Bacteria and fungi associated with isoprene consumption in soil

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Abstract

Isoprene is a reactive volatile organic compound released from the biosphere that can be abundant in the planetary boundary layer, where it can have a myriad of effects on atmospheric chemistry and secondary aerosol formation. There is currently a high degree of uncertainty in forecasts of how atmospheric isoprene concentrations will shift in response to anthropogenic land-use change and climate change. One source of this uncertainty is that studies of terrestrial isoprene fluxes have almost entirely focused on plant sources of isoprene, largely ignoring the role of soils as a sink of isoprene and the corresponding microbial consumption of isoprene. We quantified isoprene consumption rates by the microbial communities found in two distinct soils under laboratory conditions and used high-throughput sequencing to identify the bacterial and fungal taxa that increased in relative abundance with changes in isoprene mixing ratios. On average, soil microbes were capable of consuming 68% (ranging 55% to 80%) of the gaseous isoprene provided to the soils (2–200 ppbv) in a flow-through experiment. Consumption rates increased with increasing levels of isoprene with rates reaching 770 pmol g⁻¹ h⁻¹ in one of the soils exposed to the highest mixing ratio of isoprene (200 ppbv). Increases in isoprene levels were associated with significant shifts in the composition of both soil bacterial and fungal communities. A wide range of taxa were associated with isoprene consumption including members of the Actinobacteria, Proteobacteria, Gemmatimonadetes, and Zygomycota phyla with many of the taxa being closely related to known hydrocarbon degraders. Soils likely represent a significant sink of atmospheric isoprene and our results suggest that a wide range of bacterial and fungal taxa are capable of isoprene degradation.

Introduction

Isoprene (2-methyl-1,3-butadiene [C₅H₈]) is the second-most abundant volatile organic compound in the atmosphere (after methane) with estimated global emissions of isoprene exceeding 500 Tg yr⁻¹ (Guenther et al., 2012). The atmosphere acts as the primary sink for isoprene through oxidation with the hydroxyl radical (OH). In areas with elevated levels of nitrogen oxides, the oxidation of isoprene leads to the formation of tropospheric ozone, a major pollutant and greenhouse gas (Chameides et al., 1992). Other effects of atmospheric isoprene oxidation include the formation of tropospheric carbon monoxide, global transport of nitrogenous compounds, extended residence times of other atmospheric trace gases, and the formation of secondary organic aerosol (Granier et al., 2000; Monson and Holland, 2001; Claeyts et al., 2004). Isoprene is clearly a key trace gas given its myriad of effects on chemical reactions in the atmosphere.

There is currently a high degree of uncertainty in models of terrestrial isoprene fluxes and the associated predictions of how atmospheric concentrations of isoprene will be altered by climate or land-use changes (Arneth et al., 2011). One reason this uncertainty persists is that we still have an incomplete understanding of the controls on isoprene fluxes and fate 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.
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(Sharkey et al., 2008; Pacifico et al., 2009). 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 and Yavitt, 1997). Cleveland and Yavitt (1997) estimated the global soil isoprene sink at 20.4 Tg yr⁻¹, approximately 4% of global emissions. These authors went on to demonstrate that the isoprene sink was microbial-driven and followed microbial temperature responses with a maximum consumption rate at 30°C (Cleveland and Yavitt, 1998). Likewise, two later studies combined the dynamics of isoprene plant emissions with soil consumption rates in enclosed ultraviolet light-depleted mesocosms containing high levels of isoprene (Pegoraro et al., 2005, 2006). They also concluded that soil acts as a significant atmospheric sink of isoprene (3% of global emissions). However, all previous studies have relied on closed static chambers that measured consumption of isoprene at levels far higher than what is typically measured in ambient air with consumption rates only measured over short time periods until the isoprene was exhausted within the closed chamber.

Several soil microorganisms have been shown to consume isoprene as their sole carbon and energy source in vitro including members of the Actinobacteria (Cleveland and Yavitt, 1998), Nocardioides (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 Sordariomycetes and Eurotiomycete fungal isolates are able to consume hydrocarbons that are similar in chemical composition, including short chain n-alkanes (Shennan, 2006).

Here we use a dynamic flow-through system to determine isoprene consumption rates at atmospherically-relevant levels over the course of 45 days combined with high-throughput sequencing to identify the 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 levels provided to the soil microorganisms with significant consumption even at very low isoprene mixing ratios, (2) in both soils, isoprene consumption would 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 groups).

Materials and methods

Soil collection

Two distinct soils were collected in October 2013 near the University of Colorado Mountain Research Station (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. From beneath adjoining Populus tremuloides (quaking aspen) and Pinus contorta (lodgepole pine) stands, approximately 12 mineral soil samples, each 5 cm deep, were collected and mixed together, yielding one composited soil sample from each of the two vegetation types. Soils were collected after the leaves had dropped from the deciduous P. tremuloides. During the microbial decomposition of litter from P. tremuloides and P. contorta isoprene is emitted at similar rates (Gray and Fierer, 2012). However, since P. tremuloides had recently dropped its leaves (and P. contorta had not), we expected that those microorganisms able to consume isoprene would be more abundant, or at least more active, in the soil beneath the decomposing P. tremuloides leaves. The two distinct soils were stored at 4°C, sieved to 2 mm and homogenized within 24 h of collection. Subsamples of the two soils were sent to the Soil, Water, and Plant Testing Laboratory at Colorado State University, Fort Collins, CO, USA for analyses of soil edaphic characteristics (Table 1).

<table>
<thead>
<tr>
<th>Soil Collected Beneath:</th>
<th>pH</th>
<th>NO₃ (ppm N)</th>
<th>%N</th>
<th>%C</th>
<th>C:N</th>
<th>P (ppm)</th>
<th>K (ppm)</th>
<th>Texture Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pinus contorta</em></td>
<td>5.0</td>
<td>5.0</td>
<td>0.194</td>
<td>6.24</td>
<td>32.2</td>
<td>5</td>
<td>174</td>
<td>Sandy Loam</td>
</tr>
<tr>
<td><em>Populus tremuloides</em></td>
<td>5.6</td>
<td>1.5</td>
<td>0.480</td>
<td>9.09</td>
<td>18.9</td>
<td>9</td>
<td>256</td>
<td>Sandy Loam</td>
</tr>
</tbody>
</table>

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Soil incubations

Approximately 20 g of each soil was added to individual 125 ml glass jars, with 6 replicate jars per soil and per experimental mixing ratio of isoprene (specifically 0, 2, 20, 200 ppbv) for a total of 48 jars. At each isoprene level, two 125 ml glass jars were included without soil as controls. De-ionized (DI) water was added to each soil to bring the soil up to 60% of water holding capacity (WHC). Blank jars received 10 ml of DI water. Each 125 ml jar was placed inside of a 500 ml glass chamber containing 50 ml of DI water to keep relative humidity near 100% during the experiment. A Teflon lined cap, with Swagelok brass bulkhead fittings for
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Table 2. Isoprene mixing ratios in the supplied gas mixtures

<table>
<thead>
<tr>
<th>Sampling Period (every 9 days)</th>
<th>Experimental Chambers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 ppbv</td>
</tr>
<tr>
<td>Actual Source Tank Mixing Ratios</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>&lt; 0.2 ppbv</td>
</tr>
<tr>
<td>2</td>
<td>&lt; 0.2 ppbv</td>
</tr>
<tr>
<td>3</td>
<td>&lt; 0.2 ppbv</td>
</tr>
<tr>
<td>4</td>
<td>&lt; 0.2 ppbv</td>
</tr>
<tr>
<td>5</td>
<td>&lt; 0.2 ppbv</td>
</tr>
<tr>
<td>Autoclaved</td>
<td>&lt; 0.2 ppbv</td>
</tr>
</tbody>
</table>

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Isoprene quantification

Isoprene levels in the outlet flow of each chamber were measured 5 times during the 45-day experiment (approximately every 9 days) and once at the end of the 8-day sterilized soil experiment. Isoprene levels were determined by collecting gas samples onto multi-stage solid adsorbent cartridges. The cartridges were made inhouse: 9 cm long, 0.64 cm o.d., glass tubes packed with 0.14 g each of Carboxen 1000 and Carboxen 1016 solid adsorbent (Sigma–Aldrich) or stainless steel tubes packed with 0.22 g each of Carboxen 1000 and Carboxen 1016. Air from the chambers’ outlet flow was routed to the adsorbent cartridges, which were temperature controlled in an incubator set at 40°C to prevent water vapor from adsorbing onto the cartridges (Karbiwnyk et al., 2002). This temperature was determined not to influence the efficiency of isoprene adsorption onto the cartridge. The sampling flow rate (approximately 30 ml min⁻¹) was controlled using needle valve rotameters and exact flow through each cartridge was measured downstream of the cartridge with an Agilent ADM1000 flowmeter. Sampling times and volumes ranged from 8 min and ~240 ml for the 200 ppbv samples to 60 min and ~1800 ml for the 2 ppbv and 0 ppbv samples. Sample cartridges were kept at 4°C until they could be analyzed (within 14 days) by thermal desorption (Perkin–Elmer ATD400) with compound separation on a gas chromatography–flame ionization detector (GC-FID) instrument (Hewlett-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 from 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 were analyzed by manual integration using PeakSimple software (SRI Instruments, Torrance, CA) and authenticated isoprene standards. Isoprene mixing ratio results from the soils incubated with no isoprene administered are not included in the analyses as they were determined to be below the conservative estimated detection limit of the setup (0.2 ppbv). The measured isoprene mixing ratios 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:
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\[ J = \left( x_b - x_s \right) \times \frac{Q}{a} \times W \]

where \( J \) is the isoprene flux to the soil in nmol of isoprene per gram of dry weight soil (gdw) per min, \( x_b \) is the average measured isoprene mixing ratio in the blank chamber in ppbv, \( x_s \) is the measured isoprene mixing ratio in the soil chamber in ppbv, \( Q \) 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 of the soils to the different isoprene levels, 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, we collected triplicate 0.1 – 0.2 g subsamples from each of the 48 soil samples yielding a total of 144 samples. Genomic DNA was extracted from these soil samples 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 DNA recovery. 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 targeting 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 sequences were demultiplexed with quality filtering and operational taxonomic unit (OTU) clustering at the 97% similarity level conducted using the USEARCH/UPARSE pipeline (Edgar, 2010, 2013). Singleton sequences were removed before OTU determination. The remaining 16S rRNA 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 across the samples. Taxonomic identities were assigned using the RDP classifier (Wang et al., 2007) with a confidence threshold of 0.5 against either the Greengenes August 2013 database (DeSantis et al., 2006) for bacteria and archaea, or the UNITE December, 19 2013 database (Koljalg et al., 2013) for fungi. The raw sequence data from this study have been deposited and made publicly available in Figshare (doi: 10.6084/m9.figshare.1318807).

Data analysis

To determine whether increasing isoprene levels resulted in differences to bacterial and fungal community composition, bacterial and fungal OTU relative abundance data were square-root transformed to minimize the influence of rare taxa followed by the determination of the pair-wise distances between communities using the Bray-Curtis distance metric. The distance matrices were analyzed by Analysis of Similarity (ANOSIM) using Primer-version 6 software (Primer-E, Plymouth, UK). Principal coordinate analysis in the R statistical software (R Core Team, 2014) was used to visualize the community data. To identify which taxa responded to increases in isoprene mixing ratios, multiple regression analyses were conducted in R (P-values uncorrected for multiple comparisons). Levels of isoprene in the flow of the autoclaved chambers were compared to that of the autoclaved blanks using T-tests to determine whether there was a significant abiotic flux of isoprene in the sterile soils.

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 1) with consumption rates reaching 770 pmol g\(^{-1}\) h\(^{-1}\) at the highest isoprene mixing ratio. The rate of consumption reported here represents the ability of the soil microbial population to consume isoprene at the soil surface, and does not necessarily represent the ability of soil microbes to influence canopy-level isoprene fluxes. The isoprene levels provided to the soils (2 and 20 ppbv) spanned the range of isoprene present in the atmosphere with the 200 ppbv level being higher than most soils would be exposed to under field conditions. For instance, Wiedinmyer et al. (2005) measured ground level isoprene averaging 10.7 ppbv with a maximum of 35.8 ppbv across 5 sites in Illinois and Missouri. In rural Texas,
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Ground level isoprene mixing ratios averaged 2.6 ppbv with a range of 0.3 to 10.2 ppbv (Wiedinmyer et al., 2001). We detected considerable isoprene consumption at all isoprene levels, even at the lowest mixing ratio (2 ppbv) where 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 administered.

The fraction of isoprene consumed ranged from 55% to 80% across all isoprene levels administered at the last sampling event (Figure 1). Previous field research using dynamic flow soil chambers near this study’s soil collection site measured consumption rates as high as 7.4 nmol m\(^{-2}\) h\(^{-1}\), which equated to 67% of ambient isoprene mixing ratios as measured at the inlet and outlet of the chambers (Gray et al., 2014). The average isoprene consumption rate in the field study was 2.0 nmol m\(^{-2}\) h\(^{-1}\) under the flow conditions used in that study or an estimated 33% of the measured ambient mixing ratio. The rates of consumption in the field study falls within the range of consumption rates measured in this study, however flow rates likely impact the overall measured consumption rates and thus do not necessarily represent what occurs in the soil under natural conditions. The cause of the lower average consumption rates in the field study could be due to the different flow rates used or possibly to the drier soil and colder temperatures in the field, which have been shown to decrease isoprene consumption in soils (Cleveland and Yavitt, 1998; Pegoraro et al., 2005). Our measured consumption rates from this lab-based study were comparable to those measured by Cleveland and Yavitt (1998). In that study, isoprene consumption was measured at 24 pmol g\(^{-1}\) h\(^{-1}\) in a soil with stated ambient isoprene mixing ratios of approximately 10 ppbv, while we measured average rates of 7.7 and 62 pmol g\(^{-1}\) h\(^{-1}\) at 2 and 20 ppbv, respectively. The consistency in these findings across studies using different techniques suggests that soil microorganisms are able to consume a substantial percentage of the isoprene in the ambient air above soils.

Isoprene consumption in soils is primarily a microbially-mediated process as evidenced by a complete lack of consumption in the sterilized soils (Table 3). Our results agree with other studies that saw little to no isoprene consumption in autoclaved soils (Cleveland and Yavitt, 1998). Also, the consumption of isoprene in soils has been shown to follow first order reaction kinetics suggesting that the levels of isoprene tested here and in other studies (ranging from 0 ppbv to 1500 ppbv) is below the Michaelis constant (\(K_m\)) for Michaelis-Menten kinetics (Cleveland and Yavitt, 1998; Pegoraro et al., 2005). This is strong evidence that the ability for the soil microbial community to consume available isoprene is well below saturation, even at the higher isoprene concentrations tested. It is likely that the microbial biomass able to consume isoprene is large relative to the supply of isoprene, the enzyme responsible is very efficient or some combination of the two. As this was a laboratory-based study, we are unable to extrapolate our results to predict the global consumption of isoprene within soils in general due to the numerous environmental controls on microbial activities (e.g. moisture and temperature) and constraints on isoprene diffusion rates into soil. However, this work adds further support to the argument that it is important to consider the microbial metabolism of isoprene in soils when trying to describe and predict isoprene dynamics in the atmosphere (Cleveland and Yavitt, 1997; Pegoraro et al., 2005).
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Table 3. Mean isoprene mixing ratios in the autoclaved soils and in the ‘no soil’ control chambers at the end of an 8-day incubation

<table>
<thead>
<tr>
<th>Autoclaved Soil</th>
<th>Experimental Chambers</th>
<th>Mean Isoprene Mixing Ratios in ppbv (p-value from t-test between soil and control)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 ppbv</td>
<td>20 ppbv</td>
</tr>
<tr>
<td><strong>Populus tremuloides</strong></td>
<td>2.3 (0.83)</td>
<td>23.7 (0.64)</td>
</tr>
<tr>
<td><strong>Pinus contorta</strong></td>
<td>2.4 (0.24)</td>
<td>23.5 (0.73)</td>
</tr>
<tr>
<td><strong>No soil control</strong></td>
<td>2.3</td>
<td>22.8</td>
</tr>
</tbody>
</table>

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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 2). 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 basidio-

mycete fungi, while the *P. contorta* soil was dominated by ascomycetes. The distinctiveness of these microbial communities could be explained by the differences in associated plant species or soil edaphic factors which differed dramatically between the two soils (Table 1). Although these two soils harbored distinct bacterial and fungal communities, the microbial communities in each of these soils varied across the isoprene mixing ratio gradient (Figure 3), and many of the bacterial and fungal taxa that responded to the isoprene additions were similar across the two distinct soils. We found significant increases in the relative abundances of various bacterial and fungal taxa with increasing isoprene levels, but isoprene exposure had no significant effect on archaeal taxa. Those bacterial and fungal 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 2). Likewise, we are unable to distinguish whether the taxa increasing in abundance with increasing isoprene levels were directly consuming isoprene or if they were indirectly stimulated by 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).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>0 ppbv</th>
<th>2 ppbv</th>
<th>20 ppbv</th>
<th>200 ppbv</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proteobacteria</strong></td>
<td>34.00</td>
<td>33.41</td>
<td>37.57</td>
<td>38.28</td>
</tr>
<tr>
<td>Alpha-</td>
<td>13.45</td>
<td>13.48</td>
<td>15.74</td>
<td>16.08</td>
</tr>
<tr>
<td>Gammaproteobacteria</td>
<td>5.70</td>
<td>5.30</td>
<td>5.86</td>
<td>5.83</td>
</tr>
<tr>
<td>Delta-</td>
<td>7.08</td>
<td>7.34</td>
<td>7.32</td>
<td>7.75</td>
</tr>
<tr>
<td>Betaproteobacteria</td>
<td>7.20</td>
<td>6.72</td>
<td>8.12</td>
<td>8.11</td>
</tr>
<tr>
<td>Acidobacteria</td>
<td>17.07</td>
<td>17.08</td>
<td>17.09</td>
<td>16.82</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>14.57</td>
<td>14.84</td>
<td>12.18</td>
<td>11.74</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td>14.30</td>
<td>14.84</td>
<td>12.87</td>
<td>12.49</td>
</tr>
<tr>
<td>Planctomycetes</td>
<td>7.28</td>
<td>6.77</td>
<td>6.95</td>
<td>6.64</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>2.46</td>
<td>2.49</td>
<td>3.63</td>
<td>4.41</td>
</tr>
<tr>
<td>Gemmatimonadetes</td>
<td>2.36</td>
<td>2.64</td>
<td>2.64</td>
<td>2.70</td>
</tr>
<tr>
<td>Chloroflexi</td>
<td>1.75</td>
<td>1.68</td>
<td>1.59</td>
<td>1.64</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Fungi</th>
<th>0 ppbv</th>
<th>2 ppbv</th>
<th>20 ppbv</th>
<th>200 ppbv</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascomycota</td>
<td>39.96</td>
<td>42.14</td>
<td>38.52</td>
<td>39.77</td>
</tr>
<tr>
<td>(undetermined)</td>
<td>12.39</td>
<td>15.94</td>
<td>12.69</td>
<td>13.72</td>
</tr>
<tr>
<td>Archaeozooyctes</td>
<td>0.49</td>
<td>0.66</td>
<td>0.54</td>
<td>0.44</td>
</tr>
<tr>
<td>Dolioldymycetes</td>
<td>11.03</td>
<td>11.39</td>
<td>9.53</td>
<td>9.39</td>
</tr>
<tr>
<td>Leotymycetes</td>
<td>2.97</td>
<td>2.84</td>
<td>3.51</td>
<td>3.81</td>
</tr>
<tr>
<td>Eurozymycetes</td>
<td>1.27</td>
<td>0.95</td>
<td>1.28</td>
<td>1.32</td>
</tr>
<tr>
<td>Basidiomycota</td>
<td>48.76</td>
<td>44.72</td>
<td>49.31</td>
<td>46.42</td>
</tr>
<tr>
<td>Agaricomycots</td>
<td>34.27</td>
<td>32.56</td>
<td>36.12</td>
<td>33.86</td>
</tr>
<tr>
<td>Tremellicmycots</td>
<td>10.75</td>
<td>8.80</td>
<td>8.82</td>
<td>7.85</td>
</tr>
<tr>
<td>Wollfinmycots</td>
<td>0.04</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>(undetermined)</td>
<td>1.47</td>
<td>1.36</td>
<td>1.85</td>
<td>2.07</td>
</tr>
<tr>
<td>Microtrichymycots</td>
<td>2.07</td>
<td>1.79</td>
<td>2.17</td>
<td>2.36</td>
</tr>
<tr>
<td>Zygomycota</td>
<td>6.60</td>
<td>8.39</td>
<td>6.62</td>
<td>8.42</td>
</tr>
<tr>
<td>Glomeromycota</td>
<td>4.42</td>
<td>4.53</td>
<td>5.13</td>
<td>5.15</td>
</tr>
<tr>
<td>Chytridymycota</td>
<td>0.24</td>
<td>0.22</td>
<td>0.19</td>
<td>0.22</td>
</tr>
<tr>
<td>Microbiota</td>
<td>0.02</td>
<td>0.00</td>
<td>0.03</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Figure 2

Changes in relative abundance for the most abundant bacterial and fungal phyla.

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 after a 45-day incubation under varying mixing ratios of isoprene (0, 2, 20, 200 ppbv). 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 mixing ratios.

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Our results suggest that the bacterial communities responded more to the increased isoprene levels than the fungal communities (Figure 3). 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 2). 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 are Actinobacteria (i.e. *Mycobacterium sp.*, *Nocardia sp.*, and *Rhodococcus sp.* (Cleveland and Yavitt, 1998; Shennan, 2006)), and we found that the relative abundance of this phylum increased significantly with increasing isoprene levels (Figure 2). Likewise, taxa within the Alpha-, Beta-, and Gammaproteobacteria sub-phyla have been shown 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 levels, suggesting that the ability to degrade isoprene is likely more widely distributed than previously thought.

Figure 4 highlights the subset of those bacterial taxa that exhibited the strongest increases in relative abundance with increasing isoprene exposure. Of those, *Sporichthya sp.* had the strongest response to isoprene (a 33-fold increase) with an average relative abundance of 0.01% in the *P. tremuloides* soil with no added isoprene, increasing to 0.36% under 200 ppbv of isoprene (Figure 4). Many of the taxa highlighted in Figure 4 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 a 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 [15N]DNA-based stable isotope probing technique, Bell et al. (2011) found Sphingomonadaceae and Caulobacteraceae to exhibit the highest percentage of enrichment.
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Soil microbes associated with isoprene consumption

Previous work has focused on isoprene catabolism by bacteria with the assumption that soil bacteria are likely the most important degraders of isoprene (Shennan, 2006; Acuna Alvarez et al., 2009). However, we observed that several fungal groups also increased in relative abundance across the isoprene mixing ratio gradient (Figure 2). The fungal phylum with the most 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 be 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 levels 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, 1973), Pseudomonas variotii (Lowery et al., 1968), Penicillium janczewskii (McLee et al., 1972), and Penicillium ochrochloron (Cerniglia, 1973). There were 5 taxa between the two soils in the Trichocomaceae family that increased in relative abundance with increased isoprene, three of which (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 levels along with the previous research on related taxa consuming similar types of hydrocarbons suggests that most of the taxa observed to increase in abundance across the isoprene mixing ratio gradient are likely directly involved in the consumption of isoprene.

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Figure 4
Distinct OTUs increasing in relative abundance with increasing isoprene levels.

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

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

We show that soil microbial communities are capable of consuming a consistently large proportion of the available isoprene across atmospherically relevant levels. However, additional research is needed to understand field rates of isoprene consumption and how such rates are influenced by soil edaphic factors before we can incorporate microbial isoprene metabolism into global and local models of isoprene emissions and its atmospheric fate. Furthermore, to our knowledge this is the first study to identify such a wide range of microbial taxa that are likely capable of isoprene consumption, including a number of fungal taxa that have not previously been considered to be important isoprene degraders. Further research is necessary to directly determine whether those taxa that increased in relative abundance across the isoprene gradient are capable of direct isoprene catabolism and the kinetics associated with this process. Also, it is important to investigate isoprene consumption in additional soils to determine whether the putative isoprene degraders identified here are also common isoprene degraders in other soil types.

References


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Contributions
• Contributed to conception and design: CMG, DH, NF
• Contributed to acquisition of data: CMG
• Contributed to analysis and interpretation of data: CMG
• Drafted and/or revised the article: CMG, DH, NF
• Approved the submitted version for publication: CMG, DH, NF

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Competing interests
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Data accessibility statement
The following datasets were generated:
• DNA sequences: doi: 10.6084/m9.figshare.1318807

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