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Short communication

Monoterpene emissions from an understory species, *Pteridium aquilinum*

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ABSTRACT

Monoterpene emissions from the dominant understory species *Pteridium aquilinum* (Bracken fern) in a mixed temperate forest were measured in the field during the summers of 2006, 2007 and 2008. The results showed that Bracken fern emitted monoterpenes at different rates depending if the plants were located in the understory or in open areas. Understory plants emitted monoterpene levels ranging from 0.002 to 13 μgC g_{dw}^{-1} h^{-1}. Open area plants emitted monoterpene levels ranging from 0.005 to 2.21 μgC g_{dw}^{-1} h^{-1}. During the summer of 2008 greenhouse studies were performed to complement the field studies. Only 3% of the greenhouse Bracken fern plants emitted substantial amounts of monoterpenes. The average emission, 0.15 μgC g_{dw}^{-1} h^{-1} ± 0.9 μgC g_{dw}^{-1} h^{-1}, was much lower than that observed in the field. The factors controlling monoterpene emissions are not clear, but this study provides evidence of the potential importance of understory vegetation to ecosystem total hydrocarbon emissions and emphasizes the need for longer-term field studies.

1. Introduction

Studies of biogenic volatile organic compounds (BVOCs) from vegetation have focused on emissions of tree species that dominate the landscapes of some terrestrial biomes. Understory vegetation has been neglected by these studies and its contribution to regional BVOC emissions is unknown.

Within the BVOC's, monoterpenes (MT – $C_{10}H_{16}$) stand out as a class due to their various ecological and atmospheric roles (Loreto et al., 2009; Fuentes et al., 2000). Ecologically, monoterpenes are known to deter herbivores and/or attract their predators. These compounds are also known to serve as signaling compounds to attract pollinators (Sell, 2003; Theis, 2006). From the atmospheric point of view, monoterpenes influence atmospheric composition through their role as precursors of ozone and aerosol formation (Finlayson-Pitts and Pitts, 2000; Hewitt, 1999). Monoterpenes can be stored in specialized structures in the plant; the amount of terpenes stored in a plant can vary from 1–3% up to 15–20% of dry mass (Penuelas and Llusia, 2001). Monoterpenes are released to the atmosphere mostly by diffusion through cellular compartments and, thus, temperature plays an important role in monoterpene emissions because of its influence on vapor pressure and diffusion processes. As temperature increases, emissions exponentially increase. However, while some monoterpene emissions depend only on temperature, other monoterpene emissions are triggered by photosynthetically active radiation (PAR) as well (Ortega et al., 2008; Kesselmeier and Staudt, 1999). Other factors that affect monoterpene emissions and storage in plants are herbivore attacks, drought, soil composition, and atmospheric CO_2 concentration among others (Rapparini et al., 2001; Staudt et al., 2000; Litvak and Monson, 1998).

PAR and temperature are normally the main drivers of monoterpene emissions from vegetation; therefore the plant's physical location plays an important role in determining emission magnitudes. The location, specifically whether a plant is in the understory or in an open area, will determine the temperature and light conditions that may promote monoterpene emissions. *Pteridium aquilinum* (Bracken fern) is a useful example of a common understory species. This organism is one of the most widespread plants in the northern hemisphere (Moran, 2004), and can also be found in some southern hemisphere regions. Bracken fern is a herbaceous perennial that grows in both open and understory environments, usually in deciduous forests (Atkinson, 1989; Gilliam and Roberts, 2003; Roberts and Gilliam, 1995; Royo and Carson, 2006). Studies of the chemical composition of Bracken fern fronds have shown that the leaves contain terpenoid compounds and, therefore, are a potential source of atmospheric hydrocarbons (Jones et al., 1991). Despite its broad distribution, *Pteridium's* potential emissions and their unknown impact on atmospheric chemistry have been

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neglected by atmospheric chemists; thus, measurements of terpenoid emissions from this plant are lacking in the literature. The broad range of light environments in which Bracken fern will grow suggests that monoterpene emissions, if present, will be variable. If environmental variables like PAR and temperature elicit the emission of monoterpenes, it is expected that open areas will produce more emissions than understory areas.

The objectives of this work were to determine (1) if *P. aquilinum* emits monoterpenes, (2) factors, including landscape configuration, that trigger any detected emissions, and (3) if detected, are emissions of this understory species substantial enough to influence atmospheric chemistry.

2. Methods

2.1. Study sites

The study took place at the University of Michigan Biological Station (UMBS, 45° 32′ 59.9″ N, 84° 39′ 36″ W, 238 m elevation) and the National Center for Atmospheric Research (NCAR) greenhouse in Boulder, Colorado (40° 2′ 6″ N, 105° 14′ 35″ W, 1625 m elevation). The study was divided into three phases; the first two phases were completed at the UMBS during the summers of 2006 and 2007 and the last phase was completed at NCAR during the summer of 2008.

2.2. Sampling and analytical techniques

The emissions of monoterpenes from Bracken fern for all three study phases were determined using a dynamic plant enclosure system (Helmig, 1997; Pollmann et al., 2005). Monoterpene emissions were adsorbed in a Volatile Collector Trap (VCT, ARS Inc., Gainesville, FL). After each measurement, fronds were cut, and leaf area and dry weight were measured to express the emissions by dry weight.

PAR measurements were made using sensors (LI-COR Quantum Sensors) placed outside the enclosure. Leaf temperature was measured with a probe that was placed inside the bag (LCD External Temperature Probe, L-TMB-M002, Onset Computer Corporation, Bourne, MA). Ambient temperature was measured with a second sensor (HOBO U12, Temp/RH Probe, Onset Computer Corporation, Bourne, MA). PAR and temperature sensors were connected to the HOBO U-12 data logger and measurements were taken every minute during the measurement period.

Monoterpenes were extracted from the VCT using the technique described by Matsunaga et al. (2009) and then analyzed with a gas chromatograph coupled with a flame ionization detector (SRI Model 310, SRI instruments, Menlo Park, CA). An aliquot of the extraction was injected into a low polarity column (Restek, MXT®-5, 5% diphenyl and 95% dimethyl polisiloxane, 30 m, 0.53 mm ID, stainless steel, Restek Corporation, Bellephonte, PA).

2.3. Study phases

The first phase (UMBS, August 2006) focused on whether Bracken fern emitted monoterpenes. We measured 4 plants in the open area during two consecutive days, 2 plants/day. The measurements were completed through the day over a course of 3 h for a total period of 9–12 h; 3–4 samples total.

The second phase objectives (UMBS, summer 2007) were to examine factors triggering monoterpene emissions from Bracken fern and to determine if landscape configuration influenced emissions. The distribution of Bracken fern in open and understory areas at UMBS provided an opportunity to investigate plants exposed to very different light and temperature environments. Four 10 m × 10 m plots were established, two plots were located in open areas and two in understory areas. Fifteen plants within each plot were randomly selected for measurements; not all the plants were measured. Monoterpene emission rates were measured from a total of 40 *P. aquilinum* plants. Two plants were measured the same time each day; one in the open and one in the understory area. Emissions were measured through the day over a course of 3 h for a total period of 9 h. The goal was to have 3 measurements/plant/day, but some days samples were lost due to power failure. The results of this phase were used to design the study's third phase.

The third phase (summer 2008) examined factors that elicited the emissions observed in the field. To reduce variability associated with genetic factors, 150 rhizomes of Bracken fern were collected at the UMBS field site and transported to NCAR's greenhouse. Bracken reproduces asexually via rhizomes to produce clones (Klekowsky, 2003). Once the plants matured, monoterpene emissions were measured under controlled PAR and temperature (25–28 °C and 500–750 μmol m^{-2} s^{-1}). These measurements were made using the same techniques and sensors used in the field.

2.4. Data analysis

The statistical analyses reported in this paper were generated using SAS software 9.2 (SAS Institute, Cary, NC). Regression analysis and analysis of variance (ANOVA) used a 95% confidence interval.

3. Results

3.1. Emission surveys

3.1.1. 2006 campaign

3.1.1.1. Survey field measurements. The survey phase in 2006 included 16 measurements from 4 plants (Fig. 1). Average total monoterpene emissions were 2.48 ± 0.31 μgC g_{dw}^{-1} h^{-1}, and the minimum and maximum values were 0.41 μgC g_{dw}^{-1} h^{-1} and 3.93 μgC g_{dw}^{-1} h^{-1}, respectively. The emissions were associated with an average PAR of 1332 μmol m^{-2} s^{-1} and temperature of 34.1 °C.

3.1.2. 2007 campaign

3.1.2.1. Monoterpene variability. The 2007 campaign (early June-late August) measurements were made at irregular intervals during that time period. Bracken fronds were just expanding when the first measurements were taken and were senescing during the final measurements. Plants measured on the same day were in the same developmental stage. To facilitate understanding of emission magnitudes during the field campaign, we considered 3 periods: Early Summer (June), Mid-Summer (July), Late Summer (August). Fig. 2 and Table 1 show the fern emissions during those periods as

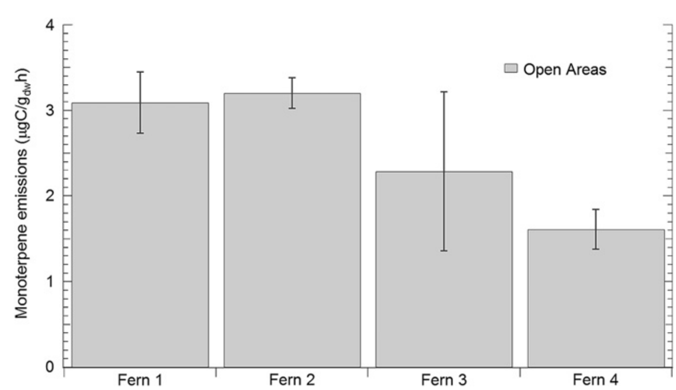

Fig. 1. Average monoterpene emissions of *Pteridium aquilinum* plants during Summer 2006. All plants were located in open areas. Whisker length = Standard error of the mean.

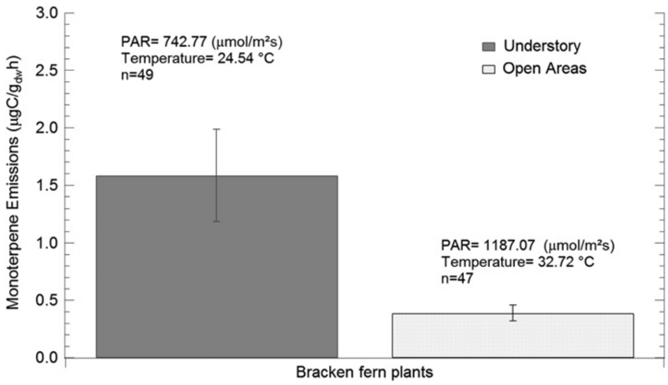

Fig. 2. Comparison of average monoterpene emissions of *Pteridium aquilinum* plants in open and understory areas during Summer 2007. Whisker length = Standard error of the mean.

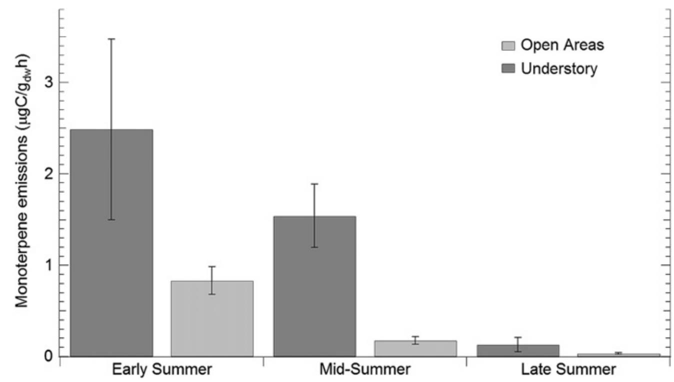

Fig. 3. Comparison of average monoterpene emissions of *Pteridium aquilinum* plants in open and understory areas during early, mid and late summer periods. Summer 2007. Whisker length = Standard error of the mean.

well as the averaged PAR and temperature. The magnitude of the emissions decreased over the summer.

3.1.2.2. Terpenoid emissions and landscape structure. To determine if there was a significant difference between the emissions of the plants located in the understory and those located in open areas, a one-way ANOVA was performed with an $\alpha = 0.05$ ($F_{1,\ 96} = 8.97$ $p = 0.0035$; Fig. 3). Results showed a significant difference between the emissions of the different sites. Average total monoterpene emissions in the understory were 1.6 ± 0.4 μgC g_{dw}^{-1} h^{-1} and 0.40 ± 0.07 μgC g_{dw}^{-1} h^{-1} in the open areas.

3.1.2.3. Factors triggering the emissions: PAR or temperature?. The results of this study showed emission variations across the summer and a difference between plants in open areas and in the understory. In studies of other plants species, this variation has often been explained by the effects of PAR, temperature or both. To determine which variables influenced Bracken emissions, regression analyses were performed separately on open and understory areas (Table 2). Results show lack of correlation with PAR (Fig. 4) or temperature (Fig. 5) in both areas; temperature and PAR explain only a fraction of the variability in emissions.

3.1.3. 2008 campaign

3.1.3.1. Greenhouse measurements. The greenhouse-cultivated Bracken fern rhizomes developed into 100 full fronds of which just 3 were found to be terpenoid emitters. The average emission of total monoterpenes for those 3 plants was 0.15 μgC g_{dw}^{-1} h^{-1} ± 0.9 μgC g_{dw}^{-1} h^{-1} and was associated with a PAR level of 750 μmol m^{-2} s^{-1} and a temperature of 27.8 °C.

3.2. Impact of *P. aquilinum* emissions in a Michigan forest

The contribution of Bracken fern to total monoterpene emissions in the UMBS forest landscape was estimated using the Model of Emissions of Gases and Aerosols from Nature (MEGAN, v2) (Guenther et al., 2006) to extrapolate enclosure measurements to the landscape scale. The average observed Bracken fern total monoterpene emission factor for the 2006 and 2007 field campaigns results in an emission of 0.11 μg m^{-2} h^{-1} when extrapolated to the landscape scale using a the measured LAI of 0.054 m^2 m^{-2} which is representative of a Northern Michigan forest The total monoterpene emission rate associated with Bracken was less than 1% of the total emission estimated for the forest canopy (154 μg m^{-2} h^{-1}). It can be concluded that there is no

Table 2
Linear regression results of PAR and temperature on Bracken fern monoterpene emissions.

		d.f.	F	$p_{\alpha=0.05}$	R^2
Open Areas	Temperature	46	1.81	0.177	0.040
	PAR	46	6.53	0.014	0.126
Understory Areas	Temperature	43	1.33	0.255	0.030
	PAR	43	0.77	0.388	0.018

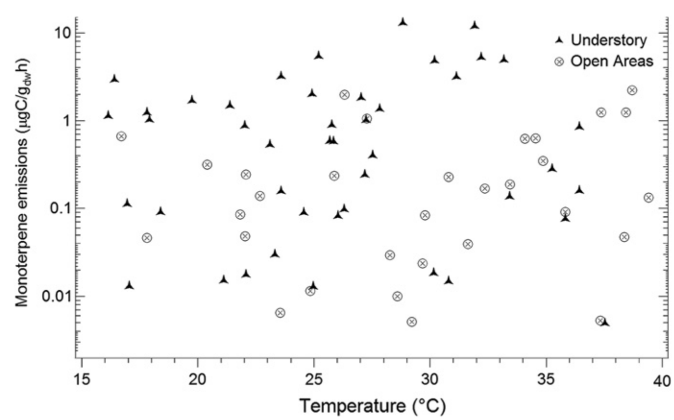

Fig. 4. Linear regression analysis of Bracken fern emissions and temperature from Summer 2007. Open Areas: d.f. = 46, $F = 6.53$, $p_\alpha = 0.05 = 0.01$, $R^2 = 0.126$. Understory Areas: d.f. = 43, $F = 0.77$, $p_\alpha = 0.05 = 0.383$, $R^2 = 0.18$.

Table 1
Average values of monoterpene emissions, PAR and temperature during Summer 2007.

	Understory			Open Areas		
	Early Summer	Mid-Summer	Late Summer	Early Summer	Mid-Summer	Late Summer
Emissions	2.48 ± 0.997	1.54 ± 0.34	0.127 ± 0.08	0.83 ± 0.15	0.175 ± 0.043	0.03 ± 0.01
PAR (μmol m^{-2} s)	957.8 ± 99.03	668.5 ± 137.2	504.0 ± 182.22	1450.8 ± 183.4	815.85 ± 106.66	1335.2 ± 147.8
Temperature (μgC g_{dw}^{-1} h)	26.08 ± 2.08	23.69 ± 0.95	23.47 ± 4.34	32.94 ± 3.61	29.34 ± 1.83	37.7 ± 2.43
N	17	20	10	18	19	12

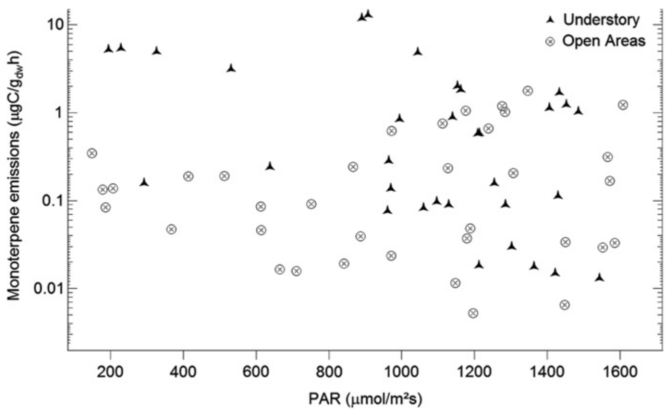

Fig. 5. Linear regression analysis of Bracken fern emissions and temperature from Summer 2007. Open Areas: d.f. = 46, $F = 1.88$, $p_\alpha = 0.05 = 0.177$, $R^2 = 0.040$. Understory Areas: d.f. = 43, $F = 1.33$, $p_\alpha = 0.05 = 0.255$, $R^2 = 0.030$.

significant contribution by Bracken fern to the total monoterpene emissions in this Michigan forest.

4. Conclusions

P. aquilinum is capable of producing and emitting monoterpenes. The factors that control these emissions are complex and were not resolved by this study. Statistical analysis showed no significant relationship between emissions and PAR or temperature. The emission variations may be attributed to other factors including air humidity, soil nutrient content, plant–insect interactions, stress or other factors not measured in this study (Hewitt and Street, 1992).

During the 2007 campaign, emissions from understory plants were significantly higher than those in open areas. Initially we were expecting higher emissions in open areas. However, those plants were exposed to harsher sun and temperature conditions. It is possible that plants in open areas allocated more resources to produce biomass in order to increase fitness, and thus had fewer resources to allocate to secondary compounds.

Emissions can be affected by the plant life cycle. Monoterpene emissions are affected by the allocation of resources between growth and production of secondary metabolites. It is known that the chemistry of Bracken fronds changes with time (Alonso-Amelot et al., 2001). Emissions were measured in late summer during 2006 and throughout the summer of 2007. Different stages of development may result in different terpenoid concentrations which contribute to the large variability of the emissions.

Bracken fern biology may explain the lack of success in measuring emissions after transplanting rhizomes from the site to the greenhouse. *P. aquilinum* is a plant that allocates the majority of its biomass to the rhizomes (Whitehead and Digby, 1997). It is possible that the plants growing in the greenhouse were allocating the majority of their nutrient resources to their rhizomes and not to the production of secondary metabolites such as monoterpenes, resulting in a lack of measurable emissions.

Another conclusion from this work is that long measurement periods are necessary for characterizing emission factors. Long-term monitoring is needed to understand how and when emissions are produced. Since emissions from Bracken fern were detected on some days but not others, a survey conducted on a single day could conclude that Bracken does not emit any monoterpenes or could overestimate emissions. Measurements of BVOC emission factors have typically been made during short-duration field campaigns. This approach can fail to identify some significant emitters, and it is necessary to lengthen the duration of field campaigns or survey the same plants during different periods of their life cycle in order to produce more representative emission estimates.

Results also showed that Bracken emissions did not make a significant contribution to total monoterpene emissions from the forest ecosystem investigated. However, understory terpenoid emitters could make an important contribution in other ecosystems.

P. aquilinum is not the only understory species that has been neglected; additional herbaceous species are potentially significant contributors of terpenes and other BVOCs to the atmosphere. It is necessary to have a deeper understanding of potential emissions from understory vegetation to the atmosphere in order to determine if such species could be significant contributors to atmospheric chemical processes.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.atmosenv.2012.02.047.

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