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Synergistic effects of insect herbivory and changing climate on plant volatile emissions in the subarctic tundra

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INTRODUCTION

Climate-change-induced alterations in temperature, snow, ice-cover, and nutrient availability are having extensive ecological consequences in Arctic ecosystems (Pascual et al., 2020; Post et al., 2009). Climate warming, which proceeds at twice the rate of the global average in the Arctic (IPCC, 2014), directly and indirectly affects the emissions of volatile organic compounds (VOCs) from vegetation. The direct effect of climate warming has been shown to substantially increase VOC emissions from Arctic ecosystems.
(Faubert et al., 2010; Kramshøj et al., 2016; Lindwall et al., 2016). Additionally, climate warming is accelerating the range expansion of insect species into higher altitudes and latitudes (Ammunet et al., 2015; Jepsen et al., 2008), thus leading to increased insect herbivore pressure on plants, which also leads to increased VOC emissions from Arctic plants during periods of active insect herbivory (Li et al., 2019; Rieksta et al., 2020; Ryde et al., 2021). Moreover, reduced light availability resulting from the increasing cloudiness predicted for the Arctic (Boucher et al., 2013; Norris et al., 2016; Young et al., 2019) may decrease VOC emissions (Kramshøj et al., 2016).

Insect herbivory is a significant source of plant stress and vegetation damage that leads to increased and altered VOC emissions. While climate change has resulted in decreased abundance and diversity for many species (IPCC, 2014; Wagner et al., 2021), some insect species have increased their population sizes or range due to warmer temperatures. Thus, the damage by insect herbivores is growing, especially at higher elevations and latitudes (Høye et al., 2015; Jepsen et al., 2008). For example, Barrio et al. (2017) found that 1.4% of leaf area of the circumpolar dwarf birch (Betula glandulosa-nana complex) is currently lost due to background insect herbivory, and that with a 1°C increase in summer temperatures, insect herbivory will likely increase by 6–7%. Furthermore, areas that experience cyclic outbreaks are expected to be exposed to more pronounced outbreak events (Jepsen et al., 2008). For example, climate change has expanded the outbreak ranges for locally dominant geometrid moths, such as winter moth (Operophtera brumata) and autumnal moth (Epirrita autumnata), that periodically cause severe defoliation in Northern Fennoscandia. Range expansion occurs due to decreased cold events during winter and increased warm spring days, which leads to higher survival rates of overwintering eggs (Ammunet et al., 2015; Bale et al., 2002; Jepsen et al., 2011).

Experimental warming alone has been shown to strongly increase VOC emissions from Arctic vegetation (Faubert et al., 2010; Kramshøj et al., 2016; Lindwall et al., 2016; Valolahti et al., 2015). These studies have shown that experimental warming by 2–4°C in air, using open top chambers (OTCs), more than doubles total VOC emissions, as well as those of specific compound groups such as monoterpens and sesquiterpenes. The two main factors that modulate increased VOC emissions upon warming are the plant physiological and physiochemical controls (Niinemets et al., 2004). First, physiological controls, such as the biosynthesis of VOCs and enzyme activity, increase with temperature. A recent study by Simin et al., (2021) showed that net photosynthesis of dwarf birch (Betula nana L.) increased with leaf temperature up to 30°C, indicating a high thermal tolerance. Furthermore, Ghirardo et al., (2020) showed that 5°C of warming doubled the de novo monoterpene biosynthesis in B. nana. Second, the physiochemical controls—volatility and diffusion—are also temperature-dependent (Niinemets et al., 2004).

Similar increases in VOC emissions in response to temperature have been reported after 1–2 years of experimental warming (Lindwall et al., 2016), 6 (Kramshøj et al., 2016) to 7 years (Faubert et al., 2010), and up to 16 years of warming (Valolahti et al., 2015). The warming duration can be an important factor influencing VOC emissions because plants can acclimate by exhibiting phenotypic plasticity, such as changes in anatomical traits in response to sustained experimental manipulation of abiotic factors (Schollert et al., 2015; Tang et al., 2018). For example, the studies by Schollert et al., (2015) and Rinnan et al., (2011) showed weak effects of warming treatment on VOC emissions from B. nana branches in subarctic tundra, where plants had been exposed to manipulated warming for 20 and 18 years at a time, respectively.

Another expected result of the rapid Arctic warming is an increase in cloud cover and thickness (Boucher et al., 2013; Norris et al., 2016; Young et al., 2019). By reflecting solar radiation away from the Earth’s surface, increased cloud cover is expected to alter plant community composition and reduce photosynthesis, and subsequently, plant VOC emissions, due to the light dependence of VOC biosynthesis (Duhl et al., 2008; Peñuelas & Staudt, 2010). For example, 6 years of experimental shading decreased VOC emissions from Arctic vegetation by 65% (Kramshøj et al., 2016), which was likely a result of both reduced photosynthetically active photon flux density (PPFD) and a reduction in canopy temperature (Duhl et al., 2008; Peñuelas & Staudt, 2010). However, Schollert et al., (2015) found only a weak shading effect on VOC emissions, potentially due to acclimation of plant traits after 20 years of treatment (Schollert et al., 2015). While these studies focused on constitutive VOCs emitted without biotic stress, it is unknown whether plant volatile defenses to insect herbivory differ in plants with long growth histories under contrasting temperatures and light availabilities.

Few studies have analyzed VOC emissions in response to insect herbivory in high-latitude ecosystems (Li et al., 2019; Mäntylä et al., 2008; Rieksta et al., 2020; Ryde et al., 2021). To date, there is only one study that has assessed the interactive effects of warming and mimicked insect herbivory (methyl jasmonate application) on VOC emissions from B. nana (Li et al., 2019). The study found that mimicked herbivory increased VOC emissions and that warming synergistically amplified this response. To our knowledge, no studies have so far assessed how effects of feeding by local insect herbivores are influenced by climatic changes, such as warming and increasing cloudiness.

The main aim of this study was to assess how 30 years of in situ experimental manipulation of temperature and light availability interact with insect herbivory and affect the VOC emissions from the widespread, subarctic-arctic, deciduous shrub, B. nana. We collected B. nana VOCs in a subarctic tundra heath in Abisko, Northern Sweden, after 30 years of climatic manipulation of warming and shading and compared emissions from intact branches and branches fed to larvae of local geometrid moths. We hypothesized that (1) herbivory alone would stimulate plant VOC emissions and alter the VOC blend, due to the defensive functions of plant VOCs against herbivore attack; (2) warming would increase VOC emissions while shading would decrease plant VOC emissions, due to the light and temperature dependence of VOC biosynthesis; and (3) warming would amplify and shading would negate the effects of herbivory on VOC emissions.
2 MATERIALS AND METHODS

2.1 Study site and climate manipulation treatments

The study was carried out in Abisko, Northern Sweden during the growing season of 2018. The growing season usually extends from early June to late August. The average annual temperature is around $-0.4 \pm 1^\circ C$ (2009–2019 average; mean ± standard error of the mean), with July being the warmest (average temperature of $12.0 \pm 1.4^\circ C$) and February, the coldest ($-11.0 \pm 0.3^\circ C$) month (Abisko Scientific Research Station, 2019).

We used a long-term field experiment that was established in 1989 and has been maintained since then in the open tundra just above the treeline (68°19′33.13″N, 18°51′13.09″E; Havström et al., 1993). The experiment mimics climate warming and increased cloudiness in a randomized complete block design. It consists of six blocks, each with an area of 400 m² placed on a uniform WNW sloping heath. In this study, we used three treatments: warming (W), shading (S), and ambient control (A), yielding a total of 18 plots (each 1.2 × 1.2 m²), each at least 5 m apart. The warming treatment uses OTCs (1989–2017 made of 0.05 mm transparent polyethylene film supported by dome-shaped PVC tubes, and from 2018 onwards, 5 mm polycarbonate plates forming a hexagonal OTC) that were designed to increase air temperature by up to 3–4°C (Havström et al., 1993). The shading treatment uses dome-shaped hessian tents that reduce PPFD by 50–60% (Ellebjerg et al., 2008; Schollert et al., 2015).

To evaluate how the treatments affect temperature, the actual soil/moss surface-level temperature in the plots was monitored in 2011 for the period June 3–August 31 (Figure 1). Averaged across the growing season, the daily average daytime temperature for shading treatment was 0.4°C lower than in ambient control (Dunnett’s test, $p = 0.02$), whereas warming treatment had 3.5°C higher temperature than ambient control (Dunnett’s test, $p < 0.001$) (Figure 1).

2.2 Insect herbivory treatment

We collected a mixture of larvae of the locally dominant geometrid moths, winter moth and autumnal moth. The larvae were collected from a mountain birch forest near Abisko (68°18′32.″N, 19º12′07.3″E) and reared in the laboratory on detached birch branches until use within 10 days. A mixture of larval instars were used during the experiment.

Two healthy, unherbivorized $B. nana$ branches, with 92 ± 8 leaves/branch, were randomly selected in each plot ($n = 36$). One branch was subjected to herbivory treatment (H) by enclosing it in a transparent mesh bag (35 × 20 cm) along with three larvae while the other branch served as a control (C) and was enclosed in a mesh bag without larvae. Larvae were gently applied on leaves using a soft brush.

Herbivory treatments started on June 26, about 2 weeks after leaf-out of $B. nana$, and lasted until July 5. We measured VOCs on June 30 and July 5 (i.e., 4 and 9 days after larval addition), and July 11 (i.e., 6 days after larval removal). Before each VOC measurement, we removed the mesh bags, larvae, and their feces. After VOC measurements, the larvae were reapplied on the branches in mesh bags, as described above.

After each VOC measurement, we visually estimated the total leaf area of the branch following the method described in Li et al., (2019) and after the last VOC sampling, we counted the number of damaged leaves on each experimental branch. Destructive sampling was not possible in the long-term experiment.

![Figure 1](https://example.com/figure1.png)

**FIGURE 1** Daily temperatures for the 2011 growing season averaged between 07:00 and 18:00 for ambient control (A), shading (S), and warming (W) treatments. (a) Data for the treatment plots at the soil/moss surface-level temperature were obtained from Gemini Tinytag Data Loggers ($n = 6$ per treatment). Daily ambient air temperatures at 2 m height at the Abisko Scientific Research Station are also shown. (b) The average temperature over the growing season for each climate treatment (mean ± standard error of the mean), where asterisks denote a significant difference from the ambient control treatment (‘*’ $p < 0.05$; ‘**’ $p < 0.001$; Dunnett’s test).
2.3 | VOC measurements

VOCs were captured using a branch enclosure method described previously (Vedel-Petersen et al., 2015). Pre-cleaned (120°C for 1 h) polyethylene terephthalate (PET) bags (25 x 38 cm) were used as enclosures through which air was circulated with pumps and VOCs were trapped from outgoing air on stainless steel adsorbent cartridges (150 mg Tenax TA, 200 mg Carbograph 1TD, Markes International Limited, Llantrisant).

Each PET bag was ventilated before the measurement for approximately 5 min with an inflow rate of 1000 ml min⁻¹. Subsequently, the adsorbent cartridge was inserted via a cut corner into the PET bag and secured with wire. During the 20-min sampling period, air was circulated through the enclosure with an inflow rate of 300 ml min⁻¹ and an outflow rate of 200 ml min⁻¹. The excess air leaked out from the opening where the branch entered the bag. The incoming air was filtered for particles and background hydrocarbons, and scrubbed for ozone in order to avoid losses of the highly reactive VOCs (Kramshøj et al., 2016; Valolahti et al., 2015). After sampling, the cartridges were sealed with Teflon-coated brass caps and stored at 5°C until analysis. Blank sampling from empty PET bags was conducted in situ, to account for compounds derived from sampling materials and the analytical system. A new PET bag was used for each measurement.

Temperature and relative humidity inside the branch enclosure, as well as in ambient air at 1 m above ground, were monitored during sampling with iButtons (Hygrochron, Maxim Integrated; Table 1). Light intensity was monitored using a Photosynthetic Light Smart Sensor (S-LIA-M003, Onset Computer Corporation; Table 1).

Averaged across the three measurements, the enclosure temperatures were 21.8 ± 1.1°C in the ambient, 19.4 ± 1.0°C in the shading treatment and 24.4 ± 1.3°C in the warming treatment (Figure S1). The enclosure temperatures in the ambient were 2.39 ± 1.1°C higher than those in the shading treatment (Dunnett’s test, p = 0.09; Figure S1), and 2.65 ± 1.1°C lower than those in the warming treatment (Dunnett’s test, p = 0.06; Figure S1). During VOC measurements, the temperature and relative humidity in the branch enclosure were, on average, 5.5°C and 11.4% units higher than in the ambient area, respectively (Table 1). The shading treatment reduced the PPFD by 61–73% across the three VOC measurements, as compared with the ambient control treatment.

2.4 | VOC analysis

VOC samples were analyzed using gas chromatography-mass spectrometry (7890A Series GC coupled with a 5975C inert MSD/DS Performance Turbo EI System, Agilent) after thermal desorption (TD100-xr, Markes International Ltd, Llantrisant, UK). The carrier gas was helium (1.2 ml min⁻¹) and the oven temperature was held at 40°C for 1 min, then raised to 210°C at a rate of 5°C min⁻¹, and finally to 250°C at a rate of 20°C min⁻¹, where it was held for 8 min. An HP-5 capillary column (50 m length, 0.2 mm diameter, and 0.33 μm film thickness) was used for the separation of VOCs.

Chromatograms were analyzed using the software, PARADiSe v. 3.8. (Johnsen et al., 2017). Compounds were identified using pure standards, when available, or tentatively identified using the NIST 2014 Mass Spectral Library. VOC concentrations were quantified using external standards. For quantification of compounds where pure standards were unavailable, we used α-pinene for monoterpenes and homoterpenes, caryophyllene for sesquiterpenes, hexanal for green leaf volatiles, 1-octene for hydrocarbons, benzaldehyde for oxygenated VOCs, and toluene for other compounds. We categorized compounds into the following groups: isoprene, monoterpenes (MTs), homoterpenes (HTs), sesquiterpenes (SQTs), green leaf volatiles (GLVs), hydrocarbons (HC), oxygenated VOCs (OVOC), and other VOCs. VOC emission rates are expressed on a leaf area basis.

2.5 | Statistical analyses

All statistical analyses were performed in the R statistical framework—version 4.0.0 (RStudio Team, 2020). To assess the effects of insect herbivory and climate treatments (warming and shading) on VOC emissions, we performed linear mixed-effect models (LMM) fitted with maximum likelihood (ML), using the “lmer” function from the lme4 package (Bates et al., 2015). We performed LMM with total VOCs and VOC groups as response variables, treatment (ambient, warming, and shading), herbivory (control, herbivory), sampling date (June 30, July 5, and July 11), and their interactions entered as fixed factors, and block and plot as random factors, with plot nested in block, to encompass potential spatial variation in soil and moisture conditions, and vegetation properties along the slope. We used backward model selection using the function "drop1" and tested with a likelihood-ratio test (Chi-square test) to identify the significance of each predictor variable (significance level for interactions was set at p < 0.25). To assess the main effects, we used the “ANOVA” function from the lmerTest package (Kuznetsova et al., 2017). Due to heteroscedasticity of the variances, we applied a log (X + 1) transformation to the VOC data. Parameter estimates between ambient and each of the climate treatments and between herbivory treatments were obtained from estimated marginal means (EMMs), using the default/balanced “emmeans” function from the emmeans package. For post-hoc pairwise comparisons, we used default pairwise tests for herbivory treatments, Dunnett’s tests for climate treatments, and Tukey’s method P-value adjustments were used for post-hoc date variables from the emmeans package (Lenth et al., 2020).

To assess how the herbivory and climate treatments altered the relative contributions of individual VOCs to the total VOC emission profile (hereafter called “VOC blend”) and to identify compounds important for the blend differences, we ran the unsupervised machine learning algorithm Random Forests (RF) (Breiman, 2001, 2003). We used the “randomForest” function from the randomForest package. RF reveals if samples fall into distinct clusters and if so, how clusters are related to a certain treatment.
For assessing herbivory effects, the RF was run separately for each measurement date \((n = 18)\), and for each climate treatment within each measurement date \((n = 6)\). For assessing climate treatment effects, RF was run for all samples in total, and for each measurement date separately. For both unsupervised and supervised RF, we drew \(N_{\text{tree}} = 100,000\) bootstrap samples with \(m_{\text{try}} = 15\) variables (VOCs) randomly selected at each node. For optimal results, Breiman (2003) suggest an \(m_{\text{try}}\) value equal to the square root of the number of variables \((n = 221)\). If the unsupervised RF model showed a clear clustering between herbivory or climate treatments, we ran supervised RF with herbivory or climate treatment as the response variable and relative proportions of individual VOCs as the predictor variables. Supervised RF returns an out-of-bag (OOB) error rate and confusion matrix for each classification and selection method, filtering a subset of samples and variables at random. As RF leaves out variables at each tree, there is no need for cross-validation or a separate test set for unbiased results (Breiman, 2003). We also obtained the importance of each VOC for the classification, which is expressed as the mean decrease in accuracy (MDA). Higher MDA values mean that a specific VOC has high importance in separating the VOC blends. Paired t-tests for herbivory treatment and Dunnett’s tests for climate treatments were used to evaluate differences between emission rates of VOCs with the highest MDA.

To assess the percentage of leaves damaged by larvae at the end of all VOC measurements, we performed LMM with the percentage of damaged leaves as the response variable and climate treatment, herbivory, and their interaction as fixed factors. Similarly, to assess the change in leaf area during the insect herbivore feeding period, we performed LMM with the predicted leaf area as the response variable and climate treatment, herbivory, date, and their interaction as fixed factors.

### RESULTS

#### 3.1 Herbivory effects on leaf damage

During the feeding period, herbivory increased the percentage of damaged leaves in the sampled branch (ANOVA, \(F_{1,15} = 63, p < 0.001\)). By July 5, that is, 9 days after larval addition, experimentally added larvae, together with naturally occurring herbivorous insects, damaged \(24 \pm 2.4\%\) of leaves across all climate treatments, compared to \(2 \pm 2.3\%\) damaged leaves in controls by naturally occurring herbivorous insects (Tukey, \(p < 0.001\)). Consequently, leaf area decreased by 22% between June 30 to July 5 in the branches exposed to insect herbivory (ANOVA, \(F_{1,85} = 14, p < 0.001\)).

#### 3.2 Herbivory and climate treatment effects on VOC emissions

Of all the VOC groups, GLVs comprised the largest fraction and contributed 22–76% across all treatments (Table S1). Oxygenated VOCs were the second largest group comprising 14–41% of the emissions depending on the treatment (Table S1).

We found that herbivory, alone, increased the emissions of total VOCs (Figure 2a), monoterpenes (Figure 2b), homoterpenes (Figure 2c), other VOCs (Figure 2d), and sesquiterpenes (Figure 2e) \((p < 0.05)\), and marginally, the emissions of hydrocarbons and isoprene \((p = 0.06, 0.09\), respectively; Table S2), whereas GLVs did not increase in response to herbivory \((p = 0.70; \text{Figure 2f})\). Emission responses to insect herbivory differed between the three measurements for monoterpenes and homoterpenes (ANOVA, \(F_{2,83} = 11.2, P_{\text{HTs}} < 0.001; F_{2,83} = 11.4, P_{\text{HTs}} < 0.001\), herbivory \(\times\) date interaction; Table S3). The herbivory effects on monoterpenes and homoterpenes were absent on June 30, 4 days after the addition of larvae (Figure 2b,c). However, the monoterpene and homoterpene emission rates were increased by herbivory on July 5, 9 days after the addition of larvae, yielding sevenfold and eightfold higher emissions, respectively, compared to unherbivorized branches, when averaged across the climate treatments (Figure 2b,c). The herbivory effects on monoterpenes and homoterpenes had ceased by July 11, 6 days after the removal of larvae. However, on July 5 and July 11, the emissions of other VOCs were threefold and twofold higher in herbivory compared to control branches (ANOVA, \(F_{2,83} = 8.6, p < 0.001\) herbivory \(\times\) date interaction), respectively, when averaged across the climate treatments (Figure 2d).

VOC emission responses to herbivory differed between climate treatments for sesquiterpenes and homoterpenes (ANOVA, \(F_{2,83} = 3.9, P_{\text{SQTs}} < 0.001; F_{2,83} = 2.3, P_{\text{HTs}} = 0.09\), herbivory \(\times\) climate treatment interaction; Table S4). The herbivory effect on sesquiterpene emissions was only present in the warming treatment, in which herbivory caused threefold increases across all measurements (Figure 2e; \(p < 0.001\)). Homoterpene emissions were fourfold, 14-fold, and sixfold higher in herbivory treatments, compared to controls in the ambient, shading, and warming treatments, respectively, on July 5 \((P_{\text{control}} = 0.06, P_{\text{shading}} = 0.01, P_{\text{warming}} < 0.001)\) (Figure 2c).

<table>
<thead>
<tr>
<th>Environmental variable</th>
<th>June 30</th>
<th>July 5</th>
<th>July 11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air temperature, 1m above ground (°C)</td>
<td>9.5 ± 0.1</td>
<td>17.6 ± 0.1</td>
<td>22 ± 0</td>
</tr>
<tr>
<td>Relative humidity, 1m above ground (%)</td>
<td>57.4 ± 0.2</td>
<td>59.2 ± 0.3</td>
<td>32.6 ± 0.1</td>
</tr>
<tr>
<td>Photosynthetically active photon flux density (μmol m⁻² s⁻¹)</td>
<td>665 ± 13</td>
<td>457 ± 13</td>
<td>1014 ± 17</td>
</tr>
<tr>
<td>Enclosure temperature (°C)</td>
<td>15.6 ± 0.2</td>
<td>20.7 ± 0.2</td>
<td>29.3 ± 0.3</td>
</tr>
<tr>
<td>Relative humidity inside the enclosure (%)</td>
<td>65.9 ± 0.4</td>
<td>66.7 ± 0.4</td>
<td>50.7 ± 0.5</td>
</tr>
</tbody>
</table>

**Table 1 Environmental variables**

(mean ± standard error of the mean) recorded during measurements on June 30, July 5, and July 11.
Overall, we found that 30 years of experimental shading did not decrease the total VOC emissions or those of any of the VOC groups when compared to the ambient control (Figure 2a; Table S5). The only exception was the monoterpene emission rate on July 11, when the ambient control treatment had a twofold higher emission rate than the shading treatment (Dunnett’s test, \( p = 0.07 \); Figure 2b). Long-term warming doubled the emissions of total VOCs relative to the ambient control, when averaged across all measurements (Dunnett’s test, \( p = 0.02 \); Figure 2a). We also found that monoterpene and GLV emissions increased in response to warming on July 5 and July 11 (ANOVA, \( F_{4,81} = 1.7, P_{MTs} < 0.001; F_{4,81} = 1.5, P_{GLVs} = 0.2 \), climate treatment \( \times \) date interaction; Table S6). More specifically, warming doubled monoterpene and GLV emissions (Dunnett’s test, \( P_{MTs} = 0.08 \) and \( < 0.01 \) for July 5 and 11.)
respectively; \( P_{GLVs} = 0.01 \) and 0.04 for July 5 and 11, respectively) (Figure 2b,f).

### 3.3 Effects of climate treatments and herbivory on VOC blends

VOC blends released by control and herbivore-damaged branches could not be separated in the June 30 sampling, 4 days after the larvae addition, either across all samples or separately for the climate treatments (Figure S2a-d). On July 5, 9 days after the addition of larvae, the blends in control and herbivore-damaged branches were separated (unsupervised RF, Figure S2e–h). On July 11, 6 days after the removal of larvae, unsupervised RF could not separate the VOC blends, indicating that herbivory effects had ceased (Figure S2i–l). The VOC blends could not be separated between the climate treatments by the unsupervised RF either across all samples or separately for each measurement (Figure S3a–c), with the exception of July 11, when shading partially clustered away from the overlapping ambient and warming treatments (Figure S3d).

Supervised RF revealed a clearer separation between control and herbivory-damaged samples both across and within climate treatments, most markedly in the shading treatment (Figure 3). Overall, the compounds with the highest MDA that separated the VOC blends between control and herbivory treatments on July 5 were the homoterpenes, (E)- and (Z)- 4,8-dimethyl-1,3,7-nonatriene (DMNT), the monoterpenes, (E)-\( \beta \)-ocimene and linalool, and the nitrogen-containing VOCs, indole, benzyl nitrile, and 2-methylbutanenitrile (Figure 4a–d, for full list of MDAs for individual VOCs, see Table S7). Paired t-tests showed that all of these compounds were induced by the herbivory treatment (Figure 5). The response to herbivory was strongest in the shading treatment. For example, herbivory caused a 33-fold increase in (E)-DMNT emission rates under shading treatment, compared to six- and five-fold increases in ambient and warming treatments, respectively (Figure 5a). The induction of nitrogen-containing VOCs was also stronger in the shading treatments (Figure 5b–d). For example, the herbivory-induced increase in indole was 63-fold in the shading, 21-fold in the ambient, and 12-fold in the warming treatment (Figure 4d). The monoterpenes, (E)-\( \beta \)-ocimene and linalool, responded in a similar way (Figure 4e–f).

### 4 DISCUSSION

We assessed how field manipulations mimicking climate warming and increased cloudiness after 30 years of these treatments interact with insect herbivory and affect the VOC emissions from *B. nana* in subarctic tundra. The climate change treatments likely had both long-term effects accumulated over time and shorter term or instantaneous effects from the prevalent differences in environmental conditions.

**FIGURE 3** Supervised random forests (RF) analysis of the volatile organic compound (VOC) blends emitted by control and herbivore-damaged branches on July 5. (a–d) Multidimensional scaling plots for RF on the VOC blends for control (C) and herbivore-damaged (H) branches are shown across all climate treatments (a), and in the ambient (b), shading (c), and warming (d) treatments, separately. The out-of-bag (OBB) classification error is in the bottom right corner. A RF confusion matrix is presented in the table within each plot. The confusion matrix shows the number of correctly and incorrectly assigned branches (to either control or herbivory) and the proportional class error.
Both experimental warming by ~3°C and insect herbivory by local geometrid moths on *B. nana* branches stimulated VOC emission rates and altered the VOC blends. However, the responses were VOC group specific: sesquiterpene emissions increased synergistically in response to herbivory in the warming treatment, whereas homoterpenes increased synergistically in response to herbivory in both the shading and the warming treatment. Overall, shading treatment did not suppress VOC emissions, which was counter to our expectations. In fact, many biotic stress-associated VOCs were induced to a greater extent by herbivory in the shading treatment, than in the ambient and warming treatments, which has potential implications on atmospheric chemistry and climate.

In the Abisko area, where we conducted the study, the mean annual air temperature has increased by 2.5°C over the period 1913–2006, with the largest increases observed in winter and spring (Callaghan et al., 2013). However, Northern Fennoscandia is also projected to experience an increase in cloud cover, which could result in cooler or only slightly warmer summer temperatures (Boucher et al., 2013; Norris et al., 2016; Young et al., 2019). Thus, both warming and shading treatments provide relevant information on potential future climate effects on volatile responses to herbivory.

4.1 | Effects of herbivory and joint effects with warming and shading

The synergistic interaction we observed between warming and herbivory in our study that led to enhanced VOC emissions from *B. nana* branches when these treatments were combined has three possible explanations already highlighted by Li et al., (2019). First, increased biosynthesis of VOCs and enhanced enzyme activity under elevated temperature lead to increased emissions of *de novo* synthesized VOCs (Ghirardo et al., 2020). Second, increased volatility and diffusion enhance the release of VOCs from storage compartments upon warming (Laothawornkitkul et al., 2009). Third, insect herbivory modifies and induces the expression of genes involved in VOC biosynthesis (Dicke, 2009; Kessler & Baldwin, 2001; De Moraes et al., 2001).

To date, only one study has looked at the synergistic effects of warming and mimicked insect herbivory on VOC emission in the Arctic (Li et al., 2019). Li et al., (2019) found that warming doubled emissions of monoterpenes, homoterpenes, and sesquiterpenes, and when combined with mimicked herbivory, this further enhanced the VOC emissions (Li et al., 2019). Our study, which used local geometrid moth larvae, showed that herbivory increased homoterpene emissions fourfold in the ambient treatment, sixfold in the warming...
treatment, and 14-fold in the shading treatment. Furthermore, we found that sesquiterpenes responded synergistically to warming and herbivory, with a threefold increase in emissions. We did not find synergistic effects with monoterpenes, but a clear response to herbivory alone, and no pattern with GLVs, upon herbivory.

In our study, herbivory increased sesquiterpene emissions, especially when combined with warming. Sesquiterpene emissions are light- and temperature-dependent (Duhl et al., 2008; Peñuelas & Staudt, 2010), which together with insect herbivory explain the enhanced emissions observed in the warming treatment. In many species, enhanced sesquiterpene emissions are observed upon insect herbivory alone (Copolovici et al., 2011; Schaub et al., 2010). The weak responses of herbivory, alone, in our study may be related to the plants being subjected to multiple stress factors in nature, such as herbivores and pathogens, simultaneously or sequentially, which can antagonize or neutralize the jasmonic-acid-mediated defense responses activated by larvae feeding (Dicke, 2009; Schweiger et al., 2014).

Homoterpenes responded to herbivory in the warming treatment and especially, in the shading treatment, but only on July 5. Homoterpene response to herbivory in the shading treatment was manyfold stronger than in the ambient and warming treatments.

Furthermore, we found the clearest separation between the control and herbivore-damaged VOC blends in the shading treatment. The individual VOCs that increased most strongly in response to herbivory in the shading treatment included (E)- and (Z)-DMNT, (E)-β-ocimene, linalool, indole, benzyl nitrile, and 2-methylbutanenitrile. All these compounds have earlier been reported to be induced by herbivory in birch and other species, and play a role in direct and indirect plant defense (Dicke & Baldwin, 2010; Irmisch et al., 2014; McCormick et al., 2019; Rieksta et al., 2020; Ryde et al., 2021).

The differences in herbivory responses between the climate treatments could reflect the contrasting light environments that plants experience. More specifically, due to reduced PPFD levels in shaded environments, plants have lower photosynthetic rates, and therefore, the relative cost of replacing leaf tissue is larger (Coley, 1983). As a result, plants in the shade tend to allocate more resources to chemical defenses, to avoid damage (Salgado-Luarte & Gianoli, 2010). Leaf anatomical adaptations could also drive different herbivory responses in the climate treatments. For example, plants exposed to direct sunlight have lower specific leaf area (SLA) and thus, higher leaf toughness, which can make them less palatable to herbivores. Indeed, Schollert et al., (2015) found that warming increased the leaf thickness in B. nana. In contrast, shading increases
the SLA of *B. nana* (Graglia et al., 2001), which can make plants more palatable to herbivores, thus increasing the stress and its associated plant defenses.

### 4.2 Main effects of warming and shading

The effects of warming on VOC emissions and blends in our study were VOC group specific. Our study showed that experimental warming by \(-3^\circ\text{C}\) doubled emissions of total VOCs, monoterpenes, and GLVs. This agrees with several recent studies showing the high sensitivity of plant VOC emissions to temperature rise in the Arctic (Angot et al., 2020; Kramshøj et al., 2016a; Rinnan et al., 2020). While a recent study by Young et al., (2019) found little increase in summer temperatures over recent decades in Northern Fennoscandia, due to increased cloud cover, the high sensitivity of plant VOC emissions to the local temperature increases in the Arctic (Ghirardo et al., 2020; Seco et al., 2020) suggests that even small temperature increases may have strong impacts on plant VOC emissions, with large implications for the atmospheric chemistry processes.

Increased cloudiness is expected to decrease plant VOC emissions, due to the light dependency of many VOCs emissions (Duhl et al., 2008; Peñuelas & Staudt, 2010), but there are few empirical studies testing this hypothesis. Our finding is in contrast with the results of Kramshøj et al. (2016), who found that 6 years of shading in the West-Greenlandic tundra reduced plant VOC emissions by 61–65%. Although the shading treatment in both our study and in Kramshøj et al. (2016) reduced PPFD by \(-65\%\), effects on temperature were different (i.e., a 2.4\(^\circ\text{C}\) reduction here, in contrast to a 5.7\(^\circ\text{C}\) reduction in Kramshøj et al., 2016). Nonetheless, our results agree with earlier studies conducted on *B. nana* in the same experiment, which showed a weak effect of shading on VOC emissions (Rinnan et al., 2011; Schollert et al., 2015).

The VOC responses to the climate treatments during our measurements are a combined result of the climate conditions plants experience in a given year, during the actual measurements, and the alterations that have occurred in the ecosystem during the long-term climate treatments (Rinnan et al., 2007; Wijk et al., 2004). Plants are able to acclimate to novel environments over time, and the responses of plant physiological and biochemical traits to short-term versus long-term climatic manipulation may differ from each other (Wang et al., 2019). For example, Hartikainen et al., (2020) showed that the effects of experimental warming on the leaf structure of silver birch (*Betula pendula* Roth) were more pronounced after the second year of warming, relative to the first. Furthermore, Oksanen and Saleem (1999) found that two growing seasons after the removal of abiotic ozone stress, *B. pendula* still exhibited ozone-induced growth reduction, suggesting that birch has a long-term biochemical memory. Enhanced emissions of some VOCs over long-term warming may be an acclimation response against high temperature-induced oxidative stress (Peñuelas & Staudt, 2010). Compared to less conspicuous changes in leaf anatomy induced by warming, shading causes leaves to become clearly larger and thinner, which is a typical anatomical acclimation to reduced light availability (Graglia et al., 2001; Schollert et al., 2015). Therefore, the exhibited absent effects of shading on VOC emissions could be an acclimation response to this treatment.

### 4.3 Potential implications on atmospheric chemistry and climate

Many plant VOCs are highly reactive in the atmosphere and the oxidation products play a crucial role in the formation of secondary organic aerosols (SOA), which can increase the number of cloud condensation nuclei (CCN) and affect cloud formation processes (Mentel et al., 2013; Zhao et al., 2017).

Herbivore-induced emissions of plant VOCs contribute to SOA formation (Joutsensaari et al., 2015; Yli-Pirilä et al., 2016) and CCN formation more so than constitutive emissions (Zhao et al., 2017), due to herbivory increasing emissions rates and altering VOC composition. Both the sixfold increase in monoterpenes emissions in response to herbivory and the synergistic effects of herbivory and warming on sesquiterpene emissions, observed in our study, have potential impacts on cloud formation because monoterpenes and sesquiterpenes have been shown to have high SOA formation potentials and high cloud condensation activity (Joutsensaari et al., 2015; Mentel et al., 2013; Yli-Pirilä et al., 2016). Moreover, we observed stronger herbivory effects on emissions of some VOCs (e.g., DMNT) under shading, than under warming and ambient conditions. As the cloud cover in the Arctic continues to rise with ongoing climate change (Young et al. 2019), the subsequent shading will exert positive feedbacks on cloud formation by strengthening herbivore-induced VOC emissions.

To conclude, our study suggests that whether the future subarctic tundra in Northern Fennoscandia experiences rising temperatures or increasing cloudiness, and therefore milder temperature increases, matter very little for herbivory-related VOC emissions. In both scenarios, we show strong interactions with herbivory effects on VOC emissions with potentially positive feedbacks on cloud formation. We also show that acclimation of plants to long-term climate treatments, which has resulted in changes in anatomical traits, might strongly interact with volatile responses to insect herbivory. This finding further complicates predictions of how climate change, together with interacting biotic stresses, affects VOC emissions in the Arctic.

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AUTHORS’ CONTRIBUTIONS
TL and RR designed the experiments. JR and TL collected the data. JR performed the data analyses, with contributions from TL. JR contributed with resources. AM maintained the long-term experimental and provided long-term climate data. JR wrote the manuscript with contributions from all authors.

DATA AVAILABILITY STATEMENT
All VOC data that support the findings of this study can be found in Figshare: https://doi.org/10.6084/m9.figshare.14695473.

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