl-3-buten-2-ol, acetone, and methanol by a portable relaxed eddy accumulation system. Environmental Science & Technology 35(9): 1701-1708

Bell TH, Yergeau E, Martineau C, Juck D, Whyte LG, Greer CW (2011) Identification of Nitrogen-Incorporating Bacteria in Petroleum-Contaminated Arctic Soils by Using N-15 DNA-Based Stable Isotope Probing and Pyrosequencing. Applied and Environmental Microbiology 77(12): 4163-4171

Bending GD, Lincoln SD (2000) Inhibition of soil nitrifying bacteria communities and their activities by glucosinolate hydrolysis products. Soil Biology & Biochemistry 32(8-9): 1261-1269

Bruce A, Verrall S, Hackett CA, Wheatley RE (2004) Identification of volatile organic compounds (VOCs) from bacteria and yeast causing growth inhibition of sapstain fungi. Holzforschung 58(2): 193-198

Bunge M, Araghipour N, Mikoviny T, Dunkl J, Schnitzhofer R, Hansel A, Schinner F, Wisthaler A, Margesin R, Mark TD (2008) On-line monitoring of microbial volatile metabolites by proton transfer reaction-mass spectrometry. Applied and Environmental Microbiology 74(7): 2179-2186

Campbell BJ, Polson SW, Hanson TE, Mack MC, Schuur EAG (2010) The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environmental Microbiology 12(7): 1842-1854

Carreiro MM, Sinsabaugh RL, Repert DA, Parkhurst DF (2000) Microbial enzyme shifts explain litter decay responses to simulated nitrogen deposition. Ecology 81(9): 2359-2365

Cerniglia CE, J. PJ (1973) Crude oil degradation by microorganisms isolated from the marine environment. Zeitschrift für allgemeine Mikrobiologie 13(4): 299-306

Chen F, Ro DK, Petri J, Gershenzon J, Bohlmann J, Pichersky E, Tholl D (2004) Characterization of a root-specific Arabidopsis terpene synthase responsible for the formation of the volatile monoterpene 1,8-cineole. Plant Physiology 135(4): 1956-1966

Claeys M, Graham B, Vas G, Wang W, Vermeylen R, Pashynska V, Cafmeyer J, Guyon P, Andreae MO, Artaxo P, Maenhaut W (2004) Formation of secondary organic aerosols through photooxidation of isoprene. Science 303(5661): 1173-1176

Cleveland CC, Yavitt JB (1997) Consumption of atmospheric isoprene in soil. Geophysical Research Letters 24(19): 2379-2382

Cleveland CC, Yavitt JB (1998) Microbial consumption of atmospheric isoprene in a temperate forest soil. Applied and Environmental Microbiology 64(1): 172-177

Craine JM, Morrow C, Fierer N (2007) Microbial nitrogen limitation increases decomposition. Ecology 88(8): 2105-2113

Crowther TW, Maynard DS, Leff JW, Oldfield EE, McCulley RL, Fierer N, Bradford MA (2014) Predicting the responsiveness of soil biodiversity to deforestation: a cross-biome study. Global Change Biology:

Dalmonech D, Lagomarsino A, Moscatelli MC, Chiti T, Valentini R (2010) Microbial performance under increasing nitrogen availability in a Mediterranean forest soil. Soil Biology & Biochemistry 42(9): 1596-1606

Davies IS, Wellman AM, Zajic JE (1973) Hyphomycetes utilizing natural-gas. Canadian Journal of Microbiology 19(1): 81-&

DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, Huber T, Dalevi D, Hu P, Andersen GL (2006) Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Applied and Environmental Microbiology 72(7): 5069-5072

Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26(19): 2460-2461

Edgar RC (2013) UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature Methods 10(10): 996-+

Farag MA, Ryu CM, Sumner LW, Pare PW (2006) GC-MS SPME profiling of rhizobacterial volatiles reveals prospective inducers of growth promotion and induced systemic resistance in plants. Phytochemistry 67(20): 2262-2268

Feng XJ, Simpson AJ, Schlesinger WH, Simpson MJ (2010) Altered microbial community structure and organic matter composition under elevated CO2 and N fertilization in the duke forest. Global Change Biology 16(7): 2104-2116

Ferrera-Rodriguez O, Greer CW, Juck D, Consaul LL, Martinez-Romero E, Whyte LG (2013) Hydrocarbon-degrading potential of microbial communities from Arctic plants. Journal of Applied Microbiology 114(1): 71-83

Fog K (1988) The effect of added nitrogen on the rate of decomposition of organic-matter. Biological Reviews of the Cambridge Philosophical Society 63(3): 433-462

Folkers A, Huve K, Ammann C, Dindorf T, Kesselmeier J, Kleist E, Kuhn U, Uerlings R, Wildt J (2008) Methanol emissions from deciduous tree species: dependence on temperature and light intensity. Plant Biology 10(1): 65-75

Galbally IE, Kirstine W (2002) The production of methanol by flowering plants and the global cycle of methanol. Journal of Atmospheric Chemistry 43(3): 195-229

Granier C, Petron G, Muller JF, Brasseur G (2000) The impact of natural and anthropogenic hydrocarbons on the tropospheric budget of carbon monoxide. Atmospheric Environment 34(29-30): 5255-5270

Gray CM, Fierer N (2012) Impacts of nitrogen fertilization on volatile organic compound emissions from decomposing plant litter. Global Change Biology 18(2): 739-748

Gray CM, Monson RK, Fierer N (2010) Emissions of volatile organic compounds during the decomposition of plant litter. Journal of Geophysical Research-Biogeosciences 115: 9

Gray CM, Monson RK, Fierer N (2014) Biotic and abiotic controls on biogenic volatile organic compound fluxes from a subalpine forest floor. Journal of Geophysical Research-Biogeosciences:

Greenberg JP, Asensio D, Turnipseed A, Guenther AB, Karl T, Gochis D (2012) Contribution of leaf and needle litter to whole ecosystem BVOC fluxes. Atmospheric Environment 59: 302-311

Gu YQ, Mo MH, Zhou JP, Zou CS, Zhang KQ (2007) Evaluation and identification of potential organic nematicidal volatiles from soil bacteria. Soil Biology & Biochemistry 39(10): 2567-2575

Guenther A (2002) The contribution of reactive carbon emissions from vegetation to the carbon balance of terrestrial ecosystems. Chemosphere 49(8): 837-844

Guenther AB, Jiang X, Heald CL, Sakulyanontvittaya T, Duhl T, Emmons LK, Wang X (2012) The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1): an extended and updated framework for modeling biogenic emissions. Geoscientific Model Development 5(6): 1471-1492

Hanson PJ, Edwards NT, Garten CT, Andrews JA (2000) Separating root and soil microbial contributions to soil respiration: A review of methods and observations. Biogeochemistry 48(1): 115-146

Harley PC, Monson RK, Lerdau MT (1999) Ecological and evolutionary aspects of isoprene emission from plants. Oecologia 118(2): 109-123

Hauglustaine DA, Brasseur GP, Walters S, Rasch PJ, Muller JF, Emmons LK, Carroll CA (1998) MOZART, a global chemical transport model for ozone and related chemical tracers 2. Model results and evaluation. Journal of Geophysical Research-Atmospheres 103(D21): 28291-28335

Hayward S, Muncey RJ, James AE, Halsall CJ, Hewitt CN (2001) Monoterpene emissions from soil in a Sitka spruce forest. Atmospheric Environment 35(24): 4081-4087

Hellen H, Hakola H, Pystynen KH, Rinne J, Haapanala S (2006) C-2-C-10 hydrocarbon emissions from a boreal wetland and forest floor. Biogeosciences 3(2): 167-174

Hobbie SE (2005) Contrasting effects of substrate and fertilizer nitrogen on the early stages of litter decomposition. Ecosystems 8(6): 644-656

Hobbie SE (2008) Nitrogen effects on decomposition: A five-year experiment in eight temperate sites. Ecology 89(9): 2633-2644

Hobbie SE, Gough L (2004) Litter decomposition in moist acidic and non-acidic tundra with different glacial histories. Oecologia 140(1): 113-124

Horiuchi J, Prithiviraj B, Bais HP, Kimball BA, Vivanco JM (2005) Soil nematodes mediate positive interactions between legume plants and rhizobium bacteria. Planta 222(5): 848-857

Insam H, Seewald MSA (2010) Volatile organic compounds (VOCs) in soils. Biology and Fertility of Soils 46(3): 199-213

Isidorov V, Jdanova M (2002) Volatile organic compounds from leaves litter. Chemosphere 48(9): 975-979

Jacob DJ, Field BD, Li QB, Blake DR, de Gouw J, Warneke C, Hansel A, Wisthaler A, Singh HB, Guenther A (2005) Global budget of methanol: Constraints from atmospheric observations. Journal of Geophysical Research-Atmospheres 110(D8): 17

Jangid K, Williams MA, Franzluebbers AJ, Sanderlin JS, Reeves JH, Jenkins MB, Endale DM, Coleman DC, Whitman WB (2008) Relative impacts of land-use, management intensity and fertilization upon soil microbial community structure in agricultural systems. Soil Biology & Biochemistry 40(11): 2843-2853

Kansal A (2009) Sources and reactivity of NMHCs and VOCs in the atmosphere: A review. Journal of Hazardous Materials 166(1): 17-26

Karl TG, Spirig C, Rinne J, Stroud C, Prevost P, Greenberg J, Fall R, Guenther A (2002) Virtual disjunct eddy covariance measurements of organic compound fluxes from a subalpine forest using proton transfer reaction mass spectrometry. Atmospheric Chemistry and Physics 2: 279-291

Kesselmeier J, Ciccioli P, Kuhn U, Stefani P, Biesenthal T, Rottenberger S, Wolf A, Vitullo M, Valentini R, Nobre A, Kabat P, Andreae MO (2002) Volatile organic compound emissions in relation to plant carbon fixation and the terrestrial carbon budget. Global Biogeochemical Cycles 16(4):

Kesselmeier J, Staudt M (1999) Biogenic volatile organic compounds (VOC): An overview on emission, physiology and ecology. Journal of Atmospheric Chemistry 33(1): 23-88

Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: A meta-analysis. Ecology 86(12): 3252-3257

Kolb S (2009) Aerobic methanol-oxidizing Bacteria in soil. Fems Microbiology Letters 300(1): 1-10

Koljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Duenas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Luecking R, Martin MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Poldmaa K, Saag L, Saar I, Schuessler A, Scott JA, Senes C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiss M, Larsson KH (2013) Towards a unified paradigm for sequence-based identification of fungi. Molecular Ecology 22(21): 5271-5277

Kravchenko IK, Kizilova AK, Bykova SA, Men'ko EV, Gal'chenko VF (2010) Molecular analysis of high-affinity methane-oxidizing enrichment cultures isolated from a forest biocenosis and agrocenoses. Microbiology 79(1): 106-114

Kreuzwieser J, Schnitzler JP, Steinbrecher R (1999) Biosynthesis of organic compounds emitted by plants. Plant Biology 1(2): 149-159

Lamarque JF, Kiehl JT, Brasseur GP, Butler T, Cameron-Smith P, Collins WD, Collins WJ, Granier C, Hauglustaine D, Hess PG, Holland EA, Horowitz L, Lawrence MG, McKenna D, Merilees P, Prather MJ, Rasch PJ, Rotman D, Shindell D, Thornton P (2005) Assessing future nitrogen deposition and carbon cycle feedback using a multimodel approach: Analysis of nitrogen deposition. Journal of Geophysical Research-Atmospheres 110(D19):

Lechner M, Fille M, Hausdorfer J, Dierich M, Rieder J (2005) Diagnosis of bacteria in vitro by mass spectrometric fingerprinting: A pilot study. Current Microbiology 51(4): 267-269

Leff JW, Fierer N (2008) Volatile organic compound (VOC) emissions from soil and litter samples. Soil Biology & Biochemistry 40(7): 1629-1636

Lelieveld J, Crutzen PJ, Dentener FJ (1998) Changing concentration, lifetime and climate forcing of atmospheric methane. Tellus Series B-Chemical and Physical Meteorology 50(2): 128-150

Lin C, Owen SM, Penuelas J (2007) Volatile organic compounds in the roots and rhizosphere of Pinus spp. Soil Biology & Biochemistry 39(4): 951-960

Lindinger W, Hansel A, Jordan A (1998) On-line monitoring of volatile organic compounds at pptv levels by means of proton-transfer-reaction mass spectrometry (PTR-MS) - Medical applications, food control and environmental research. International Journal of Mass Spectrometry 173(3): 191-241

Lowery CE, Jr., Foster JW, Jurtshuk P (1968) The growth of various filamentous fungi and yeasts on n-alkanes and ketones. Archiv für Mikrobiologie 60(3): 246-254

Macdonald RC, Fall R (1993) Detection of Substantial Emissions of Methanol from Plants to the Atmosphere. Atmospheric Environment Part a-General Topics 27(11): 1709-1713

Mackie AE, Wheatley RE (1999) Effects and incidence of volatile organic compound interactions between soil bacterial and fungal isolates. Soil Biology & Biochemistry 31(3): 375-385

Mantel N (1967) Detection of Disease Clustering and a Generalized Regression Approach. Cancer Research 27(2P1): 209-220

Maurer D, Kolb S, Haumaier L, Borken W (2008) Inhibition of atmospheric methane oxidation by monoterpenes in Norway spruce and European beech soils. Soil Biology & Biochemistry 40(12): 3014-3020

McLee AG, Kormendy AC, Wayman M (1972) Isolation and characterization of n-butane-utilizing microorganisms. Canadian Journal of Microbiology 18(8): 1191-1195

Melillo JM, Aber JD, Muratore JF (1982) Nitrogen and lignin control of hardwood leaf litter decomposition dynamics. Ecology 63(3): 621-626

Miqueletto PB, Andreote FD, Dias AC, Ferreira JC, Dos Santos Neto EV, de Oliveira VM (2011) Cultivation-independent methods applied to the microbial prospection of oil and gas in soil from a sedimentary basin in Brazil. AMB Express 1: 35

Monson RK, Holland EA (2001) Biospheric trace gas fluxes and their control over tropospheric chemistry. Annual Review of Ecology and Systematics 32: 547-576

Monson RK, Prater MR, Hu J, Burns SP, Sparks JP, Sparks KL, Scott-Denton LE (2010) Tree species effects on ecosystem water-use efficiency in a high-elevation, subalpine forest. Oecologia 162(2): 491-504

Moorhead DL, Sinsabaugh RL (2006) A theoretical model of litter decay and microbial interaction. Ecological Monographs 76(2): 151-174

Paavolainen L, Kitunen V, Smolander A (1998) Inhibition of nitrification in forest soil by monoterpenes. Plant and Soil 205(2): 147-154

Pacifico F, Harrison SP, Jones CD, Sitch S (2009) Isoprene emissions and climate. Atmospheric Environment 43(39): 6121-6135

Papanikolaou N, Britton AJ, Helliwell RC, Johnson D (2010) Nitrogen deposition, vegetation burning and climate warming act independently on microbial community structure and enzyme activity associated with decomposing litter in low-alpine heath. Global Change Biology 16(11): 3120-3132

Pegoraro E, Abrell L, Van Haren J, Barron-Gafford G, Grieve KA, Malhi Y, Murthy R, Lin GH (2005) The effect of elevated atmospheric CO2 and drought on sources and sinks of isoprene in a temperate and tropical rainforest mesocosm. Global Change Biology 11(8): 1234-1246

Pegoraro E, Rey A, Abrell L, Vanharen J, Lin GH (2006) Drought effect on isoprene production and consumption in Biosphere 2 tropical rainforest. Global Change Biology 12(3): 456-469

Poisson N, Kanakidou M, Crutzen PJ (2000) Impact of non-methane hydrocarbons on tropospheric chemistry and the oxidizing power of the global troposphere: 3-dimensional modelling results. Journal of Atmospheric Chemistry 36(2): 157-230

R CT (2014) R: A Language and Environment for Statistical Computing. In. R Foundation for Statistical Computing.

Ramirez KS, Lauber CL, Fierer N (2010) Microbial consumption and production of volatile organic compounds at the soil-litter interface. Biogeochemistry 99(1-3): 97-107

Rinne HJI, Delany AC, Greenberg JP, Guenther AB (2000) A true eddy accumulation system for trace gas fluxes using disjunct eddy sampling method. Journal of Geophysical Research-Atmospheres 105(D20): 24791-24798

Scheutz C, Mosbaek H, Kjeldsen P (2004) Attenuation of methane and volatile organic compounds in landfill soil covers. Journal of Environmental Quality 33(1): 61-71

Schulz S, Dickschat JS (2007) Bacterial volatiles: the smell of small organisms. Natural Product Reports 24(4): 814-842

Scott-Denton LE, Rosenstiel TN, Monson RK (2006) Differential controls by climate and substrate over the heterotrophic and rhizospheric components of soil respiration. Global Change Biology 12(2): 205-216

Sharkey TD, Wiberley AE, Donohue AR (2008) Isoprene emission from plants: Why and how. Annals of Botany 101(1): 5-18

Shennan JL (2006) Utilisation of C-2-C-4 gaseous hydrocarbons and isoprene by microorganisms. Journal of Chemical Technology and Biotechnology 81(3): 237-256

Smolander A, Ketola RA, Kotiaho T, Kanerva S, Suominen K, Kitunen V (2006) Volatile monoterpenes in soil atmosphere under birch and conifers: Effects on soil N transformations. Soil Biology & Biochemistry 38(12): 3436-3442

Spracklen DV, Bonn B, Carslaw KS (2008) Boreal forests, aerosols and the impacts on clouds and climate. Philosophical Transactions of the Royal Society a-Mathematical Physical and Engineering Sciences 366(1885): 4613-4626

Steeghs M, Bais HP, de Gouw J, Goldan P, Kuster W, Northway M, Fall R, Vivanco JM (2004) Proton-transfer-reaction mass spectrometry as a new tool for real time analysis of root-secreted volatile organic compounds in arabidopsis. Plant Physiology 135(1): 47-58

Strickland MS, Lauber C, Fierer N, Bradford MA (2009) Testing the functional significance of microbial community composition. Ecology 90(2): 441-451

Taylor BR, Parkinson D, Parsons WFJ (1989) Nitrogen and lignin content as predictors of litter decay-rates - A microcosm test. Ecology 70(1): 97-104

Timmis KN, Kertesz MA, Kawasaki A (2010) Hydrocarbon-Degrading Sphingomonads: Sphingomonas, Sphingobium, Novosphingobium, and Sphingopyxis. In: Handbook of Hydrocarbon and Lipid Microbiology. Springer Berlin Heidelberg. p 1693-1705

Treseder KK (2008) Nitrogen additions and microbial biomass: a meta-analysis of ecosystem studies. Ecology Letters 11(10): 1111-1120

Uusitalo M, Kitunen V, Smolander A (2008) Response of C and N transformations in birch soil to coniferous resin volatiles. Soil Biology & Biochemistry 40(10): 2643-2649

van Diepen LTA, Lilleskov EA, Pregitzer KS, Miller RM (2010) Simulated Nitrogen Deposition Causes a Decline of Intra- and Extraradical Abundance of Arbuscular Mycorrhizal Fungi and Changes in Microbial Community Structure in Northern Hardwood Forests. Ecosystems 13(5): 683-695

Van Ginkel CG, De Jong E, Tilanus JWR, De Bont JAM (1987) Microbial oxidation of isoprene, a biogenic foliage volatile and of 1,3-butadiene, an anthropogenic gas. Fems Microbiology Ecology 45(5): 275-279

Van Lancker F, Adams A, Delmulle B, De Saeger S, Moretti A, Van Peteghem C, De Kimpe N (2008) Use of headspace SPME-GC-MS for the analysis of the volatiles produced by indoor molds grown on different substrates. Journal of Environmental Monitoring 10(10): 1127-1133

Vlieg J, Leemhuis H, Spelberg JHL, Janssen DB (2000) Characterization of the gene cluster involved in isoprene metabolism in Rhodococcus sp strain AD45. Journal of Bacteriology 182(7): 1956-1963

Wang Q, Garrity GM, Tiedje JM, Cole JR (2007) Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and Environmental Microbiology 73(16): 5261-5267

Warneke C, Karl T, Judmaier H, Hansel A, Jordan A, Lindinger W, Crutzen PJ (1999) Acetone, methanol, and other partially oxidized volatile organic emissions from dead plant matter by abiological processes: Significance for atmospheric HOx chemistry. Global Biogeochemical Cycles 13(1): 9-17

Weintraub MN, Scott-Denton LE, Schmidt SK, Monson RK (2007) The effects of tree rhizodeposition on soil exoenzyme activity, dissolved organic carbon, and nutrient availability in a subalpine forest ecosystem. Oecologia 154(2): 327-338

Wheatley RE (2002) The consequences of volatile organic compound mediated bacterial and fungal interactions. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology 81(1-4): 357-364

Wheatley RE, Millar SE, Griffiths DW (1996) The production of volatile organic compounds during nitrogen transformations in soils. Plant and Soil 181: 163-167

White CS (1994) Monoterpenes - Their effects on ecosystem nutrient cycling. Journal of Chemical Ecology 20(6): 1381-1406

Wiedinmyer C, Friedfeld S, Baugh W, Greenberg J, Guenther A, Fraser M, Allen D (2001) Measurement and analysis of atmospheric concentrations of isoprene and its reaction products in central Texas. Atmospheric Environment 35(6): 1001-1013

Wiedinmyer C, Greenberg J, Guenther A, Hopkins B, Baker K, Geron C, Palmer PI, Long BP, Turner JR, Petron G, Harley P, Pierce TE, Lamb B, Westberg H, Baugh W, Koerber M, Janssen M (2005) Ozarks Isoprene Experiment (OZIE): Measurements and modeling of the "isoprene volcano". Journal of Geophysical Research-Atmospheres 110(D18): 22