Seasonal and Elevational Variability in Dwarf Birch VOC Emissions in Greenlandic Tundra

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Key Points:

- Strong seasonal variability of VOC emissions in dwarf birch within the growing season are likely driven by leaf phenology
- Higher VOC emission potentials from dwarf birch observed at the harsher high-elevation site than previously assumed, due to abiotic limitations
- More environmentally challenging abiotic conditions limit the VOC diversity, but not the emission rates

Supporting Information:
Supporting Information may be found in the online version of this article.

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Seasonal and Elevational Variability in Dwarf Birch VOC Emissions in Greenlandic Tundra

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Abstract Rising temperatures amplify biogenic volatile organic compound (VOC) emissions from Arctic vegetation, causing feedbacks to the climate system. Changes in climate also alter plant physiology and vegetation composition, all of which can influence VOC emissions. Moreover, leaf development and biotic stresses cause highly variable emissions during the growing season. Therefore, linking VOC emissions with plant traits and tracking responses to climate change might provide better understanding of VOC emission regulation under future conditions. We measured VOC emissions and other plant traits in populations of dwarf birch (Betula glandulosa) at two elevations in Narsarsuaq, South Greenland. The measurements were performed in warming experiments that have run since 2016. We collected VOCs using the branch enclosure method from early June until late July 2019 (n = 200). Emissions of green leaf volatiles (GLVs), oxygenated monoterpenes (oMTs), and homoterpenes followed a seasonal trend. VOC emission rates and the diversity of the VOC blend decreased at the end of the measurement period. Differences in VOC emission rates between elevations were pronounced early in the season. Majority of the traits did not explain the variation in VOC emissions. We show strong seasonal variability in VOC emissions within the growing season, which is likely driven by leaf phenology. While the diversity of VOCs was greater at the milder low-elevation site, VOC emission rates were higher or similar at the harsher high-elevation site, showing stronger VOC emission potentials than previously assumed. Seasonal variations in the emissions of VOCs are crucial for accurate predictions of current and future VOC emissions from arctic ecosystems.

Plain Language Summary Plants release mixtures of trace gases to the atmosphere, called plant volatiles. Plants are the largest source of volatile emissions to the atmosphere, which may cause feedbacks on our climate. With ongoing climate change, we expect higher plant volatile emissions from the Arctic. We used a field experiment in South Greenlandic tundra to study how contrasting environments (warmer and colder), rising temperatures, and changes in plant structure would affect the release of plant volatiles from dwarf birch during the summer growing season. Lower amounts and less diverse mixtures of volatiles were released by plants as the summer progressed. Plant structure generally had a minor effect on the volatiles released from dwarf birch. Plants at the colder site released less diverse volatile mixtures, but the amounts were higher or similar when compared to the warmer site.

1. Introduction

Plants are the largest source of biogenic volatile organic compound (VOC) emissions to the atmosphere. Observations of VOC emissions from the Arctic, previously thought to be low-VOC-emitting ecosystems, have shown that arctic vegetation is a significant VOC source; these emissions vary seasonally and increase with warming (Baggesen et al., 2021; Faubert et al., 2010; Kramshøj et al., 2016; Lindwall et al., 2016).

The Arctic has warmed nearly four times faster than the global average since 1979 (Rantanen et al., 2022), and this warming has direct and indirect effects on VOC emissions from the vegetation (Rinnan et al., 2020). Direct effects include increased rates of VOC emissions from plants due to the temperature dependence of VOCs
VOCs are climate relevant gasses, but their influence on the Earth system, particularly in the rapidly warming and changing Arctic, is poorly understood. More specifically, VOCs participate in secondary organic aerosol formation with effects on radiative forcing (Shrivastava et al., 2017; Spracklen et al., 2008). VOCs can also indirectly increase the concentrations of greenhouse gases—tropospheric ozone when interacting with nitrogen oxides, and methane through the capacity of VOCs to deplete hydroxyl radicals and thereby increase the lifetime of methane (Boy et al., 2022; Fitzky et al., 2019).

Relationships between functional and structural plant traits and VOC emissions in high-latitude ecosystems have been recently described (Seco et al., 2022; Simin et al., 2021), and these traits are also affected by climate warming (Bjorkman et al., 2018). However, alterations in plant traits in response to climate warming and how the responses might affect VOC emissions vary greatly (Baggesen et al., 2021; Rinnan et al., 2011; Schollert et al., 2015). Understanding the relationships between plant structure and function, their responses to climatic changes, and ultimately, their impacts on VOC emissions, are important for providing accurate predictions of current and future VOC emissions from arctic ecosystems, where emissions of VOCs are predicted to increase.

Constitutive VOC emissions vary between species (Rinnan et al., 2011; Schollert et al., 2017, 2017, 2017; Simin et al., 2021, 2022), within species (Yassa et al., 2012), and temporally (Baggesen et al., 2021; Llusia et al., 2010; Ryde et al., 2021). Many factors control VOC emissions, including abiotic factors, such as temperature, light intensity, and soil moisture (Ninemets et al., 2004), as well as the physiological and functional properties (traits) of plants. The volatility and diffusion of VOCs from plant cells increases with temperature and thus, VOC emissions increase in response to rising temperatures (Ninemets et al., 2004). In tundra plants, VOC emissions have been shown to strongly increase with temperature, even up to leaf surface temperatures of around 38°C (Simin et al., 2021). Emissions of some VOCs, such as many isoprenoids, are light dependent (Peñuelas & Staudt, 2010). Soil moisture also plays a role in modulating VOC emissions. Increases in green leaf volatile (GLV) emissions, in particular, have been shown in response to drought (Ambebe & Dang, 2009; Mao et al., 2004; Simin et al., 2022; Wang et al., 1998).

Abiotic factors change during the high-latitude growing season and thus, VOC emissions and their composition undergo great changes over the growing season (Baggesen et al., 2021; Hakola et al., 1998; Llusia et al., 2010; Ryde et al., 2021; Seco et al., 2022; Trowbridge et al., 2014; Valoahiti et al., 2015). Changes in phenology over a growing season also alter the emissions of VOCs (Baggesen et al., 2021; Hellen et al., 2021; Ryde et al., 2021). In downy birch (Betula pubescens), leaf growth early in the season was shown to be a strong VOC source, especially for sesquiterpenes and oxygenated sesquiterpenes (Hellen et al., 2021). In Subarctic tundra, Baggesen et al. (2021) showed that different VOC groups dominated the emission profile during different phenological states. For example, during flowering, monoterpenes were emitted at higher rates, whereas during green-up and seed dispersal, acetaldehyde and sesquiterpene emissions were higher.

Plants adjust their physiological properties/phenotype in response to abiotic (e.g., temperature, irradiance, soil moisture) and biotic stressors (e.g., herbivory, pathogens). Plant traits—the morphological, anatomical, physiological, biochemical, and phenological characteristics of plants—reflect overall plant fitness, function, and responses to environmental conditions. Some relationships between VOC emissions and functional traits have been identified (Fernandez-Martinez et al., 2018; Simin et al., 2021). For example, the global positive relationships between the emissions of isoprenoids and foliar nitrogen content have been attributed to the nitrogen dependence of the Rubisco enzyme (Fernandez-Martinez et al., 2018). However, a local study of Hawaiian plant communities assessing the relationships between traits, such as foliar nitrogen, specific leaf area (SLA), photosynthesis, and isoprenoid emissions, did not find any relationships (Llusia et al., 2010). In a study on Subarctic dwarf birch (Betula nana), Simin et al. (2021) found a negative relationship between 2-hexenal emissions and SLA, and a positive relationship between 3-carene emissions and SLA. Thus, high variation in traits within species may affect VOC emissions and blend compositions.

Here, our aim was to assess seasonal variations in VOC emissions under in situ climatic conditions and evaluate relationships between VOC emissions and various plant traits. We measured VOC emissions in natural populations of dwarf birch (Betula glandulosa), a dominant species in tundra ecosystems, through the growing season...
of 2019 at two elevations in Narsarsuaq, South Greenland. The measurements were carried out in experiments, where open-top chambers (OTCs) have been used to warm permanent plots since 2016, resulting in plant growth under ambient, as well as elevated temperatures. Our research questions were:

1. How do VOC emissions change during the growing season at two elevations and in control and warming treatments?
2. Does climate warming alter plant traits of dwarf birch and do the traits differ between the elevations?
3. Which traits are important for explaining within-species variation in VOC emission rates? Do the responses differ between VOC groups?

2. Materials and Methods

2.1. Study Site and Experimental Design

The field campaign was carried out in long-term warming experiments at two tundra sites (low elevation fen at 50 m a.s.l. (61°11'N, 45°22'W), high elevation heath at 450 m a.s.l. (61°09'N, 45°23'W)) in South Greenland. The experiments were established in 2016 and data for this study were collected during the 2019 growing season. Vegetation at the low-elevation tundra fen is dominated by mosses, Salix spp., Carex spp., and Betula glandulosa Michx. Vegetation at the high-elevation tundra heath is dominated by the dwarf shrubs, B. glandulosa, Salix glauca L., Vaccinium uliginosum L., Empetrum nigrum L., and lichens (Høye et al., 2018). Soils at the sites are haplic podzols with underlying granitic bedrock (Jones et al., 2010; St Pierre et al., 2019). The sites are part of a global warming and species removal experiment in mountain ranges (Prager et al., 2022). Each site hosts a warming experiment with eight blocks placed in homogeneous vegetation (Figures 1a and 1b). Each block is seven by seven meters with a 2 × 2 m plot in each corner of the block (Figure 1c). Three treatments (warming, removal, warming + removal) and ambient control are randomly assigned to plots in each block. For this study, only the warming and ambient control plots were used. Transparent hexagonal OTCs made of fiberglass (1.14 mm thickness; eplastics.com) with an inside diameter of 1.4 m centered on an area of 2 × 2 m were used to accomplish warming of the canopy temperature (Prager et al., 2022). In 2016 and 2017, OTCs increased canopy temperature by ~2.0°C at high elevation and ~1.2°C at low elevation (unpublished data). The OTCs were set up annually at the beginning of the growing season (~May) and removed at the end of the growing season (~September). To assess the seasonal variability of VOC emissions from the dominant dwarf shrub, B. glandulosa, we measured VOCs at both elevations between June 4 and July 31.

We selected one B. glandulosa branch from the control and warming plots in six (June 4–20 measurements, n = 12/elevation) and eight (July 7–31, n = 16/elevation) blocks. Each branch was measured seven times across the period.

2.2. VOC Measurements

We used a branch enclosure technique described previously (Schollert et al., 2017; Vedel-Petersen et al., 2015) to sample VOCs. Pre-cleaned (120°C for 1 hr) polyethylene terephthalate (PET) bags (25 × 38 cm, ~1 L volume) were used as enclosures through which clean air was circulated with pumps (Stewart-Jones & Poppy, 2006). VOCs were trapped from outgoing air on stainless steel adsorbent cartridges (150 mg Tenax TA, 200 mg Carbograph 1TD, Markes International Limited, Llantrisant, UK). Each PET bag was ventilated for ~5 min at an inflow rate of 1000 ml min⁻¹ before the measurement. The inflow air into the PET bag was pulled through a particulate and activated carbon filter (F03-C2-100 with Filter MXP-95-054, Wilkerson Corp., Richland, Michigan, USA) which removes ambient VOCs from the inflow air, and a copper tube coated with potassium iodide, which filters ozone to avoid losses of the highly reactive VOCs (Kramshøj et al., 2016; Valolahi et al., 2015). During the 20-min sampling period, purified air was circulated through the enclosure at an inflow rate of 300 ml min⁻¹. The sample outflow (200 ml min⁻¹) passed through an adsorbent cartridge secured in the corner of the PET bag and the excess air leaked out of the opening where the branch entered the bag. After sampling, the cartridges were sealed with Teflon-coated brass caps and stored at 5°C until analysis.

Blank sampling was conducted in situ using the same approach but with empty PET bags to account for compounds derived from sampling materials and the analytical system. A new PET bag was used for each measurement.
2.3. VOC Analysis

VOC samples were analyzed using gas chromatography-mass spectrometry (7890A Series GC coupled with a 5975C inert MSD/DS Performance Turbo EL System, Agilent Technologies, Santa Clara, CA). In short, cartridges were desorbed at 250°C for 10 min (TD100-xr, Markes International Ltd, Llantrisant, UK) and introduced to an HP-5 capillary column (50 m length, 0.2 mm diameter, 0.33 μm film thickness, Agilent Technologies, Santa Clara, CA) with a split flow of 20 ml min⁻¹. VOCs were separated and analyzed as in Simin et al. (2022) and Rieksta et al. (2023a). Chromatograms were analyzed using PARADISe v. 6.0 software (https://ucphchemometrics.com); Johnsen et al., 2017; Quintanilla-Casas et al., 2022). Compounds were identified using pure standards, when available, or tentatively identified against mass spectra in the 2014 NIST Mass Spectral Library. VOC concentrations were quantified using external standards that were analyzed in the same manner alongside the samples. Where pure standards were unavailable, we used the most structurally related standard compound as the reference.

We categorized compounds into the following groups: isoprene, monoterpene (MTs), oxygenated monoterpenes (oMTs), homoterpenes (HTs), sesquiterpenes (SQTs), oxygenated sesquiterpenes (oSQTs), and GLVs. Compounds not belonging to above mentioned groups, were further classified into hydrocarbons (HCs) or oxygenated VOCs (OVOCs). VOCs that did not belong to any of the groups mentioned, were classified as other VOCs. VOC emission rates were calculated according to Ortega and Helmig (2008) and expressed on the basis of leaf dry mass (ng g⁻¹ dw h⁻¹). More details of the classification of the VOCs in different VOC groups can be seen in Methods S1 in Rieksta et al. (2023a).

2.4. Plant Traits

Plant traits were determined in the same branches as used for the repeated VOC measurements during the growing season (n = 16/elevation) after the last VOC measurement (July 30–31). A total of seven plant traits were measured to assess whether they were altered by warming, if they differed between the elevations, and whether they were correlated to VOC emissions.

Specific leaf area (SLA) is the one-sided area of a fresh leaf, divided by its oven-dry mass, and expressed as cm² g⁻¹ (Perez-Harguindeguy et al., 2013). Leaves were removed from the petiole and scanned at 300 dpi using a CanoScan LiDE220 Color Image Scanner (Canon USA, Inc. New York, USA). Leaf area was determined using ImageJ (v. 1.52a; Wayne Rasband, National Institutes of Health, Bethesda, USA; Rasband, 1997).

Leaf carbon and nitrogen contents were measured using a Eurovector CN elemental analyzer (Euro Vector, Pavia, Italy) in oven-dried and ground leaf material collected on July 30–31 (n = 16/elevation). We determined a “chemical fingerprint” from near-infrared reflectance (NIR) spectroscopy analysis in the leaf material, which shows variations in leaf chemical composition. More specifically, NIR detects different chemical bonds present in the chemical compounds of leaves without classifying them into specific compounds or groups (Foley et al., 1998). Each sample was analyzed as five subsamples that were then averaged across a spectral range of 4,000–10,000 cm⁻¹ on an Antaris II Fourier-transform NIR analyzer.
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We also measured the rates of net photosynthesis ($A_N$; μmol CO₂ m⁻² s⁻¹), transpiration (E; mmol H₂O m⁻² s⁻¹), stomatal conductance ($G_s$; mol H₂O m⁻² s⁻¹), and vapor pressure deficit based on leaf temperature (VpdL; kPa). These traits were measured three times at each elevation, on July 7–9, July 23–24, and July 30–31. We measured at a fixed chamber temperature (20°C), CO₂ level (400 μmol CO₂ mol⁻¹), and photosynthetically active photon flux density (PPFD; 1,000 μmol m⁻² s⁻¹). Measurements of $A_N$ were conducted using a portable photosynthesis system (LI-6400 XT, LI-COR Inc., Lincoln, NE) with an LED light source (6400-02B), mounted on the standard 3 × 2 cm leaf chamber, and a CO₂ mixer (6400-01 Liss 12g CO₂ cartridges were used). For $A_N$ measurements, we used a side branch from the same individual that was used for VOC measurements (n = 16/ elevation). As in Simin et al. (2021), the leaf chamber was enclosed around the stem of the branch and $A_N$, $G_s$, transpiration, and VpdL were recorded after the $A_N$ reached a steady state. This took between 5 and 20 min after clamping, depending on how similar the ambient environmental conditions were to the controlled ones inside the leaf chamber.

2.5. Environmental Variables

Air temperature and relative humidity during the VOC measurements were measured with iButtons at the canopy height (Hygrochron, Maxim Integrated, San Jose, USA) in one of the warming and control plots (Table S1 in Supporting Information S1). PPFD was monitored at the canopy height in the control and warming plots, using The Photosynthetic Light Smart Sensor coupled with a Hobo Micro Station (S-LIA-M003, Onset Computer Corporation, Bourne, MA, USA). Air temperature and relative humidity were also always measured inside the enclosure during VOC sampling. PPFD in the warming plots was only measured on July 7–July 31.

Soil moisture was measured around the base of each plant. During each VOC measurement, we recorded five measurements per plant with a Theta-probe (Sensor type ML3, Delta-T Devices Ltd, Cambridge, United Kingdom) and averaged these for one value per plant.

2.6. Statistical Analysis

All statistical analyses were performed using the R statistical framework—version 4.0.3 (RStudio Team, 2021). To assess the effects of warming, elevation, and seasonality (measurement week) on VOC emission rates, we used linear mixed-effect models (LMM) fitted with maximum likelihood (ML) using the “lmer” function from the lme4 package version 3.1.3 (Kuznetsova et al., 2017). LMMs were chosen to encompass the potential spatial variation in the study area, which were accounted for as random effects in the models (Johnson, 2014).

We performed LMMs with total VOCs and VOC group emission rates as the response variables; warming (control, warming), elevation (low, high), measurement week (June 4–5, June 13–14, June 19–20, July 7–9, July 15–18, July 23–24, July 30–31), and their interactions entered as fixed factors; block and plant ID were assigned as random factors, with plant ID nested in block. We employed a backward model reduction method using function “drop1” and chisq-test for model selection, and simplified the models by removing statistically non-significant interactions (significance level for interactions was set at $P < 0.25$). To assess the main effects (warming, elevation, week), we used the “ANOVA” function from the LmerTest package version 3.1.3 (Kuznetsova et al., 2017). Due to the heteroscedasticity of the variances, we applied a log (x+1) transformation to the VOC emission rates. Parameter estimates between the ambient control and warming, and between the elevations were obtained from estimated marginal means (EMMs) using the default/balanced function “emmeans” from the emmeans package version 1.75 (Lenth et al., 2020).

To assess if VOC emission rates changed over the measurement period, we used contrasts from the LMMs as described above on VOC emission rates standardized to a temperature of 30°C using the VOC grouping and coefficients for each VOC group from The Model of Emissions of Gases and Aerosols from Nature version 2.1 (MEGAN2.1) (Guenther et al., 2012). This was to minimize the effects of short-term temperature variations and to allow for assessment of long-term temperature effects due to the warming treatment.
or the inherent temperature differences of the two elevations, which may have affected the vegetation characteristics. Similar LMMs and the custom contrast tests between the first and the last measurement week were used to assess the effects of warming, elevation, and week on the measured environmental variables (air temperature, soil moisture, relative humidity, and PPFD).

To assess changes in the VOC blends across the measurement period, elevations, and warming, ANOVA simultaneous component analysis (ASCA) was used to assess and interpret the effect of the design on the totality of VOC emission profiles (Bertinetto et al., 2020). ASCA is a multivariate version of ANOVA to analyze datasets with an underlying experimental design. Inference of individual design factors was established by permutation testing, using 1000 random permutations. An initial three-factor interaction model was the basis for backward elimination, to reduce the model to only sustain significant terms. Similar to the univariate models, emission rates of the individual VOCs were log-transformed and further scaled to unit standard deviation. Data from the June 5 campaign showed deviating behavior in a non-biologically meaningful way, due to a relatively large proportion of zeros in the VOC abundance matrix. For clearer interpretation of the data, all data from this time point were removed from the analysis.

The chemical fingerprint data based on NIR spectroscopy were summarized using a Principal Component Analysis (PCA) after Multiplicative Scatter Correction (MSC) using SIMCA (Sartorius AG, version 16.0.1., Göttingen, Germany). The first three principal components (PC), which explained 57%, 19%, and 11% of the variation, respectively, were used as the variables describing the chemical composition.

To investigate how elevation and warming affected plant traits (SLA, foliar nitrogen, C:N ratio, \( A_N \), VpdL, \( E \), \( G_s \), and the PCs from the NIR analysis), we used LMMs with the plant trait as the response variable and elevation, warming, and their interaction as fixed factors. Block and plant ID, nested in block, were entered as random factors. For \( A_N \), VpdL, transpiration, and \( G_s \), the week was also entered as random factor. However, these traits did not differ between measurement weeks and as such, we pooled the data for further analysis. Model selection and parameter estimates were obtained, as described above.

We ran multiple regression analyses with VOC group as a response variable and plant traits (SLA, foliar nitrogen, foliar carbon, C:N ratio, \( A_N \), VpdL, \( E \), \( G_s \), GCC, and the first three PCs from the NIR analysis) as predictor variables. Prior to regression analysis, we ran correlation tests to test for collinearity of predictor variables, and removed C:N ratio, \( G_s \), and \( E \) from further analyses. For the final model, we calculated the variance inflation factors (VIF) of included predictors in the models using the “vif” function in the usmd package (Naimi et al., 2014). The threshold for VIF was set to 5 and all traits met these requirements and were kept in the models. To account for the trait variables being measured on different scales, all traits were z-transformed using the “scale” function in R. The majority of trait data only had one time point and for the traits with several measurement times, we used only the mid-growing season data from July 7–9.

3. Results

3.1. Environmental Conditions During Measurements

Across the measurement period, ambient air temperature and soil moisture differed between elevations. Air temperature at the canopy height was, on average, 1.6°C ± 0.4°C cooler at high elevation compared to low elevation (\( t_{21} = -4.2, P < 0.001 \); Figure 2a). Soil moisture was, on average, two times higher at low (33% ± 1.8%) than high elevation (14% ± 1.9%, \( P < 0.001 \); Table S1 in Supporting Information S1). Relative humidity and PPFD did not differ between elevations during measurements (Figure 2b).

The canopy air temperatures were similar during the first (June 4–5) and the last measurements (July 30–31) at each elevation (\( P > 0.05 \)). Soil moisture, however, increased by 6% and 14% from first to last measurement at high and low elevation, respectively (high: \( t_{152} = 3.4, P = 0.01 \); low: \( t_{152} = 7.4, P < 0.001 \)).

The warming treatment increased the canopy air temperature by 5°C on average during VOC measurements (\( t_{20} = -13.3, P < 0.001 \)). The OTCs used for warming reduced the PPFD by 12% at high elevation (\( t_{21} = 1.90, P = 0.07 \); Table S1 in Supporting Information S1) and did not change the PPFD at low elevation (\( t_{2} = 0.18, P = 0.95 \)). Soil moisture did not change in response to warming at either elevation (Table S1 in Supporting Information S1).
3.2. Plant Trait Differences Between the Elevations and Treatments

The SLA, AN, C:N ratio, E, GS, and VpdL, were greater at low than high elevation ($P < 0.01$; Figures 3a and 3b), whereas the reverse was true for foliar nitrogen and GCC ($P < 0.01$; Figure 3a). Foliar nitrogen was 12% greater at high than low elevation ($P < 0.02$; Figure 3a). Scores of PC1–PC3 (the first three Principal Components from the NIR data describing the chemical fingerprint of the leaves) differed between elevations ($P < 0.05$; Figure 3c).

Foliar nitrogen content marginally increased in response to warming at high elevation ($t_{22} = −1.8, P = 0.08$) and decreased by 23% under warming at low elevation ($t_{22} = −1.8, P < 0.01$; Figure 3a). Warming increased the foliar C content at both elevations ($P < 0.05$) and increased C:N ratio by 37% at low elevation only ($t_{21} = −3.8, P = 0.01$; Figure 3).

3.3. Seasonality Effects on VOC Emission Rates and VOC Blends

Several VOC groups exhibited a seasonal pattern, with the highest emission rates in the early season (June) and lowest emissions at the end of the measurement period (July 30–31; Figures 4 and 5). Despite the temper-
Figure 3. Trait differences between the elevations and treatments. (a) Leaf traits measured at the end of the VOC measurements (July 30–31), n = 16/elevation: specific leaf area (SLA), foliar nitrogen, foliar carbon, (C:N ratio, green chromatic coordinate (GCC). (b) Traits measured with a portable photosynthesis system during July 7–9, July 23–24, and July 30–31: A_{\text{n}} (net photosynthesis), E (transpiration), G_{s} (stomatal conductance), and VpdL (vapor pressure deficit). As there were no differences between the measurement weeks, averages over the three measurements are shown. (c) Scores of PC1-PC3 (first three Principal Components from NIR analysis describing the chemical fingerprint of the leaves). Symbols show mean ± standard error of the mean.
The emission rates of GLVs, HCs, OVOCs, SQTs, oSQTs, and other VOCs did not differ between elevations (ANOVA, \( P > 0.05 \); Figures 4 and 5), and marginally greater for the monoterpenes (\( P = 0.09 \)). However, LMMs for these groups indicated statistically significant week × elevation interactions (Figure 4), except for the HTs. Total VOC emissions were 75% and 60% greater at high elevation than at low elevation during June 19–20 (\( t_{145.6} = 2.0, P = 0.04; \) Figure 4a) and July 23–24 measurements (\( t_{118.2} = 3.8, P < 0.001; \) Tables S4–S5 in Supporting Information S1), respectively, but there were no significant differences during other weeks. oMT emissions differed between elevations during all measurement weeks, except June 4–5 (\( t_{118.6} = 0.1, P = 0.9 \)). MT emissions were 60% greater at high elevation than at low elevation during July 23–24 (\( t_{17.9} = 2.7, P = 0.01 \)) and marginally greater during July 7–9 (\( t_{17.9} = 1.8, P = 0.08 \); Tables S4–S5 in Supporting Information S1), whereas the emissions did not differ between elevations during other weeks. The emission rates of GLVs, HCs, OVOCs, SQTs, oSQTs, and other VOCs did not differ between elevations (ANOVA, \( P > 0.05 \); Figure S2 in Supporting Information S1).

3.4. Elevation Effects on VOC Emissions and Blends

Overall, emission rates were greater at high than low elevation for total VOCs, HTs, isoprene, and oMTs (ANOVA, \( P < 0.05 \); Figures 4 and 5), and marginally greater for the monoterpenes (\( P = 0.09 \)). However, LMMs for these groups indicated statistically significant week × elevation interactions (Figure 4), except for the HTs. Total VOC emissions were 75% and 60% greater at high elevation than at low elevation during June 19–20 (\( t_{145.6} = 2.0, P = 0.04; \) Figure 4a) and July 23–24 measurements (\( t_{118.2} = 3.8, P < 0.001; \) Tables S4–S5 in Supporting Information S1), respectively, but there were no significant differences during other weeks. oMT emissions differed between elevations during all measurement weeks, except June 4–5 (\( t_{118.6} = 0.1, P = 0.9 \)). MT emissions were 60% greater at high elevation than at low elevation during July 23–24 (\( t_{17.9} = 2.7, P = 0.01 \)) and marginally greater during July 7–9 (\( t_{17.9} = 1.8, P = 0.08 \); Tables S4–S5 in Supporting Information S1), whereas the emissions did not differ between elevations during other weeks. The emission rates of GLVs, HCs, OVOCs, SQTs, oSQTs, and other VOCs did not differ between elevations (ANOVA, \( P > 0.05 \); Figure S2 in Supporting Information S1).

When standardized to 30°C, the differences between elevations were only significant for the emissions of HTs and oMTs, while there were no significant differences for other groups or total VOCs. Across all measurement

Figure 4. Seasonal trend and elevation effects on VOC groups. Estimated marginal means (EMMs) of the emission rates for (a) total VOCs and the most abundant VOC groups, (b) OVOC (oxygenated VOCs), (c) GLVs (green leaf volatiles), as well as two VOC groups with strong elevation differences, (d) oMT (oxygenated monoterpenes) and (e) HT (homoterpenes). Symbols show mean ± standard error of the mean. Note the different y-axis and the lines connecting measurements are a guide to the eye. \( P \)-values for the main effects of week, elevation, and their interactions are listed in Table S2 in Supporting Information of the measurement week and elevation, and their interactions are listed in the connecting measurements are a guide to the eye.

The ASCA model results showed that the VOC blends differed between the measurement weeks (\( P = 0.01 \)), and there was a week × elevation interaction (\( P = 0.01 \); Figure S1 in Supporting Information S1). The relative abundance and diversity of VOCs showed a downwards trend, that is, both were higher in early June and decreased toward the final measurement in late July (Figure 6). Among the VOCs that were highest early in the season compared to the late season, were the HTs, MTs (ocimene and norbornane), oMTs ((+)-borneol, linalool, thujone, and eucalyptol). Several SQTs and oSQTs had highest abundances in early season compared to late season, including \((Z, E)\)-α-farnesene, \( β \)-bisabolene, \( β \)-farnesene, aromadendrene, \( α \)-humulene, caryophyllene oxide, and nerolidol. While the majority of VOCs decreased in abundance as the growing season progressed, some VOCs increased in abundance at the end of the measurement period. Among these compounds was the GLV, 3-hexenal, the MTs, \( β \)-cymene and \( α \)-tricyclene, the oMTs, myrtenal, pinocarvone, and fenchone, and the SQT-calamenene (Figure 6).

Atmospheres not differing between the first (June 4–5) and last measurements (July 30–31), the emissions of total VOCs, OVOC, GLVs, oMTs, and HTs, were lower at the end of the growing season at both elevations (\( P < 0.01 \); Figures 4a–4e; Table S2 in Supporting Information S1). The total VOC emissions decreased by 53% (\( t_{160} = 3.1, P < 0.01 \); Figure 4a) but overall, the decrease was VOC group specific (Table S3 in Supporting Information S1; Figure 5). For the most abundant VOC groups emitted by dwarf birch, oxygenated VOCs and GLVs, emission rates were 76% and 63% lower, respectively, in the last relative to the first measurement (OVOCs: \( t_{160} = 5.4, P < 0.0001 \); GLVs: \( t_{167} = 3.1, P < 0.01 \); Figures 5a and 5b). HT emissions ((E)- and (Z)-DMNT) were 94% lower in the last than in the first measurement (\( t_{147} = 9.4, P < 0.0001 \); Figure 5g).

Emissions of SQTs and oSQTs did not differ between elevations, and MT and HC emission rates were only lower at the end of the measurement period at the low-elevation site (\( P = 0.02 \); Figure 5).
weeks, the emissions of HTs and oMTs were 70% and 74% greater at high elevation, compared to low elevation, respectively ($P < 0.001$).

The blends of the individual VOCs differed between elevations (ASCA, $P = 0.001$, Figure S3a in Supporting Information S1). Low elevation had higher relative abundances of many GLVs, HCs, and MTs (Figure S3b in Supporting Information S1). For example, low elevation had higher abundances of toluene and benzene, as well as the MTs, $\beta$- and $p$-cymene, myrcene, $\gamma$-terpinene, and $\alpha$-phellandrene. High elevation had greater abundance of indole and benzothiazole (Figure S3b in Supporting Information S1). A substantial part of the effect of elevation was seasonal with 29.9% of the variance accounted for by the interaction effect between week and elevation (ASCA, $P = 0.001$; Figure S1 in Supporting Information S1).

3.5. Warming Effects on VOC Emissions and Blends

Warming treatments did not increase the emissions of the majority of VOC groups, except for GLVs, which increased by 60% on average ($t_{20.6} = -3.4$, $P = 0.01$), and oMTs, which doubled in response to warming ($t_{21.0} = -3.0$, $P = 0.001$). Isoprene, a minor compound in the emission profile of dwarf birch, increased by 30% upon warming ($t_{21.8} = -1.95$, $P = 0.01$). Standardized emissions of “stress VOCs” (MEGAN classification, a group which mainly consists of GLVs) were 54% higher in warming compared to control treatments ($t_{25} = 0.46$, $P = 0.01$).

However, the volatile blends differed between the control and warming treatments. The overall effect of warming on the VOC blend explained 1.7% (ASCA, $P = 0.03$; Figure S4a in Supporting Information S1). The warming treatment had higher relative abundances of many GLVs, MTs, and oMTs, whereas the controls had higher relative abundances of HTs, HCs, and SQTs (Figure S4b in Supporting Information S1). Among individual VOCs that contributed most to the blend differences between treatments were (E, Z)-2,4-hexadiene, norbornane, $\alpha$-terpineol, (E)-DMNT, tridecane, octane, nonane, and calamenene.

3.6. Functional and Chemical Traits Explaining Variation in VOC Emissions

$A_N$, $VpdL$, SLA, foliar nitrogen, and foliar carbon did not covary with emissions of any of the VOC groups ($P > 0.05$; Figure S5 in Supporting Information S1). The chemical fingerprint of the leaves (PC2 and PC3 from NIR analysis) was related to the emissions of GLVs (PC2), as well as hydrocarbons and OVCs (PC3) ($P < 0.05$; Figures 7a–7c). Emissions of hydrocarbons and OVCs also negatively correlated with GCC (Figures 7c and 7d).

4. Discussion

4.1. Seasonal Effects and Elevational Differences in VOC Emissions and Blend

Here, we show a clear seasonal trend in VOC emissions for many VOC groups irrespective of the prevailing air temperature, the main abiotic driver of VOC emissions. The ambient temperatures did not differ between the first measurement in early June and the last measurement in late July. However, the emissions of GLVs, oxygenated VOCs, oxygenated monoterpenes, and homoterpenes were 63%–94% lower in the last measurement compared to the first, which coincides with a 40% decline in $B. \text{glandulosa}$ GLV emissions over a similar time frame reported in another study at the same site (Simin et al., 2022). The decline in the emission rates at the end of the measurement period is likely associated with leaf senescence. Leaf senescence has earlier been shown to decrease
Figure 6. Effects of seasonality on VOC blend throughout the measurement period from early June to end of July. PC1 loadings of week x elevation interaction from ANOVA simultaneous component analysis, which shows the main variance related to the week and elevation and is distributed across VOC groups. Bars show loadings for individual VOCs that are colored by VOC group. The majority of VOCs have positive values representing early season, whereas only few VOCs are negative, and represent late season. Most abundant VOCs and VOCs contributing to the differences between early and late season are presented in the graph with label. PC1 captured 29.9% of the variance. The arrow indicates the direction from highest VOC diversity in early season (June 13–14) to lowest VOC diversity in the late season (July 30–31).
isoprene emissions in aspen (Populus tremula L.; Sun et al., 2012). The subarctic coastal climate in the study area has long, cold winters, and short, warm-to-cool summers. The first signs of senescence during our measurement period were observed during the July 7–9 measurements (J. Rieksta, personal observation). Furthermore, Sun et al. (2012) found that senescence-driven degradation processes in leaves, rather than temperature, were the main drivers of reduced emissions, which is in agreement with our findings.

The emission rates of many VOC groups peaked in mid-June, which was then followed by a decline. In contrast, Schollert et al. (2017) found that the highest VOC emissions from dwarf birch (Betula nana L.) in West Greenland occurred much later, that is, July 7–8, when emissions of most VOC groups in our study were already declining. The onset of the season in a particular year, abiotic and biotic conditions of the site, and the ambient temperature in the days prior to VOC measurements play an important role in VOC emissions (Rinnan et al., 2020), and thus,
direct comparisons between the two studies cannot be made. The higher emissions observed early in the season are considered a defense mechanism; because younger leaves have a higher nutritional quality, they are more attractive to herbivores and pathogens (Bracho-Nunez et al., 2011; McCall & Fordyce, 2010).

The emission peak observed in the early season was less clear for sesquiterpenes, oxygenated sesquiterpenes, and other VOCs. While oxygenated monoterpenes exhibited a clear peak in mid-June, monoterpenic emission rates were approximately similar across the measurement period. The emission rates for these groups did not differ between the first and the last measurement. High within-species variation (i.e., plasticity) and/or genetic differences between individuals (e.g., potential hybridization of B. glandulosa with other Betula spp. (de Groot et al., 1997; Thörsson et al., 2007) could have contributed to large variation in the VOC emissions, thus blurring potential seasonality (Deepak et al., 2018; Makhnev et al., 2012). These findings are in contrast to those by Hellén et al. (2021), who found higher sesquiterpene and oxygenated sesquiterpene emissions early in the season for downy birch (Betula pubescens). This mismatch could be because the measurements were performed at different developmental phases. Hellén et al. (2021) measured soon after bud burst, when high emissions of VOCs are considered residuals from the buds containing almost 100-fold more extractable terpenoids than the leaves (Lievonen, 1982), while our study focused on fully expanded leaves.

We found more pronounced emission rate differences between elevations in the early than late season for total VOCs, oxygenated VOCs, GLVs, oxygenated monoterpenes, and homoterpenes. Greater emissions at high elevation sites earlier in the season are likely a result of differences in the leaf developmental stage (Baggesen et al., 2021; Hellén et al., 2021; Ryde et al., 2021). Due to harsher environmental conditions at high elevation compared to low elevation, leaf development lags behind. We found higher GCC values—a proxy for leaf greenness—at high than low elevation, indicating greener, younger leaves at high elevation. Thus, higher emission potentials in younger leaves likely resulted in greater emission rates early in the season at high elevation (Bracho-Nunez et al., 2011).

We also found a clear seasonal trend in the chemical composition of the VOC blend. Over the growing season, the VOC diversity gradually decreased at both elevations, likely due to leaf senescence. The homoterpenes, (E)- and (Z)-DMNT, monoterpenes, ocimene and norbornane, and the oxygenated monoterpenes, (+)-borneol, linalool, thujone, and eucalyptol, had higher abundances early than late in the season. Linalool was among the most abundant VOCs observed in silver birch early in the growing season (Hellén et al., 2021), which is in agreement with our results on dwarf birch. On the other hand, we showed that ocimene was among the most abundant VOCs in the early season, whereas high ocimene emissions in late season have been associated with senescence in silver birch (Hellén et al., 2021; Vuorinen et al., 2005). In mountain birch, ocimene was the most abundant VOC emitted across three sites in the Subarctic (Rieksta et al., 2020) and thus, birch species-specific differences in VOC composition might lead to contrasting results. The high abundance of herbivore-induced VOCs early in the season, such as (E)- and (Z)-DMNT, (Z, E)-farnesene, and linalool, could indicate more activated defense against herbivores and pathogens in younger leaves (Bracho-Nunez et al., 2011; McCall & Fordyce, 2010). However, we did not observe any herbivores or pathogens on the measured branches.

While the elevations differed in abiotic conditions, with the high-elevation site having 3°C lower ambient temperatures than the low-elevation site, VOC emissions were greater or similar at high elevation compared to low elevation. For the homoterpenes, oxygenated monoterpenes, and isoprene (a minor compound in the birch VOC profile), emission rates were greater at high elevation, whereas the emissions of GLVs, hydrocarbons, oxygenated VOCs, sesquiterpenes, and oxygenated sesquiterpenes, were similar overall at high and low elevations. Simin et al. (2022) also found that GLV emission rates of dwarf birch growing outside the experimental plots on the same site were significantly greater at high elevation compared to low elevation, and negatively correlated with soil moisture content. However, the diversity of individual VOCs was greater at low than high elevation. The similar or greater emissions at high elevation compared to low elevation indicate that plants in abiotically harsher environments might not be limited by those abiotic conditions when it comes to emission potentials. The lower herbivore pressure and higher metabolic constraints on growth and reproduction, due to the harsher environmental conditions at high elevation, may limit the VOC diversity (Descombes et al., 2020; Kergunteuil et al., 2019).
Climate manipulation experiments at high latitudes generally show positive warming effects on VOC emissions (Faubert et al., 2010; Ghirardo et al., 2020; Kramshøj et al., 2016; Lindwall et al., 2016; Rinnan et al., 2020; Valolahti et al., 2015). However, weak or complex effects of warming on VOC emissions have also been reported (Rieksta et al., 2021, 2023a; Rinnan et al., 2011; Schollert et al., 2015). In our data, most VOCs were unaffected by warming, but GLV emission rates doubled in response to warming and this response remained even when emissions were standardized by temperature. This finding suggests that factors other than temperature alone are driving GLV emissions. Plants may have sustained high enzymatic activity due to warmer temperatures in the warmed plots, thus supporting higher GLV emissions (Niinemets et al., 2004).

Previous studies on dwarf birch showed no effects of warming on the VOC blend (Rieksta et al., 2021, 2023a) when analyzed using random forests, a simple machine learning technique. However, using ASCA, which allows for the investigation of interaction effects on VOC blends, we were able to detect differences in the VOC blends. Our results show that warming caused higher relative abundances of many GLVs, monoterpenes, oxygenated monoterpenes, oxygenated VOCs, and oxygenated sesquiterpenes. However, several sesquiterpenes had higher relative abundances in the control treatment, for example, (−)-α-bourbonene, calamene, α-copaene, and γ-cadinene. Sesquiterpene emissions are light and temperature dependent (Peñuelas & Staudt, 2010). OTCs are known to reduce photosynthetic photon flux density by up to 25% in alpine study sites (Hollister et al., 2022) and thus, potentially suppress emissions of light-dependent compounds, such as some sesquiterpenes. Our findings agree with earlier studies on dwarf birch biogenic VOC emissions reporting no significant effects of warming by OTCs (Rinnan et al., 2011; Schollert et al., 2017).

4.2. Changes in Traits in Response to Warming and Differences Between Elevations

Climatic changes, such as increased temperatures, can modify plant phenotypes over time and thus, have an effect on VOC emissions (Lindwall et al., 2016; Rinnan et al., 2011; Schollert et al., 2015). From all of the traits assessed in this study, the specific leaf area (SLA), photosynthesis ($A_{\text{p}}$), carbon to nitrogen (C:N) ratio, transpiration (E), stomatal conductance ($G_s$), and vapor pressure deficit (VpdL), were greater at low than high elevation, confirming milder and less stressful conditions for dwarf birch at low elevation. Foliar nitrogen content and green chromatic coordinate (GCC)—a proxy for leaf greenness—were greater at high than low elevation. Lower GCC values at low elevation suggest that earlier onset of leaf senescence occurred at low elevation.

Only a few traits responded to the 4 years of experimental warming. Foliar nitrogen content marginally increased in response to warming at high elevation and decreased by 23% at low elevation, which might affect leaf palatability. Warming increased the foliar C content at both elevations, whereas the changes in C and N contents led to 37% higher C:N ratio under warming than control only at the low-elevation site, suggesting decreased nutrient quality (Xu et al., 2020) with warming. These results indicate that warming may alter plant traits differently at low and high elevation.

4.3. The Trait Effects on VOC Emissions

The effects of plant traits on VOC emissions are variable (Fernández-Martínez et al., 2018; Llusià et al., 2010). In our study, trait effects on specific VOC groups were generally weak, except for three VOC groups that are among the major groups in the VOC profile of dwarf birch: GLVs, hydrocarbons, and oxygenated VOCs. The chemical fingerprint of the leaf was related to the emissions of these VOC groups, suggesting a potential trade-off investing resources into volatile versus non-volatile metabolites (Agrawal & Fishbein, 2006). A proxy for leaf senescence, the GCC, was negatively correlated with the emissions of OVOCs and other VOCs, may indicate that senescence reduces emissions of OVOCs and other VOCs. Several high-latitude studies have reported changes in the anatomical traits of plants in response to temperature and light availability, coupled with weak or no effects of traits on VOC emissions (Rieksta et al., 2021; Rinnan et al., 2011; Schollert et al., 2015). This highlights the complexity of the interactions between the treatments mimicking climatic changes and predicting future VOC emissions. Despite few trait responses to warming and the overall weak trait-VOC emission relationships reported here, we encourage field studies linking other physical, morphological, and chemical plant traits with VOC measurements under simulated climatic scenarios. Such studies will help to disentangle processes behind the highly varying VOC emissions from plants and how they might respond to future climatic changes.
5. Conclusion

Our study from Greenlandic tundra investigated seasonal variations in VOC emission rates, VOC blends, their responses to warming, and the drivers behind the variations, which are crucial for providing accurate predictions of current and future VOC emissions from arctic ecosystems. Our study highlights three key findings. First, dwarf birch VOC emission rates and diversity decreased at the end of the growing season, illustrating strong seasonal variability within the growing season, irrespective of the prevailing air temperature. While the diversity of VOCs was greater at the milder low-elevation site, VOC emission rates were greater or similar at the harsher high-elevation site. This suggests stronger VOC emission potentials for high elevations than generally assumed due to abiotic and biotic limitations. We suggest that leaf phenology is a strong driver of seasonal trends in dwarf birch VOC emissions and diversity in tundra ecosystems, and likely the main driver in elevational differences in the emission rates early in the season. Using ASCA, which allows for investigation of the interaction effects on VOC blends, we were able to detect individual VOCs that can characterize the seasonal variability in VOC emissions. Second, 4 years of experimental warming altered only few traits but warming altered plant traits differently at low and high elevation. Finally, the studied plant traits did not explain the variations in VOC emissions, but the chemical composition of the leaf tissue and the leaf greenness (green chromatic coordinate) were related to certain VOC groups.

Data Availability Statement

Supporting information and data supporting these findings can be found in Rieksta et al. (2023b).

References


