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and Donald M. Cropek

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Abstract

Rationale. Earth scent is the odour emitted from soils. This scent, primarily comprising the alcohols geosmin and 2-methylisoborneol (MIB), has not been fully characterised, but offers high potential for use as an environmental interrogation tool. **Methodology.** We utilised our field-based, solid-phase microextraction fibre method to test the hypothesis that soil activity and soil property variation can be detected *in situ* by comparing biogenic volatile emissions. **Results.** We eliminated sources of error utilising field-based sampling with these fibres, concluding that room temperature storage for up to 7 days is acceptable with minimal loss. Variation in individual fibre affinity for both compounds was higher than expected but no measured concentrations were observed to constitute outliers. Disturbance of minor soil volumes led to significantly higher emission of both compounds over background levels. Soil texture and soil cover had a significant effect on the emission of both compounds. Simulated rainfall, producing the characteristic odour known as petrichor, initiates elevated emission of geosmin. Background (undisturbed soil) concentrations of MIB were occasionally detectable during some sampling events, but geosmin concentrations in the air were always below detection limits without soil disturbance. Virtually all background and disturbed soil samples contained much higher concentrations of MIB compared to geosmin, but geosmin variation between replicates and experimental units was much lower. **Discussion.** Soil disturbance and soil property variation can be remotely detected using emission of volatile compounds. Correlating emission from the soil with respect to disturbance events and environmental properties could yield a powerful new tool for acquiring soil information.

Preface

This study was conducted for US Army Corps of Engineers (USACE) under the US Army Engineer Research and Development Center (ERDC) Basic Research Program through Program Element 601102/Project AB2/Task 4.

The work was performed by the US Army Engineer Research and Development Center, Construction Engineering Research Laboratory (ERDC-CERL). At the time of publication, the deputy director of ERDC-CERL was Ms. Michelle J. Hanson, and the director was Dr. Andrew J. Nelson.

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COL Christian Patterson was commander of ERDC, and Dr. David W. Pittman was the director.

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Characterising earth scent

Environmental context. Everyone is familiar with the earth odour that comes from digging in the soil. We measured the chemicals comprising this odour and studied how the environment influences how much of these chemicals are released from disturbed soils. Understanding what conditions affect emission of these compounds from soils could provide new technologies for remotely assessing soil health and what people are doing to soils.

Introduction

Disturbed soils have long been known to possess a distinct odour. Primary sources of this disturbed 'earth scent' have been attributed to two terpene alcohols: geosmin and 2-methylisoborneol (MIB) (Gerber and Lechevalier 1965; Gerber 1969). Geosmin and MIB are produced by dozens of cyanobacteria, actinobacteria and proteobacteria species (Krishnani *et al.* 2008; Churro *et al.* 2020), as well as multiple species of common soil fungi (Breheret *et al.* 1999; Schnürer *et al.* 1999), some plants (Spörle *et al.* 1991; Freidig and Goldman 2014) and even amoebae (Hayes *et al.* 1991). Actinobacteria, the microbes most associated with these compounds, are dominant and ubiquitous in nature. They are one of the most diverse bacterial groups and are commonly found in soil, marine and freshwater systems. One genus alone, *Streptomyces*, accounts for approximately 5% of described bacterial species (Lewin *et al.* 2016). Geosmin biosynthesis occurs from conversion of farnesyl diphosphate (the universal C_{15} sesquiterpene precursor) via a single enzyme, geosmin synthase (Jiang *et al.* 2006; Giglio *et al.* 2011). MIB biosynthesis occurs

from conversion of geranyl diphosphate (the universal C_{10} sesquiterpene precursor) via two enzymes, geranyl diphosphate 2-methyltransferase and MIB synthase (Komatsu *et al.* 2008; Giglio *et al.* 2011).

Animals worldwide have evolved adaptations exploiting these compounds. *Aedes* mosquitoes use geosmin to locate ephemeral aquatic puddles for egg laying (Melo *et al.* 2020). Soil arthropods use geosmin and MIB to locate, consume and distribute spores of *Streptomyces* (Becher *et al.* 2020) and to locate suitable nesting sites (Huang *et al.* 2020). Predators use the odour of disturbed soil to find prey in freshly dug nests (Buzuleciu *et al.* 2016). Camels are also believed to utilise these compounds to locate wet soils up to 80 km away (Simons 2003). These biological examples indicate valuable information is likely present within the soil volatilome that can be exploited for site characterisation and monitoring.

Current research on geosmin and MIB is focused almost exclusively on detection and mitigation of these volatiles in drinking water, as they are the primary sources of off-taste and unpleasant odours in freshwater globally (Watson *et al.* 2000; Yu *et al.* 2014; Son *et al.* 2015). While previous research examined geosmin and MIB concentrations in soil, none measured these compounds *in situ*, identified the presence of a background concentration, or compared undisturbed with mechanically disturbed soils (Buttery and Garibaldi 1976; Jüttner 1990; Stahl and Parkin 1994, 1996; Rinnan *et al.* 2013). Our recent work, however, was the first to measure geosmin and MIB emission from soils *in situ*, discovered that these compounds commonly exist in the troposphere, and determined that soil disruption leads to elevated levels of both compounds in the atmosphere above the disruption (Conrady *et al.* 2021). This lead to the possibility that remote monitoring, tracking and recording of geosmin and MIB atmospheric concentrations can be indicators of site activity and/or properties. Here, we utilise our optimised solid-phase microextraction (SPME) protocol (Conrady *et al.* 2021), with method detection limits (MDL) of 0.16 ng L^{-1} for geosmin and 0.72 ng L^{-1} for MIB, to compare volatile emissions from disturbed versus undisturbed and dry versus recently wetted soils across variable *in situ* environmental conditions to test the hypothesis that soil properties influence the volatilome upon disturbance.

Experimental

Sample quality assurance

Geosmin (>97.0%) and MIB (99.3%) standards (Sigma-Aldrich, St. Louis, MO, USA) were dissolved in hexane (99.9% purity, Fisher, Waltham, MA, USA) to prepare a 0.2 µg L^{-1} stock solution. Standard concentrations of geosmin and MIB were prepared in 20 mL headspace vials (Agilent Technologies, Inc., Santa Clara, CA, USA) by injecting 10 µL of the 0.2 µg L^{-1} geosmin and MIB standard solution through the vial septum using a 10-µL syringe (Hamilton Company, Reno, NV, USA). Because our previous research developing an *in situ* detection method for geosmin and MIB identified 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fibres as having the optimal affinity for simultaneous geosmin and MIB binding among multiple fibre coatings (Conrady *et al.* 2021), these fibres were used for all experiments described here.

To determine the analyte storage conditions among DVB/CAR/PDMS SPME fibres (Supelco, Inc., Bellefonte, PA, USA) prior to *in situ* field collection, four fibres were chosen for analysis that span the range for prior usage, from 0 to 68 previous collection events. Each fibre was exposed to a standard headspace concentration of geosmin and MIB for 40 min and stored at 4° or 23°C for a period of 1 h, 3 days, or 7 days, replicated three times for each fibre/period/temperature combination. To determine the analyte variation among DVB/CAR/PDMS SPME fibres, 15 separate fibres were randomly selected for analysis. Each fibre was exposed to the same geosmin/MIB headspace standard as previously described and each was assessed in triplicate, stored in the same conditions for the same time period and analysed in the same order.

Field sampling locations

Three soil types representing common but disparate soil textures were identified in central Illinois, USA, and sample collection sites were selected where a residence bordered a row crop field. This allowed perennial grass turf (yard) to be compared directly with bare soil at the field edge in proximity and from the same soil type, as confirmed with Web Soil Survey (Soil Survey Staff *et al.* 2022). The soils at each location are as follows: sand (Chelsea loamy fine sand; Mixed, mesic Lamellic Udipsamments), silt loam (Martinton silt loam; Fine, illitic, mesic Aquic Argiudolls) and silty clay (Bryce silty clay; Fine, mixed, superactive, mesic Vertic Endoaquolls). Two of the sites (silt loam and silty clay) were located approximately 200 m from one another, while the sand site was located approximately 50 km from the other two sites. Vegetation at all sites was dominated by grasses: the silt loam and silty clay sites were composed primarily of *Festuca* and *Poa* spp., with *Digitaria* and *Festuca* spp. dominant at the sand site.

Field sample collection

Sample collection occurred randomly at the three sites from September 2020 through June 2021. Sample collections were staggered so that replicate collections at all three sites and on vegetated or bare ground occurred on similar dates throughout the sampling period. Sample collection utilised DVB/CAR/PDMS SPME fibres taped to a grooved wooden shim to keep fibres anchored to a single location at a uniform height 3–5 mm above the soil surface. At each sampling event, four fibres were placed at random locations 5 m apart on the desired cover treatment (vegetated or bare ground) while maintaining consistent soil types.

For background control samples, fibres (the same four were used across dates for consistency) were exposed to the air directly above the soil surface for 40 min following Conrady *et al.* (2021), removed, and then stored for analysis. Immediately following background sample collection, each of the four sampling locations was subjected to a replicable soil disturbance using a Corona soil ripper (Corona Tools, Corona, CA, USA). The soil ripper spikes were pressed into the soil completely and turned clockwise four complete revolutions (1440°), while removing the ripper from the soil and depressing the plunger to remove attached soil between each revolution to improve soil mixing. The dimensions of the soil disturbance were 18.4 cm in diameter to a depth of 13.3 cm (3.6 L total volume). Four fibres (the same four across dates for consistency) were attached to grooved wooden shims and centred directly over the disturbed soil at a height between 3 and 5 mm above the soil surface. After 40 min exposure to the air directly over the disturbed soil, fibres were retracted and placed into their storage boxes. All fibres were stored at room temperature for no more than 3 days before analysis. Soil temperature and moisture to a depth of 68 mm was obtained using a Delta-T WET-2 sensor probe (Delta-T Devices, Cambridge, UK). Three measurements were taken randomly within 10 cm of each soil disturbance and recorded to obtain mean soil temperature and moisture estimates for each replicate.

Soil wetting was included as a treatment in August 2022 to simulate an intense rainfall event. Two 0.25 m^2 PVC quadrat frames were placed in areas of the turf on the lawn of the Construction Engineering Research Laboratory (Champaign, IL, USA) over a Drummer silty clay loam (Fine-silty, mixed, superactive, mesic Typic Endoaquolls) (Soil Survey Staff *et al.* 2022). Dry soil emissions were collected by exposing three replicate fibres per frame near the ground surface for 40 min for each quadrat. Fibres were removed and stored for analysis. Immediately following background sampling, soils were wetted by applying 7.5 L water to each quadrat, replicating a 3-cm intense rainfall event, and three new fibres were exposed to each quadrat for 40 min, after which they were removed and stored for analysis. Again, all fibres were stored at room temperature for no more than 3 days before analysis.

Sample analyses

From a 20 $\mu g\,L^{-1}$ combined MIB and geosmin stock solution, a series of dilutions was used to create a calibration curve for both MIB and geosmin. The calibration solutions ranged from 0.05 to 20 $\mu g\,L^{-1}$ and 10 µL of each was injected into its own headspace vial, resulting in a range of internal vapour concentrations. These vials were used to monitor gas chromatography–mass spectrometry (GC-MS) system performance, instrument sensitivity to geosmin and MIB, and calculate air concentrations when using the SPME fibres. The extraction temperature was 30°C and the desorption time was 4 min. The GC inlet port contained a 0.75 mm i.d. inlet liner (Supelco) and the splitless mode was used during sample analysis to improve linear flow rates around the fibre. For all SPME analyses, fibres were desorbed at 230°C in the inlet of an Agilent 7890B/5977A GC-MS system (Agilent Technologies, Inc., Santa Clara, CA, USA). A PAL3 RSI 85 autosampler (CTC Analytics AG, Zwingem, Switzerland) was used to ensure uniformity among sample analyses (both extraction and desorption events), and the separation was performed using a HP-5MS (30 m × 0.25 mm, 0.25-µm film thickness) capillary column (Agilent). The carrier gas was helium, and the flow rate remained constant at 1.2 $mL\,min^{-1}$. The GC-MS oven temperature program was held at 50°C for 1 min, ramped at 10°C min^{-1} to 200°C and held for 1 min, and finally ramped at 20°C min^{-1} to 220°C (Conrady *et al.* 2021). For analysis of fibre variation and storage time and temperature, a solution of geosmin and MIB at 0.2 $\mu g\,L^{-1}$ was used as mentioned in section 2.1. The DVB/CAR/PDMS SPME fibres exposed during field data collection were analysed using the same GC-MS parameters as the calibration fibres above.

Data analysis

The effects of soil texture, cover, moisture, and temperature, and their interactions on volatile emissions were analysed with analysis of variance using PROC GLM in SAS Version 9.2 (SAS Institute, Cary, NC, USA). Values below detection limit were analysed as actual values to include the observations without bias and because they were clustered primarily in specific treatments where no penalty existed for inclusion of inaccurate values. Geosmin and MIB emission concentrations, soil temperature and moisture, and soil wetting data were square root transformed to achieve normality. Levene's test confirmed homogeneity of variances. Mean separation of significant effects was performed using Tukey's least significant difference (l.s.d.) with $\alpha = 0.05$. The effects of fibre storage time and temperature and their interaction, and fibre variability comparisons were compared in a similar fashion using PROC GLM in SAS, but with Bonferroni-adjusted l.s.d. comparisons at $\alpha = 0.05$ to control the experiment-wise error rate. Means and standard errors (s.e.) were calculated with the PROC MEANS statement.

Results and discussion

Fibre performance

When using SPME as a field sampling method, shipping samples from the field to the laboratory for analysis is nearly always required, and the time between sampling and analysis should be considered. For this reason, sustainability of analytes adsorbed by the sorbent must be evaluated. It is

reported in Supelco Application Note 141 (Supelco Sigma-Aldrich 1998) that their highly retentive CAR/PDMS coating keeps volatile and semi-volatile compounds on the fibre without substantial loss for up to 3 days when stored at −4°C. Therefore, we wished to test these same parameters using our fibres and standards.

Further, when SPME reliability is evaluated, many important random error sources must be considered even when a rigorous standardisation is applied. Fibre-to-fibre reproducibility and fibre storage time from the field may lead to random errors. Inter-fibre reproducibility of CAR/DVB/PDMS SPME fibres have been investigated (Tuduri *et al.* 2001; Bicchi *et al.* 2007) and new fibre coating materials have also been developed to improve the efficiency and fibre-to-fibre reproducibility (Yu *et al.* 2002; Feng *et al.* 2010; Li *et al.* 2021). Overall, their results yielded an average relative standard deviation (RSD%) of 1.2–9.0%, which they considered good reproducibility.

Here, storing fibres up to 1 week (168 h) had no negative effect on retention of bound geosmin compared to 1 h storage (Fig. 1*a*) at room temperature. For MIB, however, storage time significantly affected retention ($F = 5.79$, $P < 0.03$) as storing fibres for 1 h did not significantly differ from 3 days (72 h) of storage, but both 1 h and 3 days retained higher mean concentration of MIB compared to 1 week of storage (Fig. 1*a*). Mean MIB concentrations retained were 0.228 μg L^{-1} for 1 h, 0.218 μg L^{-1} for 3 days and 0.156 μg L^{-1} for 1 week, while mean concentrations of geosmin retained were 0.151 μg L^{-1} for 1 h, 0.168 μg L^{-1} for 3 days and 0.144 μg L^{-1} for 1 week. Storing fibres for 1 day at 4°C vs 22°C had no effect on MIB ($F = 0.78$, $P = 0.38$) or geosmin ($F = 1.72$, $P = 0.20$) retention (Fig. 1*b*). Mean peak area across all fibres was 152 000 for geosmin and 171 300 for MIB (Fig. 2), corresponding to concentrations of 0.276 and 0.153 μg L^{-1}, respectively. Fibre identity was significant for geosmin retention ($F = 2.36$, $P = 0.04$), with, for example, fibre 15 binding more geosmin than fibre 5 and fibres 7, 8, 10, 11 and 15 binding more geosmin than fibre 2 based on Bonferroni adjusted mean separation (Fig. 2*a*).

MIB binding was insensitive to fibre identity but was significantly affected by sample replicate ($F = 9.47$, $P < 0.001$), with replicate 1 (mean peak area = 184 100; standard error = 9984) and 2 (180 100; 9125) higher than replicate 3 (149 600; 10 180), based on Bonferroni-adjusted mean separation (Fig. 2*b*). This indicates that all fibres behaved in a similar fashion, fibre handling was reproducible, and fibre selection was unimportant. Systematic errors, however, may be introduced but they affect the entire sample suite (replicate 3). Fibre sample replicate did not affect geosmin retention. None of the mean geosmin and MIB concentrations were more than 2 standard deviations from the mean (no outliers observed in the data). Again, this speaks to the consistent performance of each fibre and eliminates the need to use the exact same fibre for each sampling event.

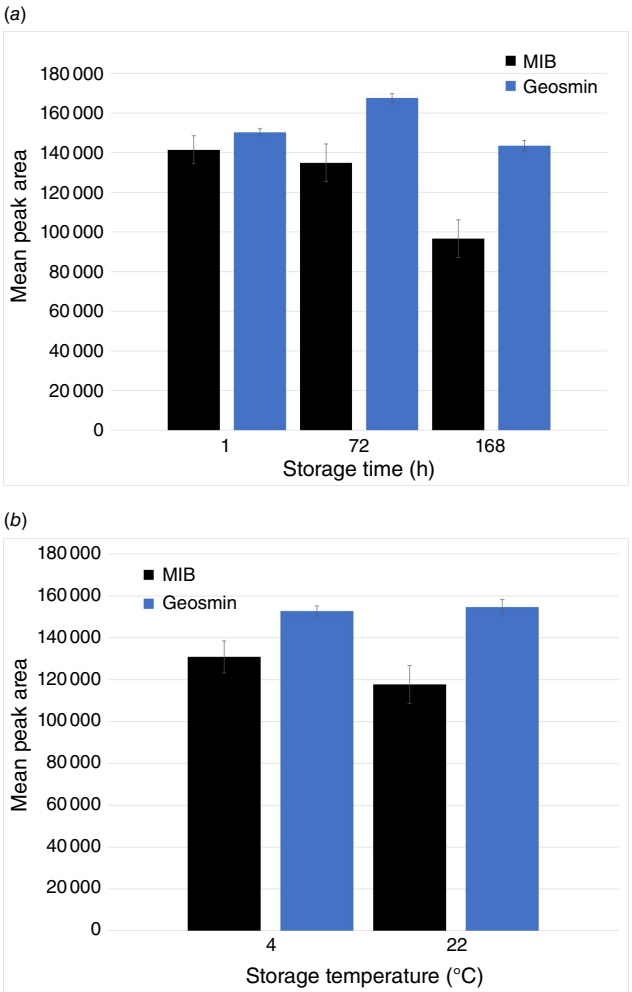

Fig. 1. Variation in geosmin and 2-methylisoborneol (MIB) retention on divinylbenzene/carboxen/polydimethylsiloxane solid-phase microextraction (SPME) fibres subjected to variable storage conditions. (*a*) Storage time at 23°C, (*b*) storage temperature for up to 1 week. Values are the means of four SPME fibres. Peak area was not converted to concentration to eliminate the added uncertainty associated with calibration curves. Bars are ± standard error.

Although these effects were significant at the statistical level, concentration calculations indicate that these differences are relatively minor, where mean MIB replicate concentrations ranged from 0.297 μg L^{-1} in replicate 1 to 0.241 μg L^{-1} in replicate 3, a difference of 0.056 μg L^{-1}. For geosmin concentrations, the mean difference between the fibre retaining the highest mean concentration of geosmin (0.176 μg L^{-1}) and the lowest (0.109 μg L^{-1}) was a difference of 0.067 μg L^{-1}. Our results indicate that field collection from remote locations or analysis delays less than a week after collection with storage at room temperature will have minimal impacts on observed results. While we observed that there is not a substantial source of variation across multiple fibres, replication with multiple fibres is recommended to ensure an accurate estimate of mean values.

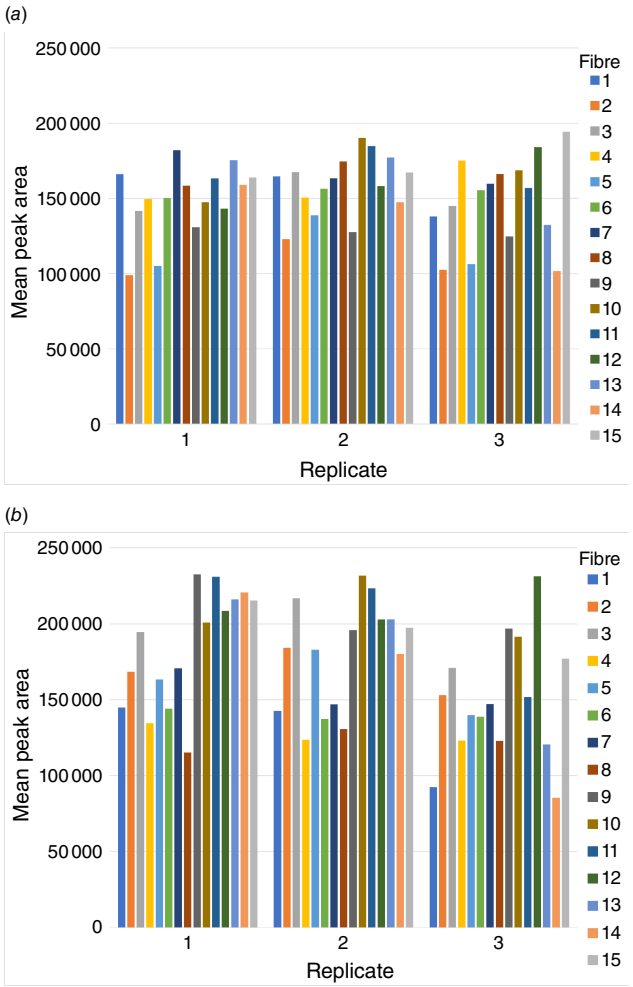

Fig. 2. Variation in geosmin and 2-methylisoborneo (MIB) retention across 15 randomly selected divinylbenzene/carboxen/polydimethylsiloxane solid-phase microextraction fibres. (*a*) Geosmin, (*b*) MIB. Peak area was not converted to concentration to eliminate the added uncertainty associated with calibration curves.

Field data

Background samples

Background control samples indicate the presence of low MIB concentrations in air above intact soils during late fall and spring (Table 1), while no detectable geosmin background was ever identified. Background MIB concentrations exhibited peaks over time (Fig. 3 and Table 1). MIB background concentration increased significantly during mid-autumn, coinciding with regional agricultural tillage activities where human activity results in widespread soil disruption (Fig. 3). Crop tillage data is not collected, but crop harvest progress data is collected by the United States Department of Agriculture, National Agricultural Statistics Service (2021). Immediately following crop harvesting, most land cover in this region is tilled to incorporate remaining crop biomass into upper soil layers to promote decomposition and protect the soil from erosion. MIB background concentrations also increased in spring, coinciding with spring tillage and planting, but not enough data were collected for a direct comparison between activities.

Disturbed soil samples

Both geosmin and MIB emission increased significantly under all environmental conditions near disturbed soils. Geosmin background concentrations were below the detection limit of 0.16 ng L^{-1} and increased to 0.32 (standard error = 0.06) ng L^{-1} following soil disturbance. Background MIB concentrations were 2.19 (0.31) ng L^{-1} and increased to 5.04 (0.68) ng L^{-1} following soil disturbance. Disturbing just 3.6 L of soil resulted in mean MIB concentrations that were 230% higher than the mean background concentrations. MIB mean percent emission change from soil disturbance [(disturbed − background/background) × 100] was 130%. Mean MIB emission from disturbed soils averaged 16-fold those of geosmin. The presence of elevated levels of these two natural biogenic compounds indicates release due to disruption of the soil structure. Soil texture had little influence on geosmin emissions, while MIB emissions from disturbed sand were significantly higher than emissions from silt loam and silty clay soils (Table 2). These observed emissions differences between soil textures may be a result of the influences of soil texture on soil microbial community composition rather than directly influencing the emission of compounds from soils (Xia *et al.* 2020). Vegetative cover on soils resulted in higher geosmin and MIB emissions compared to bare soils (Table 2). This is likely due to rhizosphere soils surrounding plant roots supporting higher relative abundances of many important Actinobacteria, including those documented to produce MIB and geosmin (Ling *et al.* 2022).

We propose one possible mechanism where these chemicals are trapped within the substructure porous soil network and released as a bolus when disrupted. If MIB is trapped within soil pore spaces, this would explain the much higher release from sandy soils upon disruption. Sandy soils have much greater macro-pore space compared to silt loam and clay soils which could result in rapid release of trapped gases (Plaster 2013). Alternatively, release of these compounds could coincide with lysing of microbial cells or a biochemical response to a new stimulus during the disturbance (West and Whitman 2022). However, it is unknown what the immediate effects of a macroscopic mechanical soil disturbance are on the cell wall integrity of soil microbes. On the other hand, actinomycetes, a primary producer of these compounds in soils, are filamentous bacteria that, like fungi, form thread-like hyphae that could be more prone to physical disruption than individual bacterial cells. Actinobacteria relative abundance increases with fine textured soil particles (Xia *et al.* 2020), and thus should generally be most abundant in the silty clay soil. Measurement of the soil microbial content or any microbiome changes due to soil disturbance, however, were beyond the scope of this study. Sorption to soil particles and mineral surfaces cannot

Table 1. Mean background concentrations of 2-methylisoborneol (MIB) and geosmin over time in central IL, USA.

Date	MIB concentration (ng L^{-1})		Geosmin concentration (ng L^{-1})	
	Mean	StdErr	Mean	StdErr
9/24/2020	BDL	–	BDL	–
9/29/2020	BDL	–	BDL	–
10/5/2020	BDL	–	BDL	–
10/8/2020	BDL	–	BDL	–
10/15/2020	BDL	–	BDL	–
10/22/2020	4.59	0.87	BDL	–
11/4/2020	6.33	0.80	BDL	–
11/12/2020	2.46	0.62	BDL	–
11/23/2020	BDL	–	BDL	–
12/1/2020	BDL	–	BDL	–
12/10/2020	BDL	–	BDL	–
12/16/2020	BDL	–	BDL	–
5/19/2021	3.41	1.07	BDL	–
5/26/2021	4.74	1.35	BDL	–
6/3/2021	7.59	1.87	BDL	–
6/10/2021	1.76	0.57	BDL	–
6/15/2021	2.59	0.72	BDL	–
6/18/2021	2.73	0.88	BDL	–
Mean	**2.19**	**0.31**	**BDL**	–

Means are of four replicate samples collected 5 m apart simultaneously (followed by standard errors). BDL, below detection limit.

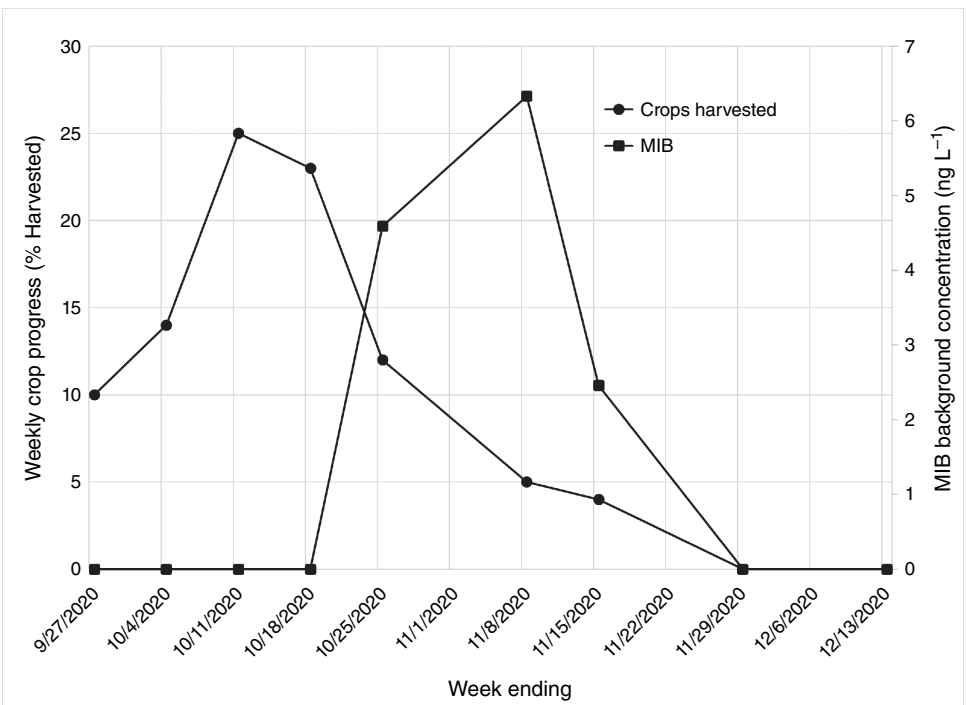

Fig. 3. Relationship between 2-methylisoborneol (MIB) background concentrations and fall agricultural activity in central IL, USA. Left *y*-axis presents crop harvest data (United States Department of Agriculture, National Agricultural Statistics Service 2021). Right *y*-axis presents background concentrations of MIB in ambient air during fall 2021. Each data point represents the mean of weekly data points collected with solid-phase microextraction fibres, each spaced 5 m apart and collected simultaneously across three soil types with four replicates per sampling date. MIB values of zero represent intervals where concentrations were below the detection limit.

Table 2. Mean net (disturbed – background) geosmin and 2-methylisoborneol (MIB) emission concentrations from disturbed soils.

	Geosmin	MIB
	Concentration (ng L^{-1})	
Soil texture		
Sand	0.27[a]	5.68[a]
Silt	0.36[a]	1.68[b]
Silty clay	0.20[a]	1.22[b]
Soil cover		
Bare	0.12[A,b]	1.59[b]
Vegetated	0.44[a]	4.12[a]

Means for each soil variable (texture and cover) in each column with the same letter are not significantly differently at $\alpha = 0.05$.
[A]Below detection limit, actual values used for analysis.

be ruled out, but because the silty clay soil has much greater surface area for binding, especially compared to the sandy soil, greater emissions should be realised from the silty clay soil if the primary storage mechanism is particle adhesion and subsequent release upon exposure. However, specific interactions with soil mineral particles are unknown and remain a likely mechanism. Because it cannot be confirmed that each of the studied soils possesses a similar microbial community composition and density, the mechanism governing storage and release of geosmin and MIB from disturbed soils cannot be confirmed. Further research is necessary to compare abundance of MIB and geosmin producers across soil and environmental gradients to determine the exact mechanism responsible for release of these compounds from disturbed soils.

Background MIB concentration was generally much higher than geosmin when detected, and this difference remained in disturbed soil emissions (16×). That geosmin occurs in lower background concentrations in the atmosphere and from disturbed soils compared to MIB was unexpected. Possible explanations include higher microbial MIB production in these soils or greater MIB lifetime in the troposphere that allows accumulation. This discrepancy could be due to higher abundance of microbes possessing MIB synthase genes relative to GeoA (Anuar *et al.* 2017) in the studied soils or greater relative transcription of the MIB synthase genes. It could also be related to chemical differences. Geosmin has a lower vapour pressure and is thus slightly less volatile than MIB (Pirbazari *et al.* 1992). However, the importance of this small difference in emissions is unknown. Given the higher and more variable concentrations of MIB in the background and lack of a background concentration of geosmin, geosmin appears to be the better indicator of soil disturbance despite its emission at much lower concentrations. Because our observations were restricted to our study area of highly disturbed soil systems in midwestern USA, it is possible that these differences are not observed in other locations. Further research is warranted to determine the extent of this phenomenon.

Our previous work noted mean MIB emission from disturbed soils averaging 10-fold higher concentration compared to geosmin (Conrady *et al.* 2021). Here, we expanded our investigation to include other soil types to provide evidence of the area of applicability. In all cases, both compounds increased significantly over background levels with only a 3.6-L disturbance.

Soil moisture had a significant effect ($F = 10.64$, $P < 0.002$) on MIB emission from disturbed soils, with wetter soils emitting higher MIB concentrations. Soil temperature had a significant effect ($F = 9.02$, $P < 0.004$) on geosmin emission from disturbed soils, with warmer soils emitting higher geosmin concentrations. Conversely, soil moisture had no effect on geosmin emission, while soil temperature had no effect on MIB emission. As higher temperature and moisture are both primary conditions influencing greater microbial growth in soils, it is surprising that emissions of both biogenic compounds were not observed with an increase in either physical factor. Further investigation of these relationships could yield a method to remotely measure soil physical properties.

MIB emission ($F = 0.26$, $P = 0.62$) was unaffected by simulated rainfall (Fig. 4). However, geosmin emission ($F = 7.68$, $P = 0.02$) increased significantly immediately following simulated rainfall. The emission concentrations observed from this experiment were lower than the average background concentration for MIB and higher (especially following rainfall) than the geosmin concentration compared to the experimental observations from sites and soils where rainfall was not simulated (Table 1). That geosmin emissions increased so much with simulated rainfall supports the hypothesis that this compound accumulates in pore spaces. As water infiltrates the soil and fills pore spaces, trapped chemicals can be displaced with the air from the pore spaces. Geosmin trapped in the pore spaces was likely forced into the troposphere where it was measured. However, as the same phenomenon was not observed for MIB, this does not support our hypothesis that MIB accumulates in pore spaces.

When dry soil is exposed to rainfall, geosmin is released and gives off a distinctive musty odor akin to damp soil or the smell associated with the first rains of the season, commonly called petrichor (Bear and Thomas 1964; Gerber and Lechevalier 1965; Joung and Buie 2015). Joung and Buie (2015) demonstrated how the effect of droplets hitting the soil surface disperse aerosols including geosmin into the atmosphere. Our results support geosmin as a component of petrichor and provide the first quantification of this phenomenon *in situ* from soils. Surprisingly, MIB has not been associated with petrichor despite a very similar chemistry and presence in the soil, and our results further confirm this distinction.

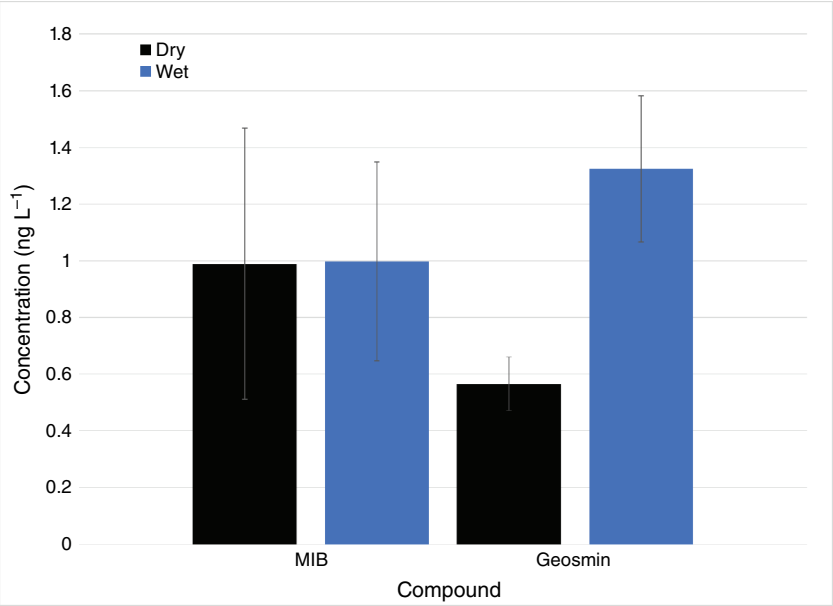

Fig. 4. Effects of simulated rainfall on mean emission of MIB and geosmin emissions from soils. Dry emissions were measured prior to soil wetting while wet emissions were measured immediately after applying water. Values for each bar are the mean concentrations collected using solid-phase microextraction fibres on two plots, replicated three times each ($N = 6$). Bars are ± standard error.

We analysed the background concentrations of geosmin and MIB at each site and noted increased concentrations after mechanical soil disruption. Chromatographic analysis of the desorbed SPME fibres allowed us to determine whether the disruption led to a change in the vapour signature in the air above the site. These observations support the concept of soil volatilome monitoring as a means of detecting soil disruption, site activity, and soil properties. Further refinement of this method could pinpoint locations and extent of site activity. Expanding data collection activities to a broader scope of soils and environmental conditions will increase correlative relationships between soil disruptions and properties, thus increasing the utility of this method as a remote soil interrogation tool.

Geosmin and MIB emission from soils is a potentially valuable soil interrogation tool. MIB is present in the atmosphere sporadically as a background signal that appears to be associated with large-scale anthropogenic soil disturbances. Emission of both compounds increases from disturbed soils, with soil texture, cover, moisture, and temperature influencing concentrations in the volatilome. Simulated rainfall confirms geosmin is a component of petrichor from soils but not MIB. Additional investigation is warranted to further refine the disturbed soil volatilome signal and identify key relationships controlling its release.

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14. ABSTRACT

Rationale. Earth scent is the odour emitted from soils. This scent, primarily comprising the alcohols geosmin and 2-methylisoborneol (MIB), has not been fully characterised, but offers high potential for use as an environmental interrogation tool. **Methodology.** We utilised our field- based, solid-phase microextraction fibre method to test the hypothesis that soil activity and soil property variation can be detected *in situ* by comparing biogenic volatile emissions. **Results.** We eliminated sources of error utilising field-based sampling with these fibres, concluding that room temperature storage for up to 7 days is acceptable with minimal loss. Variation in individual fibre affinity for both compounds was higher than expected but no measured concentrations were observed to constitute outliers. Disturbance of minor soil volumes led to significantly higher emission of both compounds over background levels. Soil texture and soil cover had a significant effect on the emission of both compounds. Simulated rainfall, producing the characteristic odour known as petrichor, initiates elevated emission of geosmin. Background (undisturbed soil) concentrations of MIB were occasionally detectable during some sampling events, but geosmin concentrations in the air were always below detection limits without soil disturbance. Virtually all background and disturbed soil samples contained much higher concentrations of MIB compared to geosmin, but geosmin variation between replicates and experimental units was much lower. **Discussion.** Soil disturbance and soil property variation can be remotely detected using emission of volatile compounds. Correlating emission from the soil with respect to disturbance events and environmental properties could yield a powerful new tool for acquiring soil information.

15. SUBJECT TERMS
2-methylisoborneol, Biogenic emissions, Earth scent, Geosmin, Soil disturbance, Soil volatilome, Solid-phase microextraction, Volatile organic compounds

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