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Executive Summary

The ultimate goal of this work was to quantify soil and volatile organic compound fluxes as a function of tree species and associated mycorrhizal associations in an intact forest, but also to describe the physical and biological factors that control these emissions. The results of this research lay the foundation toward an improved mechanistic understanding of carbon pathways, fluxes, and ecosystem function, ultimately improving the representation of forest ecosystems in Earth System models. To this end, a multidisciplinary approach was necessary to fill a critical gap in our understanding of how soil and root processes may influence whole-ecosystem carbon-based volatile fluxes in the face of a rapidly changing climate. We developed a series of novel sampling protocols and coupled a variety of advanced analytical techniques, resulting in findings relevant across disciplines. Furthermore, we leveraged existing infrastructure, research sites, and datasets to design a low-cost exploratory project that links belowground processes, soil volatile emissions, and total ecosystem carbon budgets.

Measurements from soil collars installed across a species/mycorrhizal gradient at the DOE-supported Moran Monroe State Forest Ameriflux tower site suggest that leaf litter is the primary source of belowground and forest floor volatile emissions, but the strength of this source is significantly affected not only by leaf litter type, but the strength of the soil as a sink. Results suggest that the strength of the sink is influenced by tree species-specific associated microbial communities that change throughout the season as a function of temperature, soil moisture, leaf litter inputs, and phenology. The magnitude of the observed volatile fluxes from the forest floor is small relative to total aboveground ecosystem flux, but the contribution of these emissions to volatile-mediated ecological interactions and soil processes (e.g. nitrification) varies substantially across the growing season. This research lays the foundation to answer important questions regarding the impacts of seasonality and forest composition on belowground volatile source-sink dynamics in mediating nutrient cycling and biogeochemistry dynamics—critical components of overall ecosystem functioning.

In collaboration with the Environmental Simulations Unit (EUS) at the Helmholtz Zentrum in Munich, Germany (headed by Prof. Dr. Joerg-Peter Schinitzler), we investigated carbon investment in above and belowground plant volatile compounds in response to environmental conditions and mycorrhizal associations. Using the sophisticated phytotron facility and on-line trace gas instruments, we conducted controlled laboratory experiments that showed that biotic stresses, such as herbivore feeding, can alter the magnitude of belowground volatile emissions as well as carbon allocation towards these volatiles. We saw no effect of mycorrhizae on any induced response, suggesting that microbial effects were unrelated to source-sink dynamics driving terpene emissions. Furthermore, the results suggest that even though enzyme activity responsible for root volatile synthesis is up-regulated following herbivory, the sink strength of the soil can significantly impact what is measured at the soil/atmosphere interface and thereby what enters the atmosphere. This is important as scientists may be underestimating the magnitude of belowground volatile emissions and their influence on belowground interactions due to limitations associated with current sampling techniques.

These key findings are being integrated with results from a hydroxyl radical reactivity-VOC campaign and a late season litter removal experiment to offer a comprehensive mechanistic understanding of the sources and controls over soil volatile emissions, particularly during times of the year when vegetative aboveground emissions are low (leaf senescence). Ultimately, these coupled field and laboratory experiments offer insights into seasonal dynamics of volatile

emissions and the mechanisms that control carbon allocation to these compounds with an eye towards improving carbon budgets, nutrient cycling, and terrestrial ecosystem models.

Comparison of actual accomplishments with the goals and objectives of the project

Biogenic volatile organic compounds (bVOCs) impact ecological interactions and atmospheric chemistry, but our understanding of the *sources* of bVOCs from terrestrial ecosystems and the biophysical and environmental *controls* over these emissions remains incomplete. bVOCs contribute to the production of ozone and secondary organic aerosol particles, both of which impact air quality and radiative forcing with consequences for human health. Furthermore, soil bVOCs can impact nutrient cycling, biogeochemistry, and multitrophic interactions, all of which impact community structure and ecosystem function. Because of the critical, but perhaps underappreciated role soil bVOCs play in forest systems, our research group set out to not only *quantify* bVOC fluxes from forest soils, but to *describe* the physical and biological factors that underlie the ecological processes controlling bVOC emissions. We proposed that the results of the field and laboratory studies could then be applied to improve ecosystem models and carbon budget estimates. The overarching objective of this work was to quantify the magnitude, timing and biological and environmental controls of bVOC emissions from forest soils. Below we separately address each of the proposed hypotheses, the approaches used, problems encountered/departure from planned methodology, and key project results.

Objective 1: Calculate the contribution of soil emissions to total annual ecosystem bVOC emissions.

Hypothesis 1: bVOCs released from soil represent the dominant source of VOCs from hardwood forests during periods of peak belowground C allocation (spring and fall).

Approach:
Our study site was located within the Morgan-Monroe State Forest (MMSF) at 39°190 N, 86°250 W in south-central Indiana, USA. This *c.* 80-yr-old mixed hardwood forest contains tree species that associate primarily with ecomycorrhizal (ECM) or arbuscular mycorrhizal (AM) fungi. ECM-associated trees include red oak (*Quercus rubra*), white oak (*Quercus alba*), American beech (*Fagus grandifolia*), shagbark hickory (*Carya ovata*) and pignut hickory (*Carya glabra*). AM-associated trees include sugar maple (*Acer saccharum*), tulip poplar (*Liriodendron tulipifera*) and sassafras (*Sassafras albidum*). The understory contains tree seedlings and patches of spicebush (*Lindera benzoin*), an isoprene-emitter. The climate is humid continental and the soil is characterized as mesic Typic Dystrochrepts. Additional site details can be found in Brzostek et al. (2015).

Eight plots were chosen from an experimental set-up established in 2011. Over 85% of the basal area of the experimental plots used was comprised of either ECM or AM tree species (n=4 for each mycorrhizal type) (15 x 10 m^2). For *in situ* bVOC measurements, three open-top stainless steel collars (973 cm^2) were placed in each of the eight plots in October 2013 (Fig. 1). Each collar was inserted 2 cm into the soil (*c.* 1.5 L of headspace air) approximately 3 m in three randomly chosen cardinal directions from the tree with the largest DBH in the plot. Relative water content of the soil and soil temperature were taken from just outside each collar during sampling using a HydroSense II (Campbell Scientific, Logan, UT) and a standard glass

thermometer, respectively. These data were compared to continuous micrometeorological measurements taken at the MMSF AmeriFlux eddy covariance tower, located within 100 m of the study plots. Measurements used in this study include air temperature at 2 m (Vaisala HMP-35D), volumetric soil moisture at 3 cm below the surface (Campbell Scientific Instruments CS615), and soil temperature.

To capture any seasonal variation in net bVOC flux from the forest floor in the field, we performed dynamic headspace measurements *in situ* during 4 separate campaigns: two at the beginning of the growing season prior to (16 April through 2 May) and just after leaf-out (20 May through 4 June) and two at the end of the growing season (24 September through 9 October and 21 October through 30 October). On each sampling date, one plot from each of the ECM and AM sites was randomly selected. Two teflon chamber lids were placed on top of the collars, equipped with a small diaphragm pump pushing 200 mL min^{-1} in through the chamber after passing through an activated charcoal filter and ozone scrubber. Chambers were allowed to equilibrate for 20 minutes (triple the residence time), as calculated using volume of the chamber and flow rate. Teflon tubing connected to the outlet was then attached to a Vac-U-Chamber (SKC, Eighty Four, PA) equipped with a 10 L Tedlar bag (CEL Scientific and small handheld pump (SKC, Eighty Four, PA) pulling 150 mL min-1. The Vac-U-Chamber was chosen as it directly fills the sample bag using negative pressure provided by the sample pump, avoiding any contamination that might occur from direct contact with a pumping system. Samples were taken for 30 minutes to obtain *ca.* 4.5 L of air and each bag was cleaned with successive flushes of N_2 gas before collection. The stainless steel inlet of the bags were immediately closed and bags were stored in a cool dark shed before being transported back to Indiana University that day to be analyzed on the same day. For each plot, two collars were measured simultaneously, followed by simultaneous measurements of the third chamber and an empty collar, placed on aluminum foil rather than soil, to serve as the blank for the plot. Measurements were taken from two plots each day from 10:00-14:00 to minimize effects from diurnal variation in emissions.

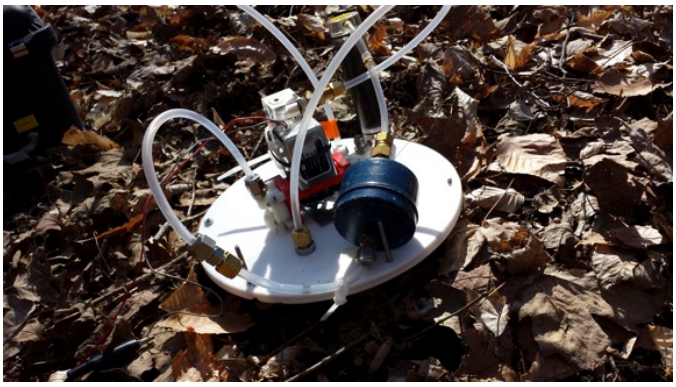

Fig. 1 Field soil collar covered with a Teflon plate equipped with inlet and outlet ports with the former connected to a pump as well as charcoal and ozone filers. All connections were made with Teflon tubing.

All samples were analyzed within 24 hours of collection using PTR-MS. Bags were connected directly to the PTR-MS and 19 compounds ranging from *m/z* (mass per charge) 31 to *m/z* 137 were selectively analyzed with a dwell time of 0.2 s. The PTR-MS drift tube was operated at 2.1 mbar and 60 °C, with a drift field of 600 V cm^{-1}; the parent ion signal was maintained at x 10^6 cps and the $O2^+$:H_3O^+ ratio was <3.5%. The scan was allowed to run until the bag was near empty, and the stable points were averaged for each collar. We solved for the volume mixing ratio (VMR; ppb) of each compound using the equations of Ellis and Mayhew (2014) and converted the mixing ratios to flux rates using the equation in Gray et al. (2014) . To compare to values in the literature, molar bVOC fluxes were calculated as total C mass fluxes. As such, all data are reported both in terms of total C flux and nmol m^{-2} h^{-1} of each bVOC compound.

Soil samples were collected near each collar on 4 June 2014 to investigate the relationships between enzyme activity and net bVOC fluxes. Soil samples were also collected from collars (n=3) in two nearby plots that were trenched in 2012, which served as a "no mycorrhizae" control. We collected one core from the top 15 cm of soil using a 5-cm-diameter soil bulk density sampler. Soils were processed within 24 hours of collection according to Brzostek et al. (2015)and stored in a -80 °C freezer until they could be analyzed for enzyme activities. We assessed chitin degrading enzymes (N-acetyl-glucosaminidase (NAG)) and phosphorous mobilizing enzymes (acid phosphatase (AP)) using a fluorometric microplate assay and lignin degrading enzymes (phenol oxidase (Phenox) and peroxidase (Perox)) using a colorimetric microplate assay following the methods described by Brzostek et al. (2015) and Saiya-Cork et al. (2002) .

Problems/Departure from Proposed Methodology:

During the 2014 field campaign, we lost 3 months of summer data due to unforeseeable equipment failure of our primary instrument, the PTR-MS. While this was unfortunate, it did not detract from testing our main hypothesis/question, in which we posited that while plant canopies are likely to be the dominant source of bVOCs in this forest once the leaves have fully expanded, soil bVOCs will be dominant during the spring and fall periods when root and mycorrhizal carbon allocation is greatest at this site (R. Phillips; *unpublished data*). As such, we were still able to take measurements in the spring (April and May) and fall (September and October), but of course, seeing how the dynamics changed in the summer would have been useful to gain a more comprehensive understanding of the ecosystem's soil bVOC dynamics.

To address this objective, we also anticipated the use of our collaborator's laser-induced fluorescence flow tube instrument to determine OH reactivity at the site, which is directly proportional to the concentration of VOCs. This approach would allow us to compare observed OH reactivity with modeled reactivity and determine whether soil VOC emission may be responsible for any observed missing reactivity, which has been cited in other systems. Unfortunately, this instrument also required maintenance during the 2014 campaign and we were unable to obtain these data. However, we were able to obtain these data the following year (described below).

Finally, to assess the degree to which soil VOCs contribute to the total VOC/carbon budget, we proposed to make concurrent soil and canopy VOC emission measurements, the latter made possible through an aboveground profiling approach leveraging existing infrastructure at the Ameriflux tower. Unfortunately, due to unforeseen space limitations at the field site, we were unable to transfer and house the PTR-MS at the tower to obtain these profile measurements during the 2014 season. However, we were able to obtain these data in both 2015 and 2016 (described below).

Key Results:

While there was significant effect of time on total bVOC flux ($F_{3,61}$=3.56, p=0.02), there was no difference in total flux between ECM and AM-dominated plots (p=0.32), nor an interactive effect between time and mycorrhizal association (p=0.73). Total net flux rates (ECM + AM) were different between April and May (p=0.004) and May and October (p=0.01), with AM plots exhibiting a *c.*185% decrease in emissions from April to May (p=0.02) while EMC plots showed *c.* 140% increase in emissions from May to October (p=0.04; Figure 2).

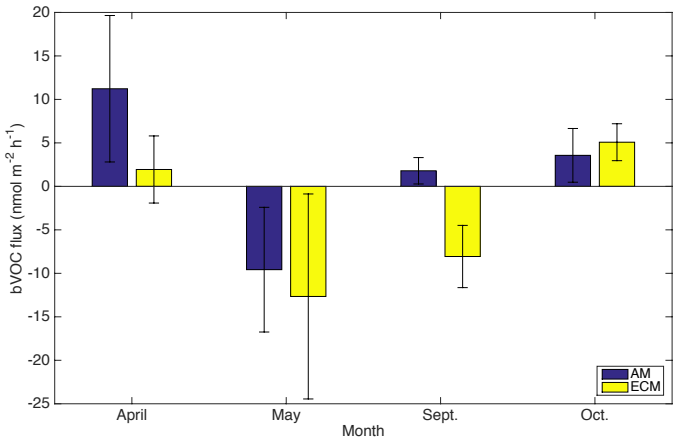

Fig. 2 Total bVOC flux of the 20 compounds of interest measured in both ECM (yellow bars) and AM plots (blue bars) during the beginning and end of the 2014 growing season.

The net flux of approximately 30% of the compounds investigated was not significantly affected by time, mycorrhizal association, or the interaction of the two variables (*m/z* 57, 59, 61, 93, 107). However, for the remaining compounds, time was a significant main effect, with the exception of *m/z* 121 (C_9 aromatics) and *m/z* 135 (C_{10} aromatics) The net fluxes of these compounds were significantly affected by the tree species/mycorrhizal associations present in the plots ($F_{1,10}$=5.68, p=0.04). In other words, when averaged over the entire sampling period, AM plots showed significantly more uptake of *m/z* 121 and 135 relative to ECM plots that exhibited net emission rates. However, these fluxes make up < 0.1% of the total net flux for AM and ECM plots over the season and are thus not a significant contribution to overall emission patterns.

There was a significant effect of mycorrhizal association on acid phosphatase (AP) ($F_{2,26}$=12.01, p=0.0002) and *N*-acetyl-glucosaminidase (NAG) activity ($F_{2,26}$=4.67, p=0.02), with ECM plots exhibiting significantly higher enzyme activity levels than both the AM-dominated and trenched plots (Fig. 6). Specifically, the AP activity in ECM plots was 33% higher than AM plots and 62% higher than the trenched plots while NAG activity was 53% higher in ECM plots relative to AM plots and 88% higher than the activity observed in the trenched plots. In contrast, we observed no difference in either of the lignin degrading enzymes, phenol oxidase (Phenox) or peroxidase (Perox) activity between the plot types (p=0.80 and p=0.16, respectively; Figure 3).

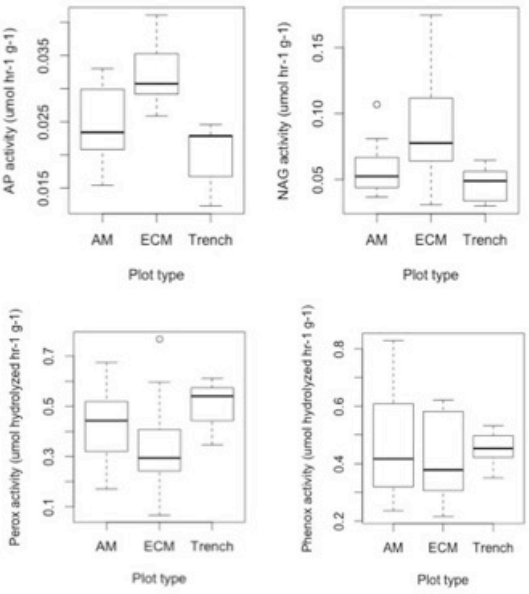

Fig. 3 Total bVOC flux of the 20 compounds of interest measured in both ECM (yellow bars) and AM plots (blue bars) in the beginning and end of the growing season.

Our data suggest that both AM and ECM-dominated forest soils are a net C sink early in the growing season following leaf-out. The small net bVOC fluxes throughout the year (on the order of hundreds of nanograms m^{-2} hr^{-1}) obscures important differences in the source-sink dynamics of individual bVOC species, particularly acetaldehyde (*m/z* 45), acetone (*m/z* 59), isoprene (m/z 69), and monoterpenes (*m/z* 137) that differ among AM and

ECM dominated plots. We are currently completing multivariate analyses investigating the relative importance of species composition (tree species litter inputs) and various abiotic factors in driving these seasonal dynamics. Preliminary results show non-methanol bVOC flux in aggregate is not significantly related to temperature (p>0.05), except in ECM-dominated plots in the fall where it explains 71% of the variability (Figure 4); these findings have important implications for bVOC modeling. While differences between ECM and AM-dominated plots suggest a microbial/biotic role in determining these flux dynamics, we must also consider the microbial communities associated with tree species litter types and how the environment interacts with these biotic factors. The results of these analyses will be presented at AGU this December and included in our in preparation manuscript listed below.

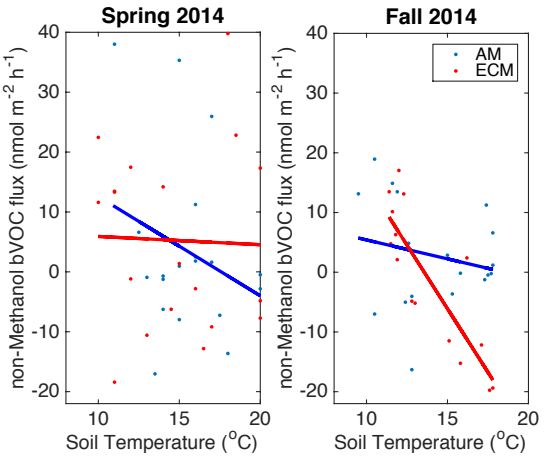

Fig. 4 Total bVOC flux of the 20 compounds of interest measured in both ECM- (red dots) and AM-dominated (blue dots) plots during the spring and fall of 2014. Soil temperature is a main driver of fluxes from ECM plots but only in the Fall.

Objective 2: Partition soil emissions into autotrophic (i.e. root and mycorrhizal-derived) versus heterotrophic sources and determine underlying biological mechanisms controlling emission dynamics.

Hypothesis 2: Soil VOCs will be derived primarily from litter-degrading fungi in AM-dominated plots and from litter-degrading fungi and root-mycorrhizal activities in ECM-dominated plots.

Problems/Departure from Proposed Methodology:

We originally proposed to remove/reduce the flux of C from roots to soil thereby altering the flow and availability of C to mycorrhizal fungi and other soil microbes via girdling. This approach was designed to enable us to examine the temporal responses of VOC emissions in response to limited autotrophic C available as a substrate for VOC production. However, we once again had instrumentation failure in the beginning of 2015, which forced us to reevaluate our goals and what we could accomplish. Together, our 2014 field data (see above) and a preliminary laboratory incubation studying assessing the relative contribution of soil bVOCs (data not shown) suggested that limiting carbon belowground via girdling would only decrease already low levels of emissions making it difficult to asses these dynamics due to low signal:noise ratios. Interestingly, the data also suggest that the soil is more of a sink than a source, and despite high levels of emissions from leaf litter alone, when added to soil in the laboratory, the emissions decreased significantly. Thus, we decided to forego the major effort of a girdling study and carried out a higher-resolution time series experiment during the leaf fall period to examine the role of the soil as a sink for leaf litter derived VOCs, thus mitigating their emissions to the atmosphere. Doing so we still addressed the relative role of litter vs. root-degrading microbial activities and did so during a critical transition period—leaf senescence. We describe the approach and results below.

We also proposed to employ a non-radioactive standard enzyme assay technique consisting of a series of protein assays, volatile collections, and GC analyses to examine the kinetic properties of terpene synthases (TPS) extracted from fungi and roots associated with both ECM and AM collected in the field. Because of instrumentation failure in the field, we decided to collaborate with our German colleagues at the Helmholtz Zentrum in Munich Germany to carry out this work in a phytotron, where PI Trowbridge was already working for six months as part of a separate fellowship. Instead of comparing ECM and AM associations, we compared ECM vs. limited ECM-associations and how those influence C allocation to above and belowground BVOC emissions before and after a biotic stress—herbivory. We describe the approach and results below.

Approach:

Field measurements of soil bVOC during senescence

During July of 2015, six soil chambers were installed in one ECM-dominated and AM-dominated plot at the Morgan Monroe State Forest site described above. The plots were chosen due to their proximity to the Ameriflux tower--so to access the profiling system--and the PTR-MS, which was transported to the field laboratory for the 2015 season. Static chamber measurements of CO_2 and bVOC emissions were taken concurrently from each collar weekly from October through November. Briefly, chambers were directly connected to the PTR-MS (as opposed to the bag sampling protocol used in 2014) and a measurement of ambient air in the chamber was sampled prior to closing the chamber. The chamber was then sealed and the PTR-MS continued to take measurements of each compound every 0.2 s for 30 minutes. The sample line was split to allow for simultaneous respiration measurements to estimate metabolic activity (LI-6400, Licor, Lincoln, NE). For a sub-sample of collars, measurements were taken throughout the sampling period where the leaf litter was taken out of the chamber in order to compare emissions from soil only, leaf litter only, and soil + leaf litter inputs at that time. We are currently collaborating with the Richardson Lab at Harvard University to quantify phenocam data and correlate our emission data to the percent of leaf area on the trees through the senescent period. These data could be useful for flux modeling where soil bVOC fluxes could be estimated by assessing the amount of leaf area present via remote sensing. When chamber measurements were not being made, the PTR-MS was connected to a canopy profiling system that sampled 7 heights for 5 minutes at each height. These observations are being used to estimate whole-ecosystem bVOC efflux using inverse methods.

Laboratory experiment assessing mycorrhizae x herbivory effects on C allocation to above and belowground bVOCs

The experiment was conducted using seedlings of two conifer species, *Picea abies* (Norway spruce) and *Pinus sylvestris* L. (Scots pine) where half of

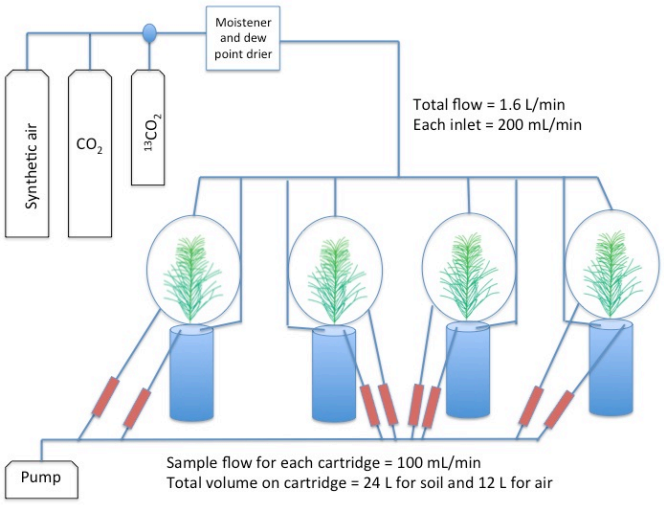

Fig. 5 Schematic of experimental design to assess mycorrhizae x herbivory effects on C allocation to above and belowground bVOC emissions.

trees for each species were assigned to the "mycorrhized" treatment and half to the "non-mycorrhized" treatment (n=22). Because it was not possible to exclude all mycorrhizal associations from nursery-obtained trees, we attempted to significantly lower the total mycorrhizal colonization rates by using a combination of microwaved forest soil and sterile potting soil. Plants remained outside in the garden at the Helmholtz Zentrum, München from April to August 2014 under mostly shaded conditions. Needles on the lower portion of each seedling were removed on 1 August so to avoid mechanical damage when fitting the soil cuvette covers during measurements, and all plants were transferred to the greenhouse on 14 August.

Both above- and belowground bVOC fluxes were measured simultaneously from four trees in parallel using the experimental set-up is illustrated in Figure 5. The set-up was installed in the Helmholtz Zentrum München Phytotron facility on a bench with 16 fluorescent lights emitting ~200 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR) that were on a 12:12 day:night timer. One tree from each species x mycorrhizal treatment was selected for each sampling period (four days) with at least one tree serving as a control for methyl jasmonate (MeJA) spraying. In total, n=5 for each species x mycorrhizal treatment that received both MeJA application and ^{13}C labeling, n=3 for each species x mycorrhizal treatment that received the control spray application and ^{13}C labeling, and n=3 for each species x mycorrhizal treatment that received the control spray application and no ^{13}C label. Teflon-coated O-rings were placed around the inside of the pot prior to installing the lids, which were cut in half with a hole in the middle to fit around the bole of the seedling and a hole on either side to serve as the inlet and outlet for belowground sampling. Care was taken to avoid any needle damage or excessive manipulation of the plants during installation. A silicone-based putty was applied around the bole and along the circumference of the pots so to ensure the chambers were gas-tight.

Cuvettes were flushed continuously with synthetic VOC-free air (21% v/v O_2, 79% v/v N_2, BASI Schoberl, Germany) mixed with CO_2 with a natural abundance of $^{13}CO2$ to create a final concentration entering each cuvette of 400 ppmv. The airstream was bubbled through pure, distilled water at 2 L min^{-1} and directed to each of the eight inlets so the final flow through the system was 200 mL min^{-1}. After sealing the pots, the inlet was attached and each cuvette was ensured to be gas tight by using a flow meter attached to the outlet. Teflon bags (~1000 cm^2 volume; Toppits, Confresco Frischhalteproduckte GmbH) were secured around the aboveground portion of the seedlings at the base of the bole and at the top using rubber bands and an inlet and outlet were created using Teflon baking paper as reinforcement for the bag and a hole punch. The flow rate of the outgoing

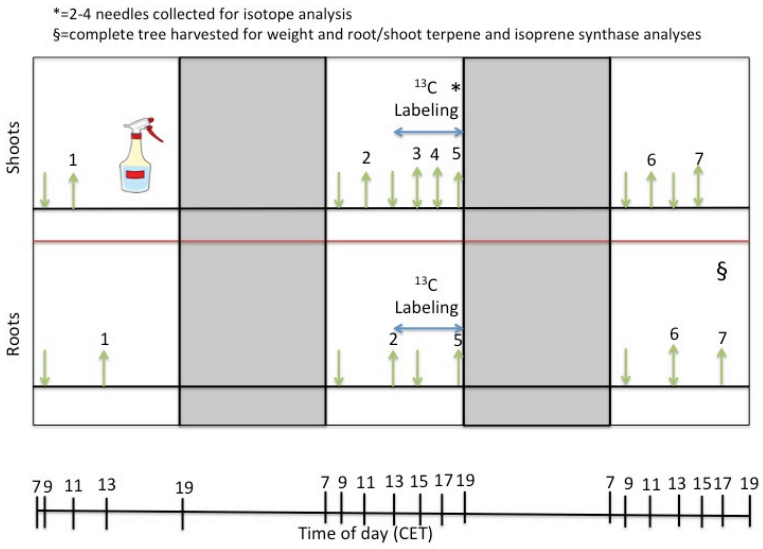

Fig. 6 Sampling regime for GC-MS analysis of above and belowground VOCs. White boxes indicate day and dark boxes indicate night. A downwards pointing arrow indicates a sampling tube was attached and the following upwards arrow indicates the sampling tube was removed. Time of day is indicated along the bottom and 13C labeling is indicated by the blue arrow.

airstream was created from a pump and controlled using mass flow controllers (MKS) set at 100 mL min^{-1}. Flow rates were checked prior to sampling and exact flows were recorded. All cuvettes and flow rates were established one day prior to sampling and left overnight to equilibrate.

For GC-MS analysis, volatile compounds were collected at 100 mL min^{-1} onto solid adsorbent cartridges (Gerstel) that contained 40 mg of Tenax TA and 10 mg of carbopack B (Supelco). Aboveground plant parts were sampled for 2 hours while belowground cuvettes were sampled for 4 hours (24 L and 12 L of air, respectively). Following sampling, each tube was capped and stored in a 98-ATEX tray (Gerstel, Germany) in the dark at 4 °C until analysis. A schematic detailing the sampling times is shown in Figure 6. A PTR-MS-TOF (IONICON, Innsbruck, Austria) was used during the whole campaign period to collect VOCs from both above and belowground chambers at 70 mL min^{-1}, switching cuvettes every 7 minutes with an integration time of 42 seconds (i.e. six data points for every measurement period).

The adsorption tubes were analyzed within one week of sampling by gas chromatography-mass spectrometry (GC-MS) (Agilent 7890A GC system and 5975C inert XL MSD with triple-axis detector MS, Agilent Technologies, Englewood, CO) equipped with a thermal desorption unit (TDU), cryoconcentrator, and multi-purpose sampler (MPS) (Gerstel, Germany). Mass spectra were used to calculate the incorporation of ^{13}C into BVOCs according to Ghirardo et al. (2010). Terpene synthase activity for above and belowground plant tissues was analyzed as described by Martin et al. (2002)

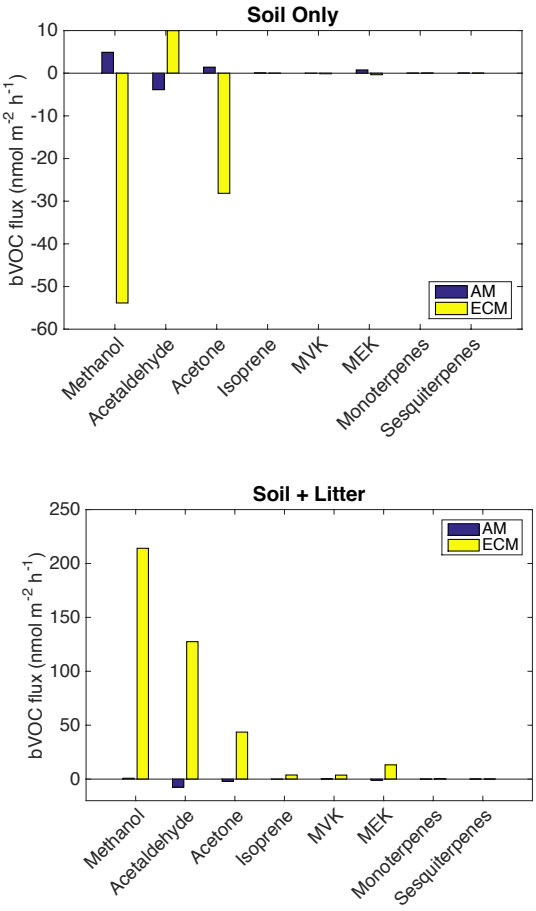

Fig. 7 Median chamber-measured fluxes from collars in an ECM (yellow bars) and AM-dominated (blue bars) plot (n=6) over entire sampling period from October – November.

Key Results:

Over the entire sampling period at MMSF (October – November) in ECM plots, soil alone appears to be large bVOC sink, but leaf litter inputs result in a net bVOC source while AM-dominated soils exhibit far lower uptake and efflux rates. We have yet to determine how varying levels of leaf litter inputs alter the source-sink dynamics during litter fall. While we expect net efflux to increase with time and litter fall, it will be telling whether the soil acts as a stronger sink as well, thus mitigating fluxes to the atmosphere. Interestingly, on average we observed higher mean CO_2 flux rates from the ECM plot collars with or without litter relative to the ACM plot (p>0.05). Once again, we will investigate this flux as a function of leaf litter input to see how respiration may or may not correlate with bVOC emission, but these data suggest that the increase in leaf biomass may drive these emissions as opposed to metabolic differences estimated via respiration measurements. Canopy profile data are still processed to investigate how

bVOC fluxes are changing through senescence and if we can detect changes in forest floor emissions when canopy vegetation is no longer a dominant source. Initial results suggest that the canopy acts as a strong bVOC source, as expected, with an unexpected source in the subcanopy likely due to the presence of spicebush, and a smaller net bVOC flux to the atmosphere from the forest floor.

From our pulse-chase laboratory experiment at the Helmholtz Zentrum, we failed to observe an effect of mycorrhizae on any induced VOC response, suggesting that microbial effects were unrelated to source-sink dynamics driving terpene emissions. We did observe significant induction in both above-and-belowground terpene emissions with the application of MeJA, albeit, the belowground emissions were orders of magnitude lower than their aboveground counterparts. In both spruce and pine, the greatest incorporation of labeled carbon in aboveground emissions was in the oxygenated monoterpenes (16% and 18%, respectively), which were also almost completely produced *de novo*. Label incorporation rates in aboveground monoterpene hydrocarbons and sesquiterpenes was significantly lower (~10%, $p<0.05$). These high emissions but low incorporation rates suggest that MeJA, an important plant stress hormone, causes biophysical changes in addition to solely up-regulating terpene synthesis, as has been often assumed. While TPS activity was up-regulated following herbivory, induced emissions belowground exhibited no incorporation of recently assimilated carbon. This result suggests either older carbon sources or some altered biophysical properties via stress signaling. Results demonstrate the critical role of existing plant carbohydrate reserves in belowground BVOC responses to herbivory.

Objective 3: Quantify soil emissions across a gradient in forest composition (tree species and their soil microbial associates)

Hypothesis 3: The magnitude of soil VOC emissions will vary by forest composition, with greater emissions occurring in forests dominated by ECM trees due to the greater fungal biomass in these soils.

Approach:
Addressing this objective requires the data collected above to explore predicted ecosystem emission factors (which are highly uncertain) against observations along the mycorrhizal gradient, specifically testing the hypothesis that soil VOC emissions will be greater in forests dominated by ECM species. We are currently working on developing a soil VOC model using maximum likelihood approaches to discriminate against multiple model formulations to arrive at minimal models of VOC efflux for different VOC species across the mycorrhizal gradient; initial results will be presented at the 2016 AGU Fall Meeting. The anticipated outcome will be parsimonious models of belowground VOC emissions that follow formulations similar to global VOC models (Guenther et al. 1995) for the purpose of improving these global models using whole-ecosystem observations. Our physiological data and C allocation dynamic results will inform mechanistically-realistic models of VOC efflux after the initial phenomenological models are parameterized and validated.

In 2015, we also participated in an international collaborative effort (the IRRONIC campaign, led by Dr. Phil Stevens at Indiana University) to assess how various analytic techniques can contribute to modeling OH reactivity and account for the observed discrepancy

between the predicted and measured oxidative capacity of the atmospheric boundary layer and troposphere to address Objective 1.

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Products developed under the award and technology transfer activities

Journal articles

Trowbridge AM, Stoy PC, Cook AA, Phillips RP (*In prep*) Net bVOC emissson from a mixed hardwood forest floor: The importance of species composition and belowground processes in driving seasonal differences. *To be submitted to Journal of Geophysical Research Spring 2017*

Trowbridge AM, Jacobs L, Stoy PC, Cook AA, Phillips RP (*In prep*) Picking up what you're putting down: How soil uptake and leaf litter inputs influence belowground bVOC emissions during leaf senescence. *To be submitted to Atmospheric Environment Spring 2017*.

Trowbridge AM, Ghirardo A, Niederbacher B, Phillips RP, Schnitzler J-P (*In prep*) Tracking the dynamics of recently-assimilated carbon into above and belowground bVOC emissions following simulated aboveground herbivory. *To be submitted to Tree Physiology Summer 2017*.

Conference papers

Trowbridge AM, Stevens P, Stoy PC, Phillips RP (2014) Up-rooting surface-atmosphere exchange models: How mycorrhizal associates may affect soil and canopy emission profiles. DOE TES and SBR joint ESS PI Meeting, Potomac, MD.

Trowbridge AM, Stevens P, Stoy PC, Phillips RP (2015) Scratching the surface of belowground volatile emissions: Determining patterns of soil bVOC source-sink dynamics. DOE TES and SBR joint ESS PI Meeting, Potomac, MD.

Trowbridge AM, Jacobs L, Stevens P, Stoy PC, Phillips RP (2016) Net bVOC emissions from a mixed hardwood forest: Importance of species composition and belowground processes in driving dynamics. DOE TES and SBR joint ESS PI Meeting, Potomac, MD.

Cook AA, Trowbridge AM, Jacobs L, Stoy PC, Stevens P, Phillips RP (2016) The role of mycorrhizal associations in controlling biogenic volatile organic carbon flux from a temperate deciduous forest. American Geophysical Union Annual Meeting, San Francisco, CA.

Trowbridge AM, Ghirardo A, Niederbacher B, Phillips RP, Schnitzler J-P (2017) Tracking the dynamics of recently-assimilated carbon into above and belowground bVOC emissions following simulated aboveground herbivory. Gordon Research Conference on Plant-Herbivore Interactions, Ventura, CA.

Networks or collaborations fostered

We developed collaborations with Phil Stevens (Indiana University), Sebastian Dusanter (Ecole des Mines de Douai), and other international participants in the IRRONIC field campaign, the Andrew Richardson group (Harvard) on phenocam observations, the Joerg-Peter Schnitzler group (Helmholtz Zentrum Munich) on stable isotope labeling, terpene synthase analyses and VOC measurement and analysis, and the Gil Bohrer group (Ohio State) on interpreting canopy VOC profile observations.

Other products or databases, educational aid or curricula, instruments or equipment
Our project objectives and results have also been disseminated to students, teachers, and managers through a number of outreach activities. In July 2015, we offered a lesson on forest/soil ecology, VOCs, the July intensive IRRONIC campaign, and PTR-MS set-up to incoming freshman research students involved in the Integrated Freshman Learning Experience program through Indiana University (IU). Later that month a similar lesson was given to two minority high school women in the Jim Holland Summer Science Research Program through IU. In October of 2015, we gave three thirty-minute lecture/demos of the bVOC project and soil/forest ecology at MMSF to foresters, soil scientists, and science educators as part of the Central States Forest Soils Workshop. Finally, in November 2015, we developed and delivered a thirty-minute lecture/demo to the School of Public and Environmental Affairs (SPEA) Masters class in Forest Ecology and Management, including two undergraduate honors students through IU.