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Phenological stage of tundra vegetation controls bidirectional exchange of BVOCs in a climate change experiment on a subarctic heath

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Abstract
Traditionally, biogenic volatile organic compound (BVOC) emissions are often considered a unidirectional flux, from the ecosystem to the atmosphere, but recent studies clearly show the potential for bidirectional exchange. Here we aimed to investigate how warming and leaf litter addition affect the bidirectional exchange (flux) of BVOCs in a long-term field experiment in the Subarctic. We also assessed changes in net BVOC fluxes in relation to the time of day and the influence of different plant phenological stages. The study was conducted in a full factorial experiment with open top chamber warming and annual litter addition treatments in a tundra heath in Abisko, Northern Sweden. After 18 years of treatments, ecosystem-level net BVOC fluxes were measured in the experimental plots using proton-transfer-reaction time-of-flight mass spectrometry (PTR–ToF–MS). The warming treatment increased monoterpene and isoprene emissions by ≈50%. Increasing temperature, due to diurnal variations, can both increase BVOC emission and simultaneously, increase ecosystem uptake. For any given treatment, monoterpene, isoprene, and acetone emissions also increased with increasing ambient air temperatures caused by diurnal variability. Acetaldehyde, methanol, and sesquiterpenes decreased likely due to a deposition flux. For litter addition, only a significant indirect effect on isoprene and monoterpene fluxes (decrease by ~50%–75%) was observed. Litter addition may change soil moisture conditions, leading to changes in plant species composition and biomass, which could subsequently result in changes to BVOC emission compositions. Phenological stages significantly affected fluxes of methanol, isoprene and monoterpenes. We suggest that plant phenological stages differ in impacts on BVOC net emissions, but ambient air temperature and photosynthetically active radiation (PAR) also interact and influence BVOC net emissions differently. Our results may also suggest that BVOC fluxes are not only a response to changes in temperature and light intensity, as the circadian clock also affects emission rates.

KEYWORDS
Arctic, BVOC, climate change, methanol, phenology, plant volatiles, terpenoids, tundra
Climate warming in the Subarctic increases the emissions of temperature-sensitive biogenic volatile organic compounds (BVOCs), both in the short and long term (Faubert et al., 2010; Lindwall, Schollert, et al., 2016; Lindwall, Svendsen, et al., 2016; Peñuelas & Staudt, 2010; Tiiva et al., 2008; Valolahti et al., 2015). Furthermore, BVOCs are highly reactive in the atmosphere (Ortega et al., 2007), contributing to the production of secondary organic aerosols and eventually can act as cloud condensation nuclei (Scott et al., 2014). Less is known about the impacts on BVOC fluxes caused by warming-induced increases in deciduous shrub abundance and leaf litter inputs to the ecosystem (Valolahti et al., 2015).

Earlier studies have documented visual changes in the high latitude vegetation community related to climate warming, such as the northward and upslope migration of the tree line and increases in deciduous shrub cover (Callaghan et al., 2004; Chapin et al., 1996; Gillespie et al., 2016; Semenchuk et al., 2016; Sturm et al., 2001). Subarctic shrubs produce a complex blend of BVOCs, and emissions are also expected to increase under a warming climate (Faubert et al., 2010; Valolahti et al., 2015). As shrub cover increases, the abundance of mosses, lichens, and bare ground decreases, while leaf litter increases. Leaf litter deposition is controlled by warming and humidity effects on litter decomposition rates (Cornellissen et al., 2001; Elmendorf et al., 2012; Hobbie et al., 2005; Walker et al., 2006) and litter quality (Rinnan et al., 2008; Rinnan & Rinnan, 2007). We expect changes in vegetation composition and leaf litter deposition to significantly impact BVOC fluxes.

BVOC emissions from leaf litter positively correlate with temperature (Greenberg et al., 2012). Litter BVOCs mainly originate from microbial activity in the litter (Gray et al., 2010), which is temperature dependent (Clein & Schimel, 1995), and degradation of plant tissues storing BVOCs (Wang et al., 2017). Moisture content of the litter also affects emissions, especially directly following rain events, when microbes rapidly accelerate litter decomposition (Greenberg et al., 2012). The bouquet of BVOCs originating from litter differs between various types of litter, both because of the differences in the chemical composition of litter and due to contrasting microbial communities residing in the litter (Gray et al., 2010; Leff & Fierer, 2008; Svendsen et al., 2018). For example, litter from deciduous and evergreen trees emit short-chained oxygenated BVOCs, and methanol is typically the most dominant compound, followed by acetaldehyde, acetone, and propanal (Gray & Fierer, 2012; Gray et al., 2010; Ramirez et al., 2010).

Traditionally, BVOC emissions have only been considered as a unidirectional flux from the ecosystem to the atmosphere (Guenther et al., 1995, 2006; Monson et al., 2012). However, laboratory (Kesselmeier, 2001; Noe et al., 2008; Rottenberger et al., 2004, 2005; Seco et al., 2008) and field (Holst et al., 2010; Seco et al., 2020; Wohlfahrt et al., 2015) experiments clearly show the potential for bidirectional fluxes of different compounds, implying that the exchange of BVOCs may be more important than earlier thought (Park et al., 2013). These studies show that BVOCs are emitted, or taken up, from all components in the ecosystem (Laathaworkitkul et al., 2009; Seco et al., 2007), but it is likely that green plant parts and flowers are the main producers (Peñuelas et al., 2014) and soils are the main consumers (Albers et al., 2018; Rinnan & Albers, 2020). The highest net BVOC emissions in arctic terrestrial ecosystems occur during the peak growing season when leaf biomass is highest simultaneously with peaks in decomposition and soil CO2 emissions (Tang et al., 2018).

Typically, BVOC emissions are measured with adsorbent tubes providing an average emission rate of a subset of volatile compounds (e.g., C5-C25 range, depending on the adsorbent) with poor time resolution because of the time-intensive sampling method. Thus, previous work of BVOC fluxes has focused on the volatile terpenoids (isoprene, monoterpene, and sesquiterpenes), because of their high contributions to total BVOC emissions, and measurements of BVOCs are typically restricted to a few snapshots, which restrict the ability to investigate short-term dynamic processes. However, since the introduction of proton-transfer-reaction mass spectrometry (PTR–MS), short-chained oxygenated BVOCs (acetone, acetaldehyde, and methanol), which are not quantitatively captured with commonly used adsorbent tubes, have received increasing attention (Seco et al., 2007).

Here, we investigate the dynamics of bidirectional BVOC fluxes in a subarctic tundra ecosystem, including variations diurnally and with plant phenological stages, and vegetation composition. The measurements covered a full growing season, from snow melt (late May) until leaf senescence (early August). We sampled concentrations and BVOC fluxes covering a wide mass range (30–347 a.m.u.), to estimate the effect of increased temperature and leaf litter addition. Our sampling site is part of an ongoing climate change experiment, with warming and litter addition treatments that have been applied since 1999. We hypothesized that: (1) the shoulder seasons (early and late summer) will have high litter BVOC emissions due to a spring pulse release after snow melt and litter thaw, and from input of fresh litter with labile C; (2) experimental warming will increase the emissions of all compounds; (3) litter addition will increase the emissions of short-chained oxygenated BVOCs, especially methanol, either directly from the litter itself (Svendsen et al., 2018), or indirectly, as a consequence of altered microbial processes (Rinnan & Rinnan, 2007); and (4) the time of day will influence BVOC fluxes as a consequence of diurnal changes in temperature and humidity, with higher net emissions as temperature increases during the day.
Evergreen and deciduous shrubs, graminoids, and herbs dominated the vegetation, while mosses and lichens covered the ground layer (Rinnan et al., 2008). The experimental area represents a heterogeneous plant community, thus large plant composition differences within treatment plots naturally appear regardless of treatment.

The experiment, established and maintained since 1999, covers a total area of 1000 m² and simulates climate warming and increased leaf litter fall. The experiment consists of four treatments replicated in six blocks, making 24 plots (120 × 120 cm each) in total. Treatments are randomized within each block and include control (C), litter addition (L), warming (W), and warming and litter addition (WL). The warming treatment was repeatedly installed during mid-June each year and achieved using dome-shaped, open-top, transparent tents made of polyethylene (0.05 mm thick, 70 cm high), which increased the daytime air temperature by 3°C and the surface parent tents made of polyethylene (0.05 mm thick, 70 cm high), which increased the daytime air temperature by 3°C and the surface

Soil samples were collected in August outside the metal frame, but inside the treated plot. A soil corer (3-cm diameter) was pressed 5 cm down into the soil three times in each plot. The cores were divided into two depths, 0–2 cm and 2–5 cm, and the three subsamples were pooled together as one sample per plot, per depth.

To analyze carbon and nutrients, 5 g of soil was extracted with 25 ml of distilled water for 1 h. The extracts were left to settle for an hour and then filtered using a vacuum pump (KNF Laboport) through Whatman GF/D glass microfiber filters. The filtered extracts were kept frozen until analysis.

BVOC fluxes were measured using a dynamic enclosure technique at the ecosystem level coupled with proton-transfer-reaction time-of-flight mass spectrometry (PTR-ToF-MS). Measurements were conducted in three of the six blocks within a maximum distance of 13 m from the PTR-ToF-MS, to limit transfer line length and minimize adsorption of BVOCs within the inlet lines. Thus, measurements were conducted on a total of 12 plots (four treatments in three blocks).

A transparent polycarbonate chamber (wall thickness 2 mm, 22 × 22 × 20 cm width × depth × height, volume 9.68 L) was placed on top of the metal frame. The exact chamber volumes were adjusted by the specific surface topography in each plot. The chamber was continuously flushed with 5.5 L min⁻¹ of ambient air taken at a height of 2.3 m, about 10 times the average canopy height. Inside the chamber, a fan circulated the air. Excess air exited the chamber from an open 1/8" Teflon line to prevent overpressure. Prior to sampling, the chamber was flushed for 10 min with ambient air to allow the chamber headspace to equilibrate, while background VOC mixing ratios (Cᵦ) in ambient air were determined. After the flushing period, a Teflon valve (Parker Hannifin, USA) was activated to direct chamber air to the PTR-ToF-MS (inlet flow = 250 ml min⁻¹) for quantification of VOC mixing ratios in the chamber (Cᵦ) during at least 8 min, or until the mixing ratios of key compounds (m/z 69, m/z 81, m/z 137, and m/z 205) were stable. After this time, Cᵦ was sampled again, and measurements continued on a different plot.
Air from the chamber was led through a 13-m long, heated 1/4” Teflon line at a flow rate of 0.25 L min⁻¹ and BVOC mixing ratios were monitored and recorded in real time using a high-resolution PTR-ToF-MS (PTR-TOF 1000 ultra, Ionicon Analytik), equipped with an ion funnel to improve sensitivity. The PTR-ToF-MS was operated at drift-tube settings of 2.30 hPa and 60°C, with a 5-s integration time at a mass range of 30–347 a.m.u. An internal 1,3-diiodobenzene standard added a constant peak at a 203.94 a.m.u. that was used to improve the mass calibration.

Fifteen measurement campaigns (c1-c15) were distributed between May 27 and August 10, 2017 to cover most of the growing season (Table S2). All plots were measured twice per campaign between 8.00 and 11:30 and then again between 12:00 and 17:00. Hereafter, the two rounds are called "morning" and "afternoon," and are used to reflect differences in time of day. Blocks were always measured in the same order, and plots were randomly measured within each block. May 27 and July 13 only consisted of one morning measurement round. Of the 15 campaigns describing the flux variation during the growing season, we selected three individual campaigns for assessment of treatment effects during three phenological stages: green-up (June 12), flowering (June 22), and seed dispersal (July 29).

### 2.5 | BVOC data processing

Raw PTR-ToF-MS data in 5-s resolution were processed using the PTrwid software tool (Holzinger, 2015). PTrwid detected peaks in the measured spectra, applied a mass scale calibration, and subsequently, calculated the mixing ratios of identified BVOCs.

The PTR-ToF-MS was calibrated regularly (3-week intervals) with a gas standard (Ionicon, Innsbruck, Austria) containing a mixture of several VOCs at ppm level, diluted in nitrogen using a liquid calibration unit (LCU; Ionicon). Five compounds (methanol, acetaldehyde, acetone, isoprene, and α-pinene) in the gas standard were used to directly calibrate the mixing ratios of the PTrwid output. α-Pinene was used as a proxy for total monoterpenes. The gas calibration standard did not include a sesquiterpene (SQT) compound, and as such, SQTs were quantified using the PTrwid output based on an instrument transmission function.

Six target compounds were selected based on their known importance and high concentrations in this experiment, and attributed to protonated masses (Yáñez-Serrano et al., 2021) from the PTrwid output: methanol (m/z 33.03), acetaldehyde (m/z 45.03), acetone (m/z 59.05), which are short-chain oxygenated BVOCs, and isoprene (m/z 69.07), monoterpenes (m/z 81.07 and 137.13), and sesquiterpenes (m/z 205.19), which are terpenoids.

From the BVOC mixing ratio time series at 5-s resolution, transition periods when the valves switched between chamber and ambient air were excluded from the data based on visual changes in isoprene concentrations (approx. 2 min). Chamber mixing ratios ($C_{out}$) for each plot were averaged (e.g., across 6 min of continuous measurement, i.e., seventy-two 5-s samples) and the average mixing ratios in the ambient air ($C_{in}$) measured directly before and after each chamber measurement were subtracted from $C_{out}$ to obtain a net change in mixing ratios. $C_{out}$ for all three replicates from each of the two daily rounds (morning and afternoon) were averaged and used to calculate a net flux per square meter of ground (mmol m⁻² h⁻¹) following Ortega and Helmig (2008), giving two calculated fluxes (one morning and one afternoon) from each treatment per campaign. Positive values indicate net emissions from the ecosystem to the atmosphere.

### 2.6 | Measurements of environmental variables

Ambient PAR was recorded every 5 s throughout the whole measurement period (May–August) with a PAR sensor (SLIA-M003, Onset Computer Corporation), except for June 8 and 12, and July 29 due to technical issues. During all BVOC campaigns, iButtons (Hygrochron DS 1923-F5 iButton, Maxim Integrated Products Inc.) recorded ambient air temperatures (1.5 m above vegetation), chamber air temperatures and relative humidity every minute. Prior to each BVOC campaign, soil moisture in the upper 8 cm of the soil (including moss, litter humus, etc.) was measured with a Theta Probe ML3 (Delta T-Devices Ltd, Cambridge, UK) in triplicate around the metal frame. Ecosystem surface temperature within the metal frame was recorded using a portable infrared thermometer (Dostmann Proscan S30, Dual Focus Infrared thermometer) prior to each BVOC measurement.

### 2.7 | GC-MS sampling and analysis

Four times during the season (May 31, June 24, July 15, and August 6), adsorbent tube sampling was carried out to measure the speciation of monoterpenes and sesquiterpenes. A parallel sample, taken from the inlet line of the PTR-ToF-MS was sampled at 200 ml min⁻¹ through a stainless steel tube filled with 150 mg Tenax TA and 200 mg Carbograph 1TD (Markes International Limited) adsorbents. The adsorbent sampling at each plot lasted 30 min and was performed concurrently with the PTR-ToF-MS measurements. Directly after sampling, the tubes were sealed with Teflon-coated brass caps and stored at 4°C until analysis.

The adsorbent tubes were analyzed within 6 weeks by gas chromatography coupled with mass spectrometry (GC-MS, Agilent 7890 A GC and 5975 C VL MSD). Compounds trapped in the tubes were thermally desorbed (UNITY2, Markes International) at 250°C for 10 min, cryofocused at −10°C, and injected onto an HP-5 capillary column using helium as the gas carrier. Column temperature started at 40°C (with a one-min hold) and increased at 5°C min⁻¹ to 210°C, then at 20°C min⁻¹ to 250°C, where remained for 8 min. Chromatograms were processed using PARADiSe software (Johnsen et al., 2017) and identified by comparing the measured mass spectra.
to known calibration standards (Table S3) or to the NIST mass spectral library. GC-MS data were used to calculate the proportions of individual terpenoid compounds in the total monoterpene and sesquiterpene fluxes measured by PTR-ToF-MS.

2.8 | Statistical analysis

The R language version 4.0.2 (R Core Team, 2020) was used for statistical processing. An analysis of variance (ANOVA) linear mixed effect model (lme4), with random and repeated factors, was used to analyze the effects of warming, litter addition, block, time of day, and phenology stage on the fluxes of our six target compounds. NDVI, ambient air temperature, and soil moisture were used as covariates in the model, because they were important explanatory variables affecting the fluxes. PAR data were not included because of the missing data and its close relationship with temperature. Plots nested within blocks (n = 3) were random factors and treatments, time of day, and campaign, together with all possible interactions were fixed factors. If phenology stages were significant, Tukey’s post hoc test was applied to test where the difference was. Owing to the violation of the assumption of normality and homogeneity of variances, a log(X) transformation was applied to isoprene, monoterpene, and sesquiterpene fluxes. For acetaldehyde, with both positive and negative flux values, log (X + (−1) × minimum) transformation was applied. Statistical significance was accepted if p < 0.05.

To assess how the biotic (the biomass of the plant functional groups, NDVI) and abiotic (soil moisture, soil nutrient concentrations, ambient air temperature) variables correlated with BVOC fluxes, a principal component analysis (PCA) was conducted using SIMCA 16.0.1 (Umetrics). The PCA was conducted on the data from the seed dispersal phenology stage, because that stage was measured closest in time to the vegetation analysis. Partial least squares (PLS) regression was used to analyze the covariance between the vegetation composition and the fluxes of the six focus compounds (in the Seed dispersal stage), making one-component PLS models for each compound (y) with the biomass data for individual plant species (x). The variable importance for the projection (VIP) of 0.5 was used to remove unimportant variables from the model stepwise starting from the variable with the lowest VIP.

3 | RESULTS

3.1 | Treatment effects on surface temperature and soil moisture

We observed a significant increase in tundra surface temperature in the warming treatment in both morning (p = 0.019) and afternoon (p = 0.002) measurements, whereas litter addition had no significant effect and there was no W × L interaction (Table S4).

Soil moisture fluctuated throughout the season from 18 vol % in c2, where L plots were especially dry, to 71 vol % during c10, where the C plots were almost waterlogged. The driest campaigns were c2, c8, and c12, where soil moisture levels ranged from 18% to 42%. Litter significantly (p < 0.0001) reduced soil moisture throughout the season (Figure S1).

3.2 | Vegetation composition, biomass, and phenology

Evergreen and deciduous shrubs dominated the vegetation inside the plots, with litter and mosses covering the ground layer independent of the treatment (Table 1). There were no significant treatment effects on the biomass of any of the plant functional groups, and the vegetation composition had large within-treatment variation as a consequence of a very heterogeneous experimental area (Table S1). A PCA assessing correlations between the variables showed a positive correlation between the biomass of graminoids and soil moisture as well as between the biomass of herbs and litter and the NH₄⁺ concentration (Figure S4).

NDVI increased gradually from May (average 0.4 for all treatments) and leveled off in July (Figure 1). We measured the highest NDVI on August 3, with an average of 0.75, and subsequently,

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C</th>
<th>L</th>
<th>W</th>
<th>WL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Graminoids (g m⁻²)</td>
<td>36 ± 6.1</td>
<td>28 ± 7.9</td>
<td>35 ± 12.3</td>
<td>40 ± 8.6</td>
</tr>
<tr>
<td>Herbs (g m⁻²)</td>
<td>33 ± 6.1</td>
<td>27 ± 1.3</td>
<td>36 ± 6.9</td>
<td>27 ± 1.1</td>
</tr>
<tr>
<td>Deciduous shrubs (g m⁻²)</td>
<td>127 ± 21.8</td>
<td>115 ± 14.0</td>
<td>117 ± 16.4</td>
<td>159 ± 41.5</td>
</tr>
<tr>
<td>Evergreen shrubs (g m⁻²)</td>
<td>149 ± 8.6</td>
<td>193 ± 18.8</td>
<td>140 ± 41.0</td>
<td>178 ± 76.5</td>
</tr>
<tr>
<td>Moss (g m⁻²)</td>
<td>72 ± 12.5</td>
<td>136 ± 43.0</td>
<td>179 ± 45.7</td>
<td>146 ± 4.1</td>
</tr>
<tr>
<td>Lichen (g m⁻²)</td>
<td>27 ± 11.8</td>
<td>11 ± 0.0</td>
<td>15 ± 3.5</td>
<td>11 ± 0.0</td>
</tr>
<tr>
<td>Litter a (g m⁻²)</td>
<td>237 ± 4.8</td>
<td>215 ± 5.8</td>
<td>231 ± 19.7</td>
<td>208 ± 3.3</td>
</tr>
</tbody>
</table>

Note: Estimated biomass in the control (C), litter addition (L), warming (W), and warming and litter addition (WL) treatments for graminoids, herbs, deciduous and evergreen shrubs, mosses, lichens, and litter (g dry biomass m⁻², mean ± SE, n = 3).
aLitter includes both litter from dead plant material and the added litter.
observed decreasing values in the following campaigns (Figure 1). In the C plots, the decrease in NDVI during late summer was more pronounced than in the other treatments. Litter addition increased NDVI throughout the season ($p < 0.001$), while warming had no significant effects.

Phenology data were collected on the dominant shrub species *V. uliginosum*, *A. polifolia*, and *E. hermaphroditum*. The greening (May 30) and leaves fully developed (June 15) stages occurred simultaneously for all three species (Figure S2). *E. hermaphroditum* completed the last four phases at a faster pace than the other two and produced ripe fruit by July 1. *V. uliginosum* and *A. polifolia* had ripe fruit on July 15 and 24, respectively (Table S5; Figure S2).

### 3.3 Soil concentrations of extractable C and N

Of all the soil characteristics we examined, only DOC (2–5 cm depth) exhibited a significant effect of treatment, with higher concentrations in the warming and litter addition treatments, but lower concentrations in the WL treatments ($p < 0.01$, Table S6).

### 3.4 Bidirectional BVOC fluxes during the growing season

Methanol and acetaldehyde had high emissions early in the season during green-up ($5.0$ and $2.0$ mmol m$^{-2}$ h$^{-1}$, respectively, averaged across all plots). Acetaldehyde stabilized to lower net emissions across the rest of the season ($0.75$ mmol m$^{-2}$ h$^{-1}$, Figure 2f,g). Methanol emissions increased slightly from $2.0$ to $3.5$ mmol m$^{-2}$ h$^{-1}$ when temperature and PAR peaked in late July, simultaneously with the onset of flower senescence and seed dispersal (Figure 2c,d).
Acetone fluxes fluctuated between net uptake (−0.2 mmol m⁻² h⁻¹) and net emission (0.8 mmol m⁻² h⁻¹) throughout the season and generally followed the ambient air temperature and PAR levels, with increasing emissions under warmer temperatures (Figure 2i,j). The highest net uptake occurred during the first half of July.

Isoprene fluxes were stable throughout most of the season (1 mmol m⁻² h⁻¹ averaged across all plots) until mid-July, when emissions (6–27 mmol m⁻² h⁻¹) and also temperature and PAR peaked (Figure 3a–d). PAR and isoprene flux also correlated positively in the PCA (Figure S3). Beside two distinct peaks during flower senescence and seed dispersal in late July, small peaks appeared during especially afternoon measurements in W plots when ambient air temperature increased.

Monoterpene emissions were low in the early season and peaked around flowering (1.5–3 mmol m⁻² h⁻¹, Figure 3f,g) where PAR also was high. From late-June onwards, monoterpene fluxes seemed to follow ambient air temperatures, similar to acetone.

The dominant monoterpenes in May were ocimene in C, L, and W plots (≈100% of total monoterpene emission; Table S7), and ocimene and camphene in WL plots (86% and 14%, respectively). In June, the dominant monoterpenes shifted to γ-terpinene and lilac aldehyde C and A in all treatments (in total 97%), except for WL plots, where γ-terpinene and α-terpeneol dominated (in total 97%). July and August revealed similar compositions between treatments and largely consisted of γ-terpinene and α-phellandrene (Table S7).

Sesquiterpene emissions were low throughout the season (0.4 mmol m⁻² h⁻¹), with increasing net emissions seen in one replicate of L plots during August (5 mmol m⁻² h⁻¹, Figure 3i,j). Sesquiterpenes followed the same treatment patterns as monoterpenes, where the compound composition of WL plots differed from the other treatments in May and June (Table S8). The dominant sesquiterpene in WL plots in May was α-selinene (100% of total sesquiterpene emission) and in June it was α-copaene (~80%) and β-copaene-4α-ol (~20%). The other treatment plots emitted both α-selinene (~50%) and β-selinene (~50%) in May, whereas noticeable emissions of β-selinene (4%–42%), α-copaene (2%–94%), and β-copaene-4α-ol (2%–24%) were observed in June. In July and August, the sesquiterpene composition was dominated by cis-muurola-3,5-diene, α-copaene, and germacrene D for all treatment plots, but in L plots, α- and β-selinene also contributed significant proportions (10%–20%). As was the case for monoterpenes, the dominant compounds remained unchanged throughout July and August.

3.5 | Effects of time of day, warming, and litter addition on BVOC fluxes during three phenological stages

Generally, there were significant differences in BVOC fluxes between morning and afternoon and between the different phenological stages (Figures 4 and 5; Table 2). Across all treatments combined, all compounds had significantly higher net emissions during morning than afternoon measurements.

Litter addition decreased isoprene emissions by more than 75% averaged across the three phenological phases (p < 0.001) and suppressed the warming treatment effect when combined, but had no
FIGURE 4  Methanol (panel a), acetaldehyde (panel b), and acetone (panel c) fluxes during three phenological stages. The fluxes are shown for green-up, flowering, and seed dispersal stages averaged for morning (8.00–11.30) and afternoon (11.30–17.00) measurements. Bars show mean ± SE, n = 3. Averaged ambient air temperature during each measurement is shown as dots. Control (C), litter addition (L), warming (W), and warming and litter addition (WL).
FIGURE 5  Isoprene (panel a), monoterpane (panel b), and sesquiterpane (panel c) fluxes during three phenological stages. The fluxes are shown for green-up, flowering, and seed dispersal stages averaged for morning (8.00–11.30) and afternoon (11.30–17.00) measurements. Bars show mean ± SE, n = 3. Averaged ambient air temperature during each measurement is shown as dots. Control (C), litter addition (L), warming (W), and warming and litter addition (WL)
effect on the other BVOC focus compounds (Figure 5a; Table 2). The same litter addition effect on isoprene was also evident across the measurement period (Figure 3e). Warming treatment had a significant increasing effect on the emissions of methanol, acetaldehyde, isoprene, and monoterpenes, resulting in a doubling of total net emissions during most phenological phases compared to plots without warming treatment (Figures 4a,b and 5a,b). The exceptions were methanol fluxes during flowering, and methanol and acetaldehyde fluxes during seed dispersal, when the warming treatment only had a minor positive effect on emissions. Ambient air temperatures affected all six focus compounds. Acetone, isoprene, and monoterpenes increased with increasing air temperatures (Figures 4c and 5a,b), whereas acetaldehyde, methanol, and sesquiterpenes displayed decreasing emissions as air temperatures increased (Figures 4a,b and 5c).

Phenological stage had a significant effect on the fluxes of methanol, acetaldehyde, isoprene, monoterpenes, and sesquiterpenes (p < 0.05; Figures 4b and 5a,c). For methanol fluxes, the significant difference between phenology stages was between flowering and seed dispersal (p < 0.03) with lower emissions during seed dispersal (Table 2).

### 3.6 Effects of plant species composition on BVOC fluxes

Results of the PLS analyses showed that methanol emission positively correlated with the biomass of *E. hermaphroditum* (Figure 6). Acetaldehyde emission positively correlated with moss biomass. Acetone emission correlated positively with the biomass of *E. hermaphroditum, Equisetum spp.*, and lichens and negatively with moss biomass.

For isoprene, the strongest positive correlations were with biomass of the herb *Tofieldia pusilla* and the graminoid *Carex vaginata*, but the relationships were not significant due to high variance (Figure 6). Monoterpene emission positively correlated with moss biomass. Sesquiterpene emission showed positive significant correlation with the biomass of *Rhododendron lapponicum* and positive correlation with the biomass of *B. nana*, although this was not statistically significant (Figure 6).

### Table 2: Statistical significance for the factors and interactions affecting BVOC fluxes during the three campaigns representing green-up, flowering, and seed dispersal

<table>
<thead>
<tr>
<th>Compound</th>
<th>Methanol</th>
<th>Acetaldehyde</th>
<th>Acetone</th>
<th>Isoprene</th>
<th>Monoterpenes</th>
<th>Sesquiterpenes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Factor</strong></td>
<td>p-values</td>
<td>p-values</td>
<td>p-values</td>
<td>p-values</td>
<td>p-values</td>
<td>p-values</td>
</tr>
<tr>
<td>Phenology</td>
<td>0.025</td>
<td>&lt;0.001</td>
<td>0.686</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.017</td>
</tr>
<tr>
<td>Litter treatment</td>
<td>0.291</td>
<td>0.510</td>
<td>0.258</td>
<td>&lt;0.001</td>
<td>0.015</td>
<td>0.105</td>
</tr>
<tr>
<td>Warming treatment</td>
<td>0.023</td>
<td>0.003</td>
<td>0.074</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.084</td>
</tr>
<tr>
<td>Time of day</td>
<td>0.012</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.002</td>
<td>0.038</td>
</tr>
<tr>
<td>NDVI</td>
<td>0.001</td>
<td>0.511</td>
<td>0.363</td>
<td>0.060</td>
<td>0.039</td>
<td>0.604</td>
</tr>
<tr>
<td>Ambient air temper</td>
<td>0.112</td>
<td>0.004</td>
<td>0.458</td>
<td>&lt;0.001</td>
<td>0.054</td>
<td>0.049</td>
</tr>
<tr>
<td>Soil moisture</td>
<td>0.679</td>
<td>0.193</td>
<td>0.054</td>
<td>&lt;0.001</td>
<td>0.520</td>
<td>0.037</td>
</tr>
<tr>
<td>Warming × time of day</td>
<td>0.215</td>
<td>0.088</td>
<td>0.064</td>
<td>0.080</td>
<td>0.508</td>
<td>0.165</td>
</tr>
<tr>
<td>Litter × time of day</td>
<td>0.988</td>
<td>0.862</td>
<td>0.643</td>
<td>0.504</td>
<td>0.599</td>
<td>0.798</td>
</tr>
<tr>
<td>Time of day × phenology</td>
<td>0.082</td>
<td>0.027</td>
<td>0.009</td>
<td>0.048</td>
<td>0.701</td>
<td>0.895</td>
</tr>
<tr>
<td>Litter × warming</td>
<td>0.238</td>
<td>0.820</td>
<td>0.225</td>
<td>0.083</td>
<td>0.312</td>
<td>0.488</td>
</tr>
<tr>
<td>Warming × phenology</td>
<td>0.128</td>
<td>0.017</td>
<td>0.461</td>
<td>0.565</td>
<td>0.166</td>
<td>0.451</td>
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<tr>
<td>Litter × phenology</td>
<td>0.704</td>
<td>0.454</td>
<td>0.362</td>
<td>0.934</td>
<td>0.717</td>
<td>0.816</td>
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<tr>
<td>Litter × warming × time of day</td>
<td>0.108</td>
<td>0.370</td>
<td>0.642</td>
<td>0.412</td>
<td>0.500</td>
<td>0.505</td>
</tr>
<tr>
<td>Warming × time of day × phenology</td>
<td>0.146</td>
<td>0.296</td>
<td>0.386</td>
<td>0.892</td>
<td>0.834</td>
<td>0.976</td>
</tr>
<tr>
<td>Litter × time of day × phenology</td>
<td>0.605</td>
<td>0.712</td>
<td>0.873</td>
<td>0.892</td>
<td>0.942</td>
<td>0.814</td>
</tr>
<tr>
<td>Litter × warming × phenology</td>
<td>0.354</td>
<td>0.974</td>
<td>0.573</td>
<td>0.078</td>
<td>0.550</td>
<td>0.757</td>
</tr>
<tr>
<td>Phenology × litter × warming × time of day</td>
<td>0.024</td>
<td>0.502</td>
<td>0.350</td>
<td>0.672</td>
<td>0.440</td>
<td>0.586</td>
</tr>
</tbody>
</table>

Note: p-values from linear mixed effect models are shown for fluxes of acetaldehyde, acetone, methanol, isoprene, monoterpenes, and sesquiterpenes. Phenology = differences between the three phenology stages (three campaigns were selected as representative of these stages). Time of day = the difference between morning and afternoon measurements. Significant p-values are shown in bold.
BVOC fluxes from the subarctic tundra heath followed the hypothesized seasonal pattern, with early season emissions dominated by short-chained oxygenated BVOCs and simultaneously low terpenoid emissions. Generally, higher terpenoid emissions were observed during the peak growing season, which coincided with the highest air temperatures and plant biomass. As expected, air temperature was an important factor controlling the fluxes, but phenology and time of day also influenced the emission rates irrespective of air temperature, which was in contrast to our hypothesis. As hypothesized, the warming and litter treatments showed a direct effect on fluxes of some compounds, but indirect effects, such as differences in plant species composition, were also important.

4.1 Short-chained oxygenated BVOCs and terpenoids have contrasting seasonal net emission patterns

Short-chained oxygenated BVOCs (acetaldehyde, acetone, and methanol) were emitted in significant quantities at the beginning of the growing season, with lower emissions during July, and increasing emissions again in late summer.

Methanol is released from leaves as a by-product of the pectin methylesterase reaction during leaf development (Fall, 2003), which might explain that the highest methanol emissions were seen during green-up. Methanol also represents between 78% and 99% (molar) of the BVOCs released from a wide variety of decomposing litter types (Gray et al., 2010), and therefore may contribute significantly to the ecosystem emissions in late growing season.

Acetone tended to be taken up by the ecosystem during peak summer, which could be explained by dry and wet deposition (Jacob, 2005) or microbial uptake. Acetone, as well as acetaldehyde and methanol, can serve as a carbon source for microbes (Albers et al., 2018; Ensign et al., 1998). Furthermore, Greenberg et al. (2012) reported that emissions from litter represented <1% of the total above-canopy ecosystem BVOC emissions, suggesting that uptake or conversion of the compounds occurs. They also reported an increase in acetone, acetaldehyde, and methanol litter emissions with increasing temperature, and thus, an increased uptake instead of a decrease in production of the compounds is a possible suggestion (Greenberg et al., 2012). The balance between emissions and uptake is variable across the season, with microbial activity and uptake dominating in July and emissions becoming increasingly dominant in late July. We also found a significantly higher soil DOC concentration in warmed plots, which supports higher microbial activity when temperature increases (Don & Kalbitz, 2005).

Terpenoids had contrasting seasonal patterns to the short-chained oxygenated BVOCs. Generally, terpenoids exhibited the highest net emissions when temperatures were highest during peak summer, but exact timing of the emission peak differed between the three selected terpenoid groups (isoprene, monoterpenes, and sesquiterpenes), and must be influenced by different factors such...
as phenology. Hakola et al. (2001) showed that monoterpene fluxes from silver birch (Betula pendula) change across plant development, with higher emissions during bud break and when leaves were fully grown (harder and darker), than during leaf development. In our study, we observed the same change in terpenoid emissions across different phenological stages, with increasing net emissions as the growing season advanced. Dani et al. (2020) showed that flowers of some plant species increase monoterpene emissions during flowering and senescence, and suggested that the increase serves as protection from seed predators. It is indeed possible that the high terpenoid emissions during flowering and seed dispersal in our study occurred to deter florivores and seed predators. However, because our measurements were on the community scale, we cannot distinguish emissions from flowers, other plant parts, and soil. Furthermore, during the seed dispersal stage, we measured the highest air temperatures, which are known to have a strong positive effect on isoprene, monoterpene, and sesquiterpene emissions (Duhl et al., 2008; Guenther et al., 1993; Lindwall, Schollert, et al., 2016; Lindwall, Svendsen, et al., 2016; Seco et al., 2020; Tiiva et al., 2008). As expected, PAR seemed to have an effect on monoterpene fluxes (Llusia et al., 2016). This is in agreement with results from Hakola et al. (2001), who discovered decreasing emissions of \( \gamma \)-terpinene, the dominant monoterpene in our study, when birch were shaded.

### 4.2 Effects of temperatures on BVOC net emissions

We expected ecosystem-level BVOC emissions to increase with increasing temperatures. Warmer temperatures increase BVOC biosynthesis rates in leaves (Laathawornkitkul et al., 2009), enhance net ecosystem production and vegetation biomass (Rinnan et al., 2008), and thus, BVOC release from vegetation. Warming also stimulates microbial decomposition (Blok et al., 2018), which enhances microbial and litter BVOC release (Li et al., 2012; Svendsen et al., 2018).

Our warming treatment had a positive significant effect on methanol and acetaldehyde fluxes, whereas ambient air temperature correlated negatively with acetaldehyde fluxes. A higher ecosystem uptake, because of increased microbial activity, than emission could explain this negative correlation (Albers et al., 2018). However, microorganisms are not only a sink for BVOCs but also a source (Lemfack et al., 2018), and the source–sink balance is affected by several factors (Peñuelas et al., 2014). Further, mineralization or transformation of these compounds into other substances, for example, in soil, may take place (Tang et al., 2019). Mineralization is a rapid process often without a lag phase (Albers et al., 2018).

After 18 years of manipulation treatments, our warming treatment significantly increased the emissions of isoprene and monoterpenes (\( \approx 245\% \) and 115%, respectively), which agrees well with previous results on monoterpene emission at the same site (Faubert et al., 2010; Valolahti et al., 2015). In contrast, the effect of warming on isoprene emissions in our study was stronger than the effect observed in earlier measurements (\(<100\% \) increase; Tiiva et al., 2008; Valolahti et al., 2015). Results from the same site after 7 years of treatments concluded that this warming effect was a direct, rather than an indirect, effect, of higher temperatures in the warming plots (Faubert et al., 2010; Tiiva et al., 2008). In our study (after 18 years), we also found a direct, positive effect of ambient air temperature with increasing isoprene and sesquiterpene emissions, regardless of treatment. This direct effect is supported by the positive correlation between isoprene flux and PAR as shown by our PCA, because PAR and vegetation surface temperature are closely interlinked (Seco et al., 2020). However, data from the same site 5 years earlier (after 13 years of consecutive summer warming), showed that the warming effect on BVOC emissions was not only a direct product of higher temperatures, but also a response to the long period with warmer conditions which had caused changes in plant species composition and increased biomass (Valolahti et al., 2015). Our results showed no changes in the aboveground plant biomass or significant effect of warming on NDVI, likely due to low statistical power when measuring only three replicate plots per treatment. Earlier measurements from the same site performed on all six replicates showed increasing greenness of the vegetation under warming (Michelsen et al., 2012; Rinnan et al., 2008), and, 2 years prior to the current study, increased total vascular plant cover with warming (Pedersen et al., 2017). Based on these earlier findings, we assume that the warming treatment still has a significant indirect effect on BVOC emissions. Thus, the initial direct effects of warming on terpenoid emissions likely become increasingly affected by biomass and plant composition changes through time (Rinnan et al., 2020).

Earlier studies from the same site have reported direct warming effects and indirect effects via vegetation changes on sesquiterpenes (Faubert et al., 2010; Tiiva et al., 2008; Valolahti et al., 2015), which contrasts with our results, because we only found a direct effect. This discrepancy may be partly due to the specific vegetation community present in the experimental plots and partly due to methodological differences. The highest sesquiterpene emissions were observed in plots L1 and W3 (see Figure 3i,j). These plots had the highest biomass of \( R. \) lapponicum, an important sesquiterpene emitter (Mofikoya et al., 2018; Valolahti et al., 2015). The PLS model for plant species biomass effects on sesquiterpene emission also revealed a positive correlation between \( R. \) lapponicum and sesquiterpene emission. Furthermore, sesquiterpenes are relatively unstable compounds and may oxidize, fragment, or degrade when collected. We used PTR-ToF-MS as the sampling method with high inlet flow, and even though the inlet line was heated, our measurement technique might lead to underestimations of sesquiterpene emissions (Duhl et al., 2008; Kim et al., 2009).

### 4.3 Litter addition effects on BVOC net emission and plant composition

To simulate the expected increases in shrub growth in a warmer future climate, additional birch litter had been applied to the experiment every autumn since 1999. We expected that a direct response
of added litter would be an increase in litter-emitted BVOCs (Gray & Fierer, 2012; Gray et al., 2010; Ramirez et al., 2010). In contrast, long-term effects could result from changes in the ecosystem, due to the extra input of nutrients and carbon from the litter (Rinnan et al., 2008; Rinnan & Rinnan, 2007), which in turn would lead to altered (likely increased) emissions from the whole ecosystem. Furthermore, litter addition increased NDVI, which indicates an increase in the greenness and likely also biomass of the vegetation (Rinnan et al., 2008). In nutrient-deficient subarctic heaths, plants regulate growth and nutrient uptake according to availability of the limiting nutrient element (Liu et al., 2020). Hence, although litter addition increased green biomass measured as NDVI, there was no treatment effect on soil available N. The increase in soil DOC concentration with litter addition and warming is consistent with earlier findings (Pedersen et al., 2017) and suggests that warming and additional input of organic matter support a more active microbial community, leading to higher DOC concentration in soil. This DOC may both stem from microbial and litter breakdown products (Don & Kalbitz, 2005) and could also be related to higher BVOC emissions from soil.

We also observed that litter addition decreased soil moisture, which may result from increased evapotranspiration and decomposition rate in response to higher vegetation biomass (Kirschbaum, 2004). Although litter addition increased the DOC content and led to slightly drier soil, the soil moisture content in this wet heath was still high (20%–40%) and decomposition was likely not limited by low moisture content. Furthermore, we did not observe a decrease in the water-loving graminoids in plots with lower soil moisture (litter-added plots), which supports our conclusion that plants in these plots were not water limited (Vendsen et al., 2016).

The point intercept analysis-based biomass estimation we performed in July showed that litter-added plots actually contained less litter than other treatment plots (Table 1), probably due to increases in the rate of microbial decomposition stimulated by additional nutrient inputs from the added litter (Rinnan et al., 2008; Rinnan & Rinnan, 2007). Our observations of lower emissions of the typical litter-emitted BVOCs (acetone, acetaldehyde, and methanol) in the litter-added plots might also be explained by an increase in microbial degradation/uptake due to enhanced nutrition. Litter decomposition rates are highest immediately after litter fall (Leff & Fierer, 2008) and temperature dependent (Harmon et al., 2009), and thus the peak release of compounds from the litter may occur after thaw, in spring, when increasing temperature boost microbial decomposition and in autumn, during defoliation. The litter addition in our experiment occurred after our last campaign, and as such, we did not capture the instant effect of the treatment, but we did see high short-chained oxygenated BVOC emissions in the early season, which is in accordance with early season litter-related emissions in a boreal pine forest reported by Aaltonen et al. (2011). Thus, the direct effect of litter addition on short-chained oxygenated BVOCs might still be relevant in our study but only in the early season.

The litter treatment had a significant negative effect on isoprene and monoterpenes fluxes, which was likely unrelated to litter addition per se, but due to differences in the vegetation composition. Willow and graminoid species are known isoprene and monoterpenes emitters (Ekberg et al., 2009; Hakola et al., 1998) and in our plots treated with litter, we saw less of these species compared to control plots, which could explain the lower isoprene and monoterpenes emissions. Our PLS models on isoprene and monoterpenes fluxes showed no significant relationships with the biomass of graminoids or willows. Furthermore, the stimulating effect of litter on microbial activity, including microbial uptake of isoprene, could also explain the negative effect observed on isoprene fluxes in litter-added plots (Gray et al., 2015).

Earlier studies on BVOC net emissions performed at the same experimental site reported no significant effect of litter addition (Faubert et al., 2010; Tiiva et al., 2008), which opposes our significant effect on isoprene and monoterpenes fluxes. Our findings support the idea that litter additions can lead to both direct and indirect effects in the longer term (Valolahti et al., 2015), mainly caused by changes in plant community composition.

4.4 | BVOC net emissions are highest in the morning

For all compounds, net fluxes measured in the morning were significantly higher than afternoon measurements during all phenological stages. This was despite the fact that temperatures were higher in the afternoon than in the morning, both during the green-up and flowering campaigns, while the seed dispersal campaign showed no difference between morning and afternoon temperatures. Thus, temperature dependency cannot explain significantly higher emissions in the morning. Instead, we propose that the difference is due to time of day patterns in ecosystem processes contributing to the emissions. Hewitt et al. (2011) and Patankar et al. (2013) showed that time of day patterns in ecosystem activity seemed to be governed to some degree by circadian rhythms irrespective of seasonal changes. Furthermore, Patankar et al. (2013) showed that assimilation of carbon, the major building block of BVOCs, peaks during morning in Cassiope-dominated vegetation, which supports a higher BVOC emission. These results are supported by Loivamäki et al. (2007) who found that isoprene synthase in poplar leaves displays time of day variations in expression, with highest expression in the morning, although they reported highest isoprene emission in the afternoon, which is in contrast with our results. Dudareva et al. (2003, 2004) showed that the emissions of some monoterpenes and sesquiterpenes follow the time of day rather than changes in temperature and PAR intensity. Furthermore, acetaldehyde, acetone, and methanol are relatively soluble in water and may get dissolved in dew drops or trapped inside stomata, and in the morning, when the dew evaporates and stomata open, these BVOCs are volatized (Seco et al., 2007).

To conclude, our results highlight the dynamic bidirectional nature of BVOC fluxes and show the complexity of factors affecting emissions and uptake of the compounds. We suggest that phenological stage and time of day have a more significant effect on
ecosystem BVOC fluxes and their chemical composition than earlier thought, although changes in temperature and PAR also interact. Indirect effects of warming and litter addition, such as changes in plant community and microbial activity, affect the bidirectional exchange of BVOCs in a subarctic tundra heath ecosystem, and we expect this influence to increase over time.

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AUTHOR CONTRIBUTIONS

NB, RR, and TH designed the experiment. NB, RR, and AM sampled BVOC, meteorological, soil, and phenology data. NB, TL, and RS analyzed the data and prepared the figures and tables. NB, RR, and TH wrote the manuscript with contributions from all the authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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