



Impacts of elevation on plant traits and volatile organic compound emissions in deciduous tundra shrubs

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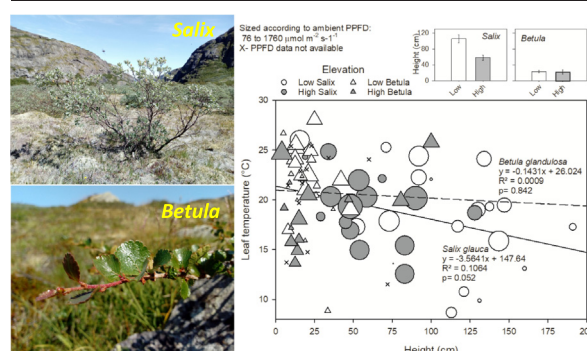
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HIGHLIGHTS

- Due to climate change, shrubs are increasing in abundance in tundra ecosystems.
- Relationship between shrub height and leaf temperature is poorly understood.
- We measured plant traits and VOC emission at two elevations in Greenland.
- Leaf temperature decreased with *Salix* shrub height, $4\text{ }^{\circ}\text{C m}^{-1}$.
- Soil moisture strongly affected the VOC emission and physiology of tundra shrubs.

GRAPHICAL ABSTRACT



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ABSTRACT

The northernmost regions of our planet experience twice the rate of climate warming compared to the global average. Despite the currently low air temperatures, tundra shrubs are known to exhibit high leaf temperatures and are increasing in height due to warming, but it is unclear how the increase in height will affect the leaf temperature. To study how temperature, soil moisture, and changes in light availability influence the physiology and emissions of climate-relevant volatile organic compounds (VOCs), we conducted a study on two common deciduous tundra shrubs, *Salix glauca* (separating males and females for potential effects of plant sex) and *Betula glandulosa*, at two elevations in South Greenland. Low-elevation *Salix* shrubs were 45% taller, but had 37% lower rates of net CO₂ assimilation and 63% lower rates of isoprene emission compared to high-elevation shrubs. *Betula* shrubs showed 40% higher stomatal conductance and 24% higher glandular trichome density, in the low-elevation valley, compared to those from the high-elevation mountain slope. *Betula* green leaf volatile emissions were 235% higher at high elevation compared to low elevation. Male *Salix* showed a distinct VOC blend and emitted 55% more oxygenated VOCs, compared to females, possibly due to plant defense mechanisms. In our light response curves, isoprene emissions increased linearly with light intensity, potentially indicating adaptation to strong light. Leaf temperature decreased with increasing *Salix* height, at $4\text{ }^{\circ}\text{C m}^{-1}$, which can have implications for plant physiology. However, no similar relationship was observed for *B. glandulosa*. Our results highlight that tundra shrub traits and VOC emissions are sensitive to temperature and light, but that local variations in soil moisture strongly interact with temperature and light responses. Our results suggest that effects of climate warming, alone, poorly predict the actual plant responses in tundra vegetation.

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1. Introduction

The Arctic is a scarcely vegetated area in the northernmost part of the world, which is experiencing a rate of surface warming twice as high as the global average (Post et al., 2019; IPCC, 2021). It is important to understand how the local tundra plants respond to this rapid climate change (Rinnan et al., 2020). Tundra plants take advantage of their unique near-surface environment and their productivity can be quantified by measuring different plant traits (Körner, 2021a). As a result of warming by climate change, tundra shrubs are increasing in height around the Arctic (Bjorkman et al., 2018) and are increasing in productivity in wet areas, such as valleys, compared to dry ones, like mountain slopes (Tape et al., 2006; Berner et al., 2020; Mekonnen et al., 2021). In contrast, low soil moisture has been shown to be a factor significantly limiting the increase in height (Bjorkman et al., 2018) and productivity (Gamm et al., 2018) of shrubs in the Arctic. Leaf temperature is important in the life of tundra plants (Körner, 2021a) and it depends on local weather conditions at any given time, such as air temperature and light availability, as well as plant species-specific leaf traits (Simin et al., 2021). However, it is unclear how the increase in plant height and variations in environmental variables affect the leaf temperature and plant-atmosphere interactions in tundra vegetation.

The leaf surface temperature of plants worldwide is remarkably similar, with a global mean of 21.4 ± 2.2 °C according to an analysis of oxygen isotope ratios in cellulose by Helliker and Richter (2008). However, the leaf surface temperatures of arctic tundra plants (which include graminoids, mosses, horsetails, herbs, and evergreen shrubs, and are often dominated by deciduous willow, *Salix* spp., and birch, *Betula nana* and *Betula glandulosa*, shrubs) are often significantly higher than the temperature of the surrounding air (Seco et al., 2020). Indeed, the surface temperature of a low arctic tundra heath vegetation, was 7.7 °C warmer than the surrounding air (Lindwall et al., 2016). Nevertheless, the increasing tundra shrub height in the Arctic (Bjorkman et al., 2018), might lead to lower top canopy temperatures as the plants grow away from the warm microclimate close to the ground, making the decoupling of air- and leaf-temperatures weaker in tall plants compared to lower ones.

S. arctica shrubs in the Canadian high Arctic increased in height by 28% after 16 years of open-top-chamber (OTC) experimental warming, compared to ambient controls (Hudson et al., 2011). The average shrub height of *B. glandulosa* has increased by 23% over 10 years under the ambient conditions of the Canadian low Arctic (Andruko et al., 2020), despite limited temperature increase in the area. We have recently demonstrated that the optimum temperature for CO₂ assimilation in arctic *Salix* and *Betula* shrubs is between 24 °C and 31 °C, which suggests that the carbon assimilation rate in these species will increase with increasing air and leaf temperatures (Simin et al., 2021). However, studies applying artificial warming using OTCs have shown contrasting photosynthetic and leaf trait responses. A 2.7 °C higher temperature by experimental warming with OTCs increased the gross photosynthesis (A_G) of *B. nana* by 27% (Schollert et al., 2017). OTC warming for 4 years had no effect on fluorescence parameters, the ratio between the variable and the maximum fluorescence (Fv/Fm) and the performance index (PI) in *S. arctica* (Mosbacher et al., 2013) or chlorophyll content in evergreen tundra shrubs (Michelsen et al., 1996). However, 16 year warming by 1–2 °C using OTCs tended to increase the specific leaf area, SLA, of *S. arctica* (Hudson et al., 2011), while 1 year experimental warming using OTCs had no significant effect on leaf surface structures in *B. nana*, such as the stomatal and glandular trichome density (Schollert et al., 2017).

One manner in which tundra shrubs interact with their environment is by emitting a significant amount of volatile organic compounds, VOCs (Kramshøj et al., 2016; Ghirardo et al., 2020; Rieksta et al., 2021; Simin et al., 2021; Swanson et al., 2021), a diverse group of reactive gases with implications for atmospheric chemistry (Glasius and Goldstein, 2016). *Salix* mainly emits isoprene, C₅H₈ (Loreto et al., 2014; Karlsson et al., 2020; Swanson et al., 2021), which is also the most widely emitted VOC in nature (Guenther et al., 2012), while the emissions from *Betula* are dominated by various hydrocarbon groups with or without oxygen, such as

alkanes, aldehydes or alcohols, and isoprenoids, such as monoterpenes and sesquiterpenes (Hakola et al., 2001; Vedel-Petersen et al., 2015; Ghirardo et al., 2020; Hellén et al., 2021). VOC emissions from tundra shrubs are highly temperature dependent (Simin et al., 2021).

Experimental warming by 3 °C has been shown to increase isoprene emissions in *Salix glauca* by 68% (Kramshøj et al., 2016) and double the total VOC emission rates in *B. nana* (Rieksta et al., 2021). However, we still lack understanding in many aspects of tundra shrub VOC emission responses to warming. For example, while the interaction of VOC emission and temperature increase has been studied extensively in the Eurasian dwarf birch, *B. nana* (Rinnan et al., 2011; Schollert et al., 2015; Vedel-Petersen et al., 2015; Schollert et al., 2017; Rieksta et al., 2021; Simin et al., 2021), similar studies are mostly missing for the north-American dwarf birch, *B. glandulosa*. A recent study focused on the volatile secondary metabolites (Seguin et al., 2021), but VOC emissions of *B. glandulosa* have not been characterized and it is unclear if it reacts to warming similarly to *B. nana*. Glandular trichomes are known to be storage organs for some VOCs (Laothawornkitkul et al., 2009; Schollert et al., 2015), so glandular trichome density is potentially an important characteristic in VOC regulation. As a dioecious species, female *Salix* plants have a higher energy investment compared to their male counterparts (Cornelissen and Stiling, 2005), because making fruits and seeds is more costly than producing pollen. Additionally, male plants have been shown to have bigger and more nutritious leaves (Cornelissen and Stiling, 2005), but it is unknown whether these differences correspond to different VOC emission blends between sexes or to differences in their responses to warming.

While the influence of temperature on VOC emissions from tundra plants has been recently studied using carbon labelling techniques (Ghirardo et al., 2020) and temperature response curves (Simin et al., 2021), the effect of light intensity remains largely unexplored. In the absence of light, emissions of the *de-novo* synthesized, light-dependent compounds should come to a halt, due to the lack of photosynthetic activity (Laothawornkitkul et al., 2009; Niinemets et al., 2015). The light dependency of VOC emissions is relevant because with the changing climate, cloud cover is expected to increase over the Arctic (Norris et al., 2016; Young et al., 2019; IPCC, 2021) and has the potential to alter CO₂ assimilation, leaf morphology, and the emission patterns of light-dependent VOCs.

Abiotic conditions that shape the environment in which plants grow, change in predictable ways with elevation. For example, for every 100 m increase in elevation, air temperature decreases by 0.8 °C in coastal areas and by 0.4 °C in continental areas, a phenomenon known as adiabatic lapse rate (Körner, 2021a). Compared to valleys, mountain slopes receive more solar radiation, due to reduced atmospheric turbidity and thinner cloud layer (Körner, 2021b). Slopes also have a lower soil moisture than valleys, which accumulate water due to the drainage, surface runoff, and seepage of snowmelt water from the mountain slopes (Körner, 2021c). According to a recent IPCC report, the future Arctic will experience higher atmospheric temperatures, more precipitation, and an increase in cloud cover (IPCC, 2021), suggesting that the elevation difference between a mountain slope and a valley can be used as a unique space-for-time substitution for climate change (Higgins et al., 2021), which is a valid alternative to a long-term warming experiment (Pickett, 1989; Elmendorf et al., 2015). Comparing elevations can, however, be obscured by differences in the timing of plant development during the growing seasons of different duration. Ryde et al. (2021) recently showed that elevational differences in chemical defenses of mountain birch (*Betula pubescens* var. *pumila*) mostly owed to differences in seasonality. As tundra plants experience phenological events (such as flowering or senescence) in fast succession throughout the growing season and as these affect plant ecophysiology, including VOC emissions (Baggesen et al., 2021), it is important to consider seasonality when assessing the effects of elevation.

As a result of intense climate change (IPCC, 2021), deciduous shrubs continue to expand across high latitudes (Mekonnen et al., 2021). Our understanding of the interactions between these expanding tundra shrubs and their environment is still limited, due to insufficient field data. The responses of plants to the environment and the effects plant have on the environment can be assessed by measuring plant traits, various morphological

and physiological metrics that describe plant functions (Lavorel and Garnier, 2002), such as the rate of photosynthesis or stomatal density.

Our main goal was to assess differences in the plant traits and VOC emission blends of the dominant *Salix* and *Betula* species at two elevations in South Greenland: a low-elevation valley and a high-elevation mountain slope. As valleys serve as a milder environment (e.g. air temperatures and soil moisture contents) than higher elevations, we expected taller plants and higher photosynthetic performance in the valley, compared to the mountain slope. To distinguish between the potential effects of phenology from the elevation effects, we categorized the measurements taken at different time points into mid- and late season data, thus introducing a seasonality factor. We treated the two sexes in *Salix* separately to account for the potential differences between them. We expected male *Salix* plants to have higher photosynthetic performance, because of the lower energy investment of male plants compared to female ones (Cornelissen and Stiling, 2005). Additionally, we investigated how leaf temperature varies with plant height and if the responses are different between *Salix* and *Betula*. We expected that the leaf temperature of the uppermost canopy would be lower, the taller the plant. Finally, we examined how the net assimilation and VOC emission rates responded to different light intensities in the dominant *Salix* and *Betula* shrubs. Since tundra plants are commonly exposed to high irradiance and employ mechanisms to deal with it (Körner, 2021a), we expected a decrease in solar radiation to have a strong negative effect on the net assimilation and VOC emission.

2. Materials and methods

2.1. Study site and species

This study was conducted at two elevations near Narsarsuaq, South Greenland: a wet tundra fen on a meltwater sediment in a valley (low-

elevation site; 50 m a.s.l.; 61.18°N, 45.37°W) and a mesic tundra heath on a northwest-facing slope (high-elevation site; 450 m a.s.l.; 61.16°N, 45.38°W; Fig. 1a).

Two dominant deciduous shrub species were studied at both elevations: the gray willow, *Salix glauca* L., distinguished between female (Fig. 1b) and male plants (Fig. 1c), and the north-American dwarf birch, *Betula glandulosa* (Fig. 1d). At the beginning of the field study, individual plants were marked for measurement from an area of ~1000 m² at both elevations. A single plant individual formed an independent sample in this study. We conducted the study on fully developed, fully irradiated leaves from the top of the canopy. On each sampling date, three to five plant individuals from each species were measured. The measurement campaign lasted between June 23 and August 11, 2019 and resulted in the collection of 20 *Salix* samples at low elevation (of which 12 were male and 8 female), 16 *Salix* samples at high elevation (of which 9 were male and 6 female), 24 *Betula* samples at low elevation, and 21 *Betula* samples at high elevation. In order to cover both elevations throughout the campaign, field measurements were alternated between elevations in the following manner: June 23–July 5, low elevation; July 8–July 19, high elevation; July 20–July 26, low elevation; July 27–August 7, high elevation; August 10–August 11, low elevation.

The average July air temperature in the area is 11 °C (2014–2021, (Mittarfearfiit, 2021)). We measured the air temperature at 15 cm height at each elevation using the HOBO U23 Pro v2 sensor (Onset Computer Corporation, Bourne, Massachusetts, USA) and the average temperature in the measurement period was 12.9 °C and 11.7 °C at low and high elevation respectively (a decrease of 1.2 °C or 10% from low to high elevation; Fig. 1e).

2.2. Leaf temperature, plant height, and soil moisture

The leaf surface temperature in the measured plant was determined from the top side using an Optris LaserSight infrared thermometer (OPTRIS

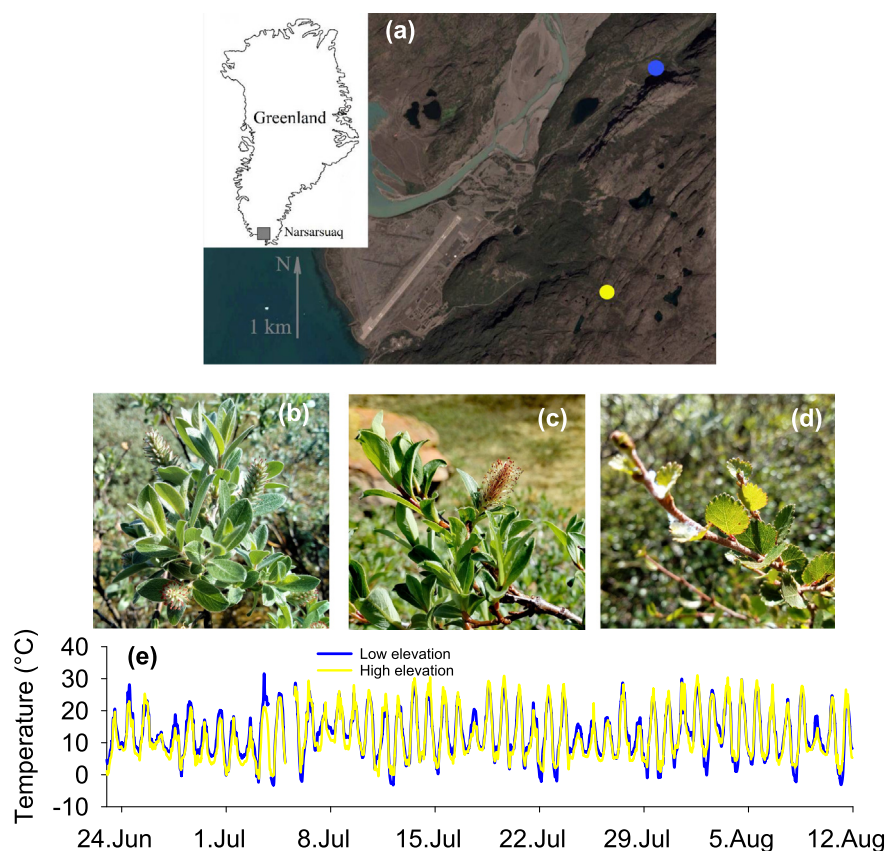


Fig. 1. (a) Map of the study area indicating low- (blue circle) and high-elevation (yellow circle) sites adjacent to the airstrip of Narsarsuaq. The base image is from © Google Earth Pro 7.3.4 (2017); Image details- Narsarsuaq, Greenland, 61.19, -45.35; 0–500 m a.s.l. Study plant species (b) *S. glauca*, female (c) *S. glauca* male, (d) *B. glandulosa*. (e) Air temperature at 15 cm height at the two elevations between June 23 and August 11, 2019.

GmbH; Berlin, Germany) and averaged (across 3 to 10 individual readings, depending on the number of leaves) to give one value per sample. Plant height was measured using a ruler from the soil surface to the measured leaves. Soil moisture was determined twice at a depth of 10 cm, within a 10 cm radius from the base of each measured plant using a Theta Probe ML3 (Delta T-Devices, Cambridge, UK) and averaged.

2.3. Gas exchange measurements

2.3.1. Instrumental setup in the field

The LI-6400 XT (LI-COR, Inc., Lincoln, Nebraska, USA) is a portable photosynthesis instrument, which measures the flux of CO₂ and H₂O in a leaf chamber using infra-red gas analyzers, and calculates gas exchange parameters, such as the rate of net CO₂ assimilation (A_N , i.e. the rate of net photosynthesis), and stomatal conductance, (G_s , Evans and Santiago, 2014). We used the LI-6400 XT with a red/blue light leaf chamber (6400-02 B; surface 6 cm²; volume 213 cm³) to measure gas exchange: A_N (μmol CO₂ m⁻² s⁻¹), G_s (mol H₂O m⁻² s⁻¹), and transpiration (Tr, mmol H₂O m⁻² s⁻¹). Water use efficiency (WUE, μmol CO₂ mol⁻¹ H₂O) was calculated as the ratio of A_N to G_s (Tenkanen et al., 2020). Vapor pressure deficit based on leaf temperature (VPD, obtained from the LI-6400 XT and based on the calculations of (Buck, 1981) was also measured. A_N external quantum sensor (LI-190 SA, LI-COR, Inc., Lincoln, Nebraska, USA) was mounted onto the sensor head of the LI-6400 XT to monitor the ambient photosynthetic photon flux density (PPFD) during gas exchange measurements.

In *Salix*, a single fully developed leaf was large enough to cover the entire leaf chamber surface, but in *Betula*, a twig containing 5–10 mature leaves was enclosed in the leaf chamber, due to the small sizes of the leaves. A *Betula* twig or a *Salix* leaf was carefully inserted into the leaf chamber to avoid physical stress and clamped around the stem. The chamber temperature was maintained at the averaged leaf temperature measured immediately beforehand and the flow rate through the chamber was kept at 400 μmol s⁻¹ (~595 ml min⁻¹). PPFD in the leaf chamber was set to 1000 μmol m⁻² s⁻¹ and a relative humidity (RH) of 60% was targeted. The reference CO₂ concentration in the leaf chamber was adjusted to 400 μmol CO₂ mol⁻¹ air using the LI-6400 XT's mixer system. After insertion, the leaves were exposed to the aforementioned conditions for 20 min to avoid stressing the leaves and to allow for acclimation (Niinemets et al., 2011). The 20-min acclimation period was determined from laboratory measurements using an online analyzer (proton-transfer-reaction time-of-flight mass spectrometer, PTR-TOF 1000 ultra, Ionicon Analytik, Innsbruck, Austria), which showed a decrease in mechanical stress-related VOCs within this period upon chamber enclosure. Following the acclimation period, photosynthetic gas exchange traits and VOC emissions (see Section 2.3.2) were measured for 20 min. After the measurement, the leaves were harvested to determine the leaf color and the leaf area, which was later used for scaling of the photosynthetic gas exchange traits.

2.3.2. Light response curves

To produce light response curves (LRCs), we measured gas exchange with a LI-6400 XT over seven PPFD intensity steps targeting 2000, 1000, 500, 250, 125, 60, and 0 μmol photons m⁻² s⁻¹ while the same twig of *Betula* or leaf of *Salix* was clamped inside the leaf chamber. The light range was chosen to reflect the natural range of peak growing season light conditions the tundra plants experience in the subarctic summer. The chamber conditions were the same as described above, except that chamber temperature was fixed at 20 °C. We measured four light response curve replicates of *Salix* at each elevation (equaling to 4 male and 4 female plants in total) and five replicates of *Betula* at each elevation.

The light response photosynthesis data were averaged per light level and fitted according to Eq. 1 (Prado et al., 1994; Prado and Moraes, 1997; Lobo et al., 2013).

$$A_N = (A_{Gmax} (1 - \exp(-k(Q - Q_{comp})))) - R_d \quad (1)$$

In Eq. 1, A_{Gmax} is the asymptomatic estimate of maximum gross photosynthesis rate (μmol CO₂ m⁻² s⁻¹), k is the initial slope of the curve (an adjustment factor; s m⁻² μmol photon⁻¹), Q is the photosynthetic photon flux density (PPFD; μmol photon m⁻² s⁻¹), Q_{comp} is the light compensation point (μmol photon m⁻² s⁻¹), and R_d is the dark respiration rate (A_N when $Q = 0$; μmol CO₂ m⁻² s⁻¹). The constants (A_{Gmax} , Q_{comp} , and k) were optimized by minimizing the residual sum of squares (SSR) using the Solver function in Excel (Lobo et al., 2013).

2.3.3. Sampling of VOC emissions

VOCs were sampled in parallel with the photosynthetic gas exchange measurements using a trace gas kit (8100–664) which functioned as a flow divider attached to the outlet of the LI-6400 XT leaf chamber (Ekberg et al., 2009; Simin et al., 2021). We installed a charcoal filter (Supelco Inc., Bellefonte, Pennsylvania, USA) on the air intake of the LI-6400 XT to prevent hydrocarbons and ozone from entering the leaf chamber (Ortega et al., 2008). A pocket pump (TOUCH; SKC Ltd., Blandford Forum, UK) pulled the air from this flow divider at 200 ml min⁻¹ through a stainless steel cartridge (Markes International, Llantrisant, UK) containing Tenax TA and Carbograph 1TD adsorbents, which trap compounds with five or more carbon atoms. Following the leaf acclimation period, VOC sampling, along with the photosynthetic gas exchange measurements, proceeded for 20 min (4 l of air sampled). The adsorbent cartridges were subsequently sealed with Teflon-coated brass caps and stored at 5 °C until analysis. Blank measurements with an empty leaf chamber were performed every sampling day under the same conditions as plant sampling to determine the compounds originating from the sampling system.

LRCs for VOC emissions were performed using an identical setup to that described in Section 2.3.2. However, VOC LRCs were carried out at different dates and times than the A_N LRCs, using the same individual plants but adjacent twigs. For isoprene emission LRCs, we modified Eq. 1, as implemented by Rasulov et al. (2011, 2015).

2.3.4. VOC analysis

Adsorbent cartridges were analyzed using gas chromatography–mass spectrometry (7890A Series GC paired with a 5975C inert MSD/DS Performance Turbo EI System, Agilent Technologies, Santa Clara, CA, USA) following a 10 min thermal desorption at 250 °C (TD100-xr, Markes International Ltd., Llantrisant, UK). The temperature of the oven was maintained at 40 °C for 3 min, followed by a raise to 210 °C at 5 °C min⁻¹, and to 250 °C at a rate of 20 °C min⁻¹, with a final hold of 8 min. An HP-5MS capillary column was used for separation of individual compounds (50 m length, 0.2 mm diameter, 0.33 μm film thickness, Agilent Technologies, Santa Clara, CA, USA) with helium as the carrier gas (1.2 ml min⁻¹).

We used PARADISE software (v3.88, Johnsen et al. (2017)) to analyze the chromatograms. We found 164 individual VOCs, which were identified according to 43 authentic standards (Table S1) or, for the compounds that we did not have standards for, tentatively identified from comparisons against mass spectra in the NIST 14 library (National Institute of Standards and Technology, Gaithersburg, MD, USA). Each VOC was classified into one of the following groups: isoprene (ISO), monoterpenes (MTs), sesquiterpenes (SQTs), other hydrocarbons (HCs), oxygenated VOCs (OVOCs), green leaf volatiles (GLVs), and other VOCs. Sample concentrations were calculated based on the known concentrations in the standard solutions, which were analyzed at the same time as the plant VOC samples, and the ratio of sample:standard peak areas. Compounds for which no standard was available were quantified using the most structurally related standard compound (see Table S2 for standard assignments).

Concentrations of VOCs in the blank samples were subtracted from concentrations in the plant VOC samples that were collected on the same date. Additionally, VOCs with high concentrations in the blanks were omitted because they originated from the LI-6400 XT system. VOC emission rates (ng

$\text{gdw}^{-1} \text{h}^{-1}$) were calculated according to Eq. 2 (Ortega and Helmig, 2008) and expressed on the basis of leaf dry weight (dw, g).

$$\text{Emission rate} = \frac{(C_{\text{out}} - C_{\text{in}}) F}{\text{dw}} \quad (2)$$

where C_{out} and C_{in} are the concentrations of VOCs in the outlet and inlet air ($\mu\text{g l}^{-1}$), respectively, and F is the inflow rate of air to the leaf chamber ($\sim 595 \text{ ml min}^{-1}$). The concentration in the filtered inlet air was presumed to be zero (Ortega and Helmig, 2008).

2.4. Specific leaf area and leaf color

The leaves harvested after the gas exchange measurements were scanned using a CanoScan LiDE220 Color Image Scanner (Canon U.S.A., Inc. New York, USA) and their total leaf area calculated using ImageJ (v. 1.52a; Wayne Rasband, National Institutes of Health, Bethesda, USA). The leaves were air-dried in paper bags at room temperature and then in an oven for 48 h, before being weighed to determine dry mass. The specific leaf area (SLA, the ratio of leaf area and dry weight) was calculated.

The green chromatic coordinate (GCC) for the leaf scans was calculated according to Eq. 3, a simple ratio (Gillespie et al., 1987; Sonnentag et al., 2011) using the red (R), green (G), and blue (B) color obtained from ImageJ:

$$\text{GCC} = \frac{G}{R + G + B} \quad (3)$$

2.5. Chlorophyll fluorescence and polyphenolic content

A portable chlorophyll fluorimeter, the Handy PEA (Hansatech Instruments Ltd., Norfolk, UK), was used to determine two dark-adapted chlorophyll fluorescence parameters, F_v/F_m and PI, from the same plant individual, but from a twig adjacent to the one used for gas exchange measurements. The dark fluorescence clips were placed on three leaves and, after 25 min, the measurements were obtained and averaged for a single sample. The same three leaves were also measured with the Dualox Scientific (Force A, Orsay Cedex, France), a portable optical sensor that estimated chlorophyll content (expressed as the chlorophyll index), flavonol content (expressed as the flavonol index), and the nitrogen balance index (NBI, a ratio of the chlorophyll and flavonol indices). Each index was averaged over the triplicate measurements for a single sample.

2.6. Leaf surface structures

Four *Betula* leaves from a twig adjacent to the one used for gas exchange measurements were harvested to obtain replicas of the leaf surface structures. These leaves were placed on a microscope slide in a drop of superglue (Loctite, Westlake, Ohio, USA), pressed for 1 min, and then carefully removed to leave a print of the leaf surface in the glue (Valkama et al., 2003). Each sample contained two adaxial (top) and abaxial (bottom) leaf replicas. Glandular trichome densities on both sides were counted for an area of $179 \mu\text{m}^2$ in four quadrants of the leaves and averaged. Stomatal density was counted and averaged similarly to glandular trichomes, but the inspected area was $29.1 \mu\text{m}^2$ and only the bottom side of the leaf was quantified, because *Betula* does not have stomata on the top side of the leaves (De Groot et al., 1997). *Salix* leaves were fully covered in leaf hairs and unsuitable for analysis using this technique, so we omitted them from the leaf surface analysis.

2.7. Statistical analyses

To separate the effects of elevation and sex from potential effects of plant phenology, we grouped the samples into mid (June 23–July 14) and

late season (July 15–August 11) based on the *Salix* GCC values, which peaked on July 14 and declined thereafter (Fig. S1).

The univariate general linear model (GLM) procedure of the software SPSS version 28.0.0 for Windows (SPSS Inc., Chicago, Illinois, USA) was used to test the effect of elevation, sex (for *Salix*), and seasonality (mid vs. late season) on plant traits, environmental variables and different VOC groups ($n = 36$ for *Salix*; $n = 45$ for *Betula*). The models included main effects and interactions. We were interested in observing the one-way and the two-way effects of the factors. The three-way interactions were omitted, due to the low number of replicates in several categories. The data were log10 transformed to meet the criteria of normal distribution, if needed, and equality of variances. Using SPSS, we also performed Pearson correlation analyses between soil moisture and leaf temperature and various plant traits and the emission of VOC groups ($n = 36$ for *Salix*; $n = 45$ for *Betula*).

Principal component analysis (PCA) was performed on the VOC emission rates using SIMCA software version 16.0.1 (Umetrics, Umeå, Sweden) to assess how the elevations, seasons, and sex (in *Salix* only) affected the VOC emission blends. Outliers were removed and the principal components (PCs) were extracted after a unit variance scaling of the variables. Clustering of samples then shows which samples are similar and which are different from each other based on the patterns in relative abundances of all individual VOCs. The scores of each PC generated by the PCA were then analyzed for differences between elevation, sex (for *Salix*), and seasonality by the GLM procedure in SPSS, as described above.

3. Results

3.1. Plant traits and environmental variables

There were no significant effects of sex on any of the measured plant traits in *Salix*, except for an interaction effect on GCC.

Soil moisture was lower at high elevation compared to low elevation ($p < 0.001$), 40% lower around *Salix* and 48% lower around *Betula* shrubs. There were no seasonality differences, nor interactions in soil moisture. Leaf temperatures were not significantly affected by elevation in either species, but were affected by seasonality, with higher temperatures in the late season than in the mid-season: 20% for *Salix* ($p = 0.016$) and 27% for *Betula* ($p < 0.001$; Table 1; Fig. 2). In *Salix*, there was also a significant elevation $\times$ seasonality interaction ($p = 0.020$; Table S3). VPD (vapor pressure deficit based on leaf temperature) was not affected by elevation in either species, but was significantly higher in the late season than in the mid-season, by 60% in *Salix* and 38% in *Betula* ($p < 0.001$).

A_N (photosynthesis) in *Salix* was 37% higher at high elevation compared to low elevation ($p < 0.001$), and not significantly affected by seasonality (Table 1; Fig. 2). In *Betula*, there were no significant elevation or seasonality effects on A_N (Table 1; Fig. 2), but the interaction of elevation and seasonality was significant ($p = 0.017$), because the A_N in low elevation *Betula* was 36% higher in the late season compared to the mid-season (Table 1).

G_s (stomatal conductance) in *Salix* was 51% lower in the late season compared to the mid-season ($p = 0.002$; Table 1; Fig. 2) especially at high elevation ($p = 0.003$ for the elevation $\times$ seasonality interaction). G_s in *Betula* was 40% lower at high elevation compared to low elevation ($p = 0.015$), 31% lower in the late season compared to the mid-season ($p = 0.023$; Table 1; Fig. 2), and there was a significant interaction because the late season high elevation plants had lower G_s than other measured plants ($p = 0.035$). In both species, G_s correlated negatively with leaf temperature and in *Betula* positively with soil moisture (Table S4).

WUE (water use efficiency) in *Salix* was not affected by elevation, but was 77% higher during the late season compared to the mid-season ($p < 0.001$; Table 1; Fig. 2) and there was a significant interaction because the two elevations differed in the opposite directions for the mid- and late seasons ($p < 0.001$). The WUE in *Salix* correlated positively with leaf temperature (Table S4). WUE in *Betula* was higher during the late season compared to the mid-season ($p < 0.001$) and was also 89% higher at high elevation

Table 1

Soil moisture, leaf temperature, vapor pressure deficit (VPD), air temperature at 15 cm above the ground, ambient photosynthetic photon flux density (PPFD), net CO₂ assimilation rate (A_N), stomatal conductance rate (G_s), transpiration rate (Tr), water use efficiency (WUE), the ratio between variable and maximum fluorescence (Fv/Fm), performance index (PI), chlorophyll index, flavonol index, nitrogen balance index (NBI) and green chromatic coordinate (GCC), for *Salix* and *Betula* at low and high elevations during the mid- and late season. The values are mean $\pm$ SE. *Salix* measurements include both sexes because there was no significant difference between them.

Environmental variable or plant trait	<i>Salix</i>				<i>Betula</i>			
	Low elevation		High elevation		Low elevation		High elevation	
	Mid-season	Late season	Mid-season	Late season	Mid-season	Late season	Mid-season	Late season
Soil moisture (%)	47.5 $\pm$ 5.9	43.3 $\pm$ 9.9	32.3 $\pm$ 4.3	26.8 $\pm$ 4.2	73.3 $\pm$ 4.5	67.2 $\pm$ 6.2	37.7 $\pm$ 5.7	35.6 $\pm$ 7.2
Leaf temperature (°C)	15.1 $\pm$ 1.2	22.3 $\pm$ 1.3	19.0 $\pm$ 0.5	19.9 $\pm$ 1.5	19.6 $\pm$ 1.4	23.6 $\pm$ 0.5	16.5 $\pm$ 1.0	22.2 $\pm$ 0.7
VPD (kPa)	0.63 $\pm$ 0.05	1.22 $\pm$ 0.13	0.87 $\pm$ 0.06	1.18 $\pm$ 0.14	1.06 $\pm$ 0.15	1.27 $\pm$ 0.09	0.84 $\pm$ 0.10	1.36 $\pm$ 0.08
Air temperature (°C)	20.0 $\pm$ 1.3	21.4 $\pm$ 1.0	27.2 $\pm$ 0.7	26.4 $\pm$ 0.9	22.7 $\pm$ 1.5	23.4 $\pm$ 0.5	21.4 $\pm$ 0.8	24.8 $\pm$ 1.1
Ambient PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	886 $\pm$ 126	946 $\pm$ 167	1333 $\pm$ 155	1113 $\pm$ 144	738 $\pm$ 133	888 $\pm$ 109	704 $\pm$ 81	1152 $\pm$ 122
A_N ($\mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$)	11.1 $\pm$ 1.1	10.2 $\pm$ 0.8	14.6 $\pm$ 2.2	14.7 $\pm$ 1.2	8.7 $\pm$ 0.7	11.8 $\pm$ 1.0	10.7 $\pm$ 1.1	8.7 $\pm$ 1.2
G_s ($\text{mol H}_2\text{O m}^{-2} \text{s}^{-1}$)	0.24 $\pm$ 0.03	0.08 $\pm$ 0.01	0.16 $\pm$ 0.02	0.16 $\pm$ 0.03	0.19 $\pm$ 0.03	0.18 $\pm$ 0.03	0.17 $\pm$ 0.03	0.07 $\pm$ 0.01
Tr ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	1.3 $\pm$ 0.1	0.7 $\pm$ 0.3	1.4 $\pm$ 0.2	1.8 $\pm$ 0.3	1.6 $\pm$ 0.2	2.0 $\pm$ 0.2	1.4 $\pm$ 0.2	0.9 $\pm$ 0.2
WUE ($\mu\text{mol CO}_2 \text{mol}^{-1} \text{H}_2\text{O}$)	51.1 $\pm$ 5.1	150.8 $\pm$ 20.0	93.5 $\pm$ 9.9	104.6 $\pm$ 13.1	60.1 $\pm$ 11.4	83.8 $\pm$ 14.5	68.0 $\pm$ 8.8	175.1 $\pm$ 56.6
Fv/Fm	0.79 $\pm$ 0.01	0.82 $\pm$ 0.01	0.79 $\pm$ 0.01	0.81 $\pm$ 0.01	0.77 $\pm$ 0.01	0.79 $\pm$ 0.01	0.77 $\pm$ 0.02	0.78 $\pm$ 0.01
PI	3.5 $\pm$ 0.5	7.0 $\pm$ 0.7	3.7 $\pm$ 0.6	5.4 $\pm$ 0.7	2.6 $\pm$ 0.2	2.3 $\pm$ 0.2	1.9 $\pm$ 0.3	2.9 $\pm$ 0.4
Chlorophyll index	42.0 $\pm$ 3.5	52.2 $\pm$ 2.6	42.4 $\pm$ 2.8	46.9 $\pm$ 1.2	28.6 $\pm$ 2.3	37.0 $\pm$ 2.2	33.9 $\pm$ 2.9	33.8 $\pm$ 1.3
Flavonol index	1.63 $\pm$ 0.12	1.65 $\pm$ 0.02	1.77 $\pm$ 0.03	1.74 $\pm$ 0.02	1.48 $\pm$ 0.16	1.69 $\pm$ 0.07	1.75 $\pm$ 0.09	1.69 $\pm$ 0.07
NBI	26.0 $\pm$ 1.5	31.8 $\pm$ 1.8	24.2 $\pm$ 1.8	27.1 $\pm$ 0.9	20.5 $\pm$ 1.8	21.1 $\pm$ 1.4	20.0 $\pm$ 1.7	20.5 $\pm$ 1.0
GCC	0.36 $\pm$ 0.002	0.35 $\pm$ 0.003	0.38 $\pm$ 0.007	0.36 $\pm$ 0.004	0.40 $\pm$ 0.008	0.41 $\pm$ 0.004	0.40 $\pm$ 0.011	0.36 $\pm$ 0.005

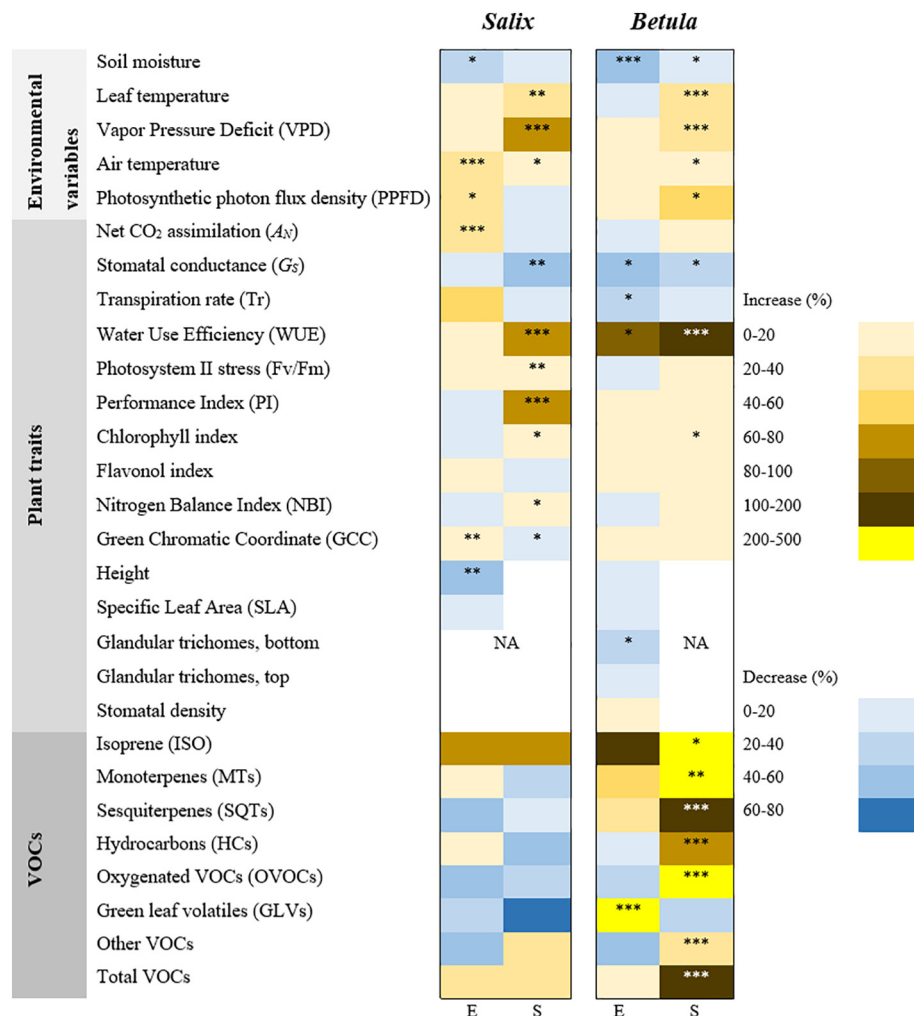


Fig. 2. Heat map showing the responses of environmental variables, plant traits and VOC emissions to elevation (E) and seasonality (S). Column “E” shows the change from low elevation to high elevation; “S” shows the change from mid- to late season; Increase and decrease (%) are represented by shades of yellow and blue color, respectively. GLMs were used to test for the effects of E, and S. $p < 0.001$ ***, $p < 0.01$ **, $p < 0.05$ *. The effects of interactions of E and S are not shown. Stomatal density, density of glandular trichomes, SLA and height were not tested for the effects of S because they were assumed not to change during the measurement period in fully developed leaves. NA, data not available.

than at low elevation ($p = 0.015$), but the interaction was not significant (Table S3).

In *Salix*, Fv/Fm and PI were 3% and 72% higher, respectively, during the late season compared to the mid-season ($p < 0.01$; Table 1; Fig. 2), and they correlated positively with leaf temperature (Table S4). In *Betula*, there were neither seasonality effects nor interactions for Fv/Fm, but there was a significant interaction effect for PI ($p = 0.019$).

The chlorophyll index was not significantly different between the two elevations in either species, but it was higher (17% for *Salix* and 13% for *Betula*) in the late season compared to the mid-season in both species ($p < 0.05$).

The flavonol index was not affected by elevation or seasonality in either species, but it did correlate positively with leaf temperature in *Betula* (Table S4). For NBI (nitrogen balance index), there were no significant differences in *Betula*, whereas in *Salix*, the index was 17% higher in the late season compared to the mid-season ($p = 0.026$; Table 1; Fig. 2).

GCC (green color coordinate) in *Salix* was 4% higher at high elevation compared to low elevation ($p = 0.004$) and 3% lower during the late season compared to the mid-season ($p = 0.024$). There was also an interaction between sex and seasonality ($p = 0.004$), with GCC in male plants decreasing by 5% from mid- to late season, while increasing by 1% in female plants (data not shown). GCC in *Betula* was not different between the elevations or seasons.

Specific leaf area (SLA) correlated negatively with leaf temperature in *Betula* (Table S4, Table S5). The density of glandular trichomes on the bottom side of *Betula* leaves was 24% lower at high elevation compared to the low elevation ($p = 0.031$; Table S5; Fig. 2).

3.2. Relationship between plant height and leaf temperatures

Salix shrubs at high elevation were 45% shorter compared to the ones at low elevation ($p = 0.005$; inset in Fig. 3; Fig. 2). For *Betula*, there was no significant difference in shrub height between elevations.

For *Salix*, there was a marginally significant relationship between the height of the plants and leaf temperature (Fig. 3). A regression line fitted

across all the data suggests that a 50 cm increase in plant height would lead to an average decrease in leaf temperature of 2 °C (or a decrease of 4 °C m⁻¹ height). In *Betula*, there was little variation in plant height within our sampled individuals and we found no relationship between plant height and leaf temperature.

3.3. VOC emissions

The most emitted VOC group in *Salix* was isoprene, as it amounted to 68% of the total VOC emission blend by mass, followed by hydrocarbons (HCs) and oxygenated VOCs (OVOCs), both at 13% (Fig. 4a, c). In *Betula*, most of the total VOC emissions consisted of OVOCs (37%), HCs (33%) and green leaf volatiles (17.5%; Fig. 4b, 3d).

In *Salix*, the total VOC emission was 28% higher at high elevation, compared to low elevation and 22% higher in late season compared to mid-season, but the differences were not significant (Fig. 2; Table S3). However, the interaction between elevation and seasonality was significant ($p = 0.023$; Table S3) because the *Salix* plants at mid-season low elevation emitted comparatively low amounts of VOCs (Fig. 4a). In *Betula*, the total VOC emission was 14% higher at high elevation and 123% higher in the late season, compared to the mid-season ($p < 0.001$; Fig. 2; Table S3), but the interaction was not significant.

Compared to the total VOC emission, isoprene emission in *Salix* showed a very similar response to elevation and seasonality (Fig. 2; Table S3), but the other VOC groups did not. Total VOC- and isoprene emissions correlated positively with leaf temperature (Table S4). Interestingly, there was a significant effect of *Salix* sex on OVOC emission ($p = 0.035$), with male plants emitting 55% more OVOCs compared to female plants (Fig. 5a, 4b; the difference is from an increase across all OVOCs).

In *Betula*, most VOC groups followed the trend of the total VOC emission and were emitted significantly more in late season, compared to the mid-season, with no significant interactions of elevation and seasonality (Fig. 2; Table S3). In addition to isoprene, SQT and OVOC emission correlating positively with leaf temperature (Table S4). The emission of GLVs was an exception to this trend, as high elevation *Betula* plants emitted 235% more GLVs compared to the low elevation ones ($p = 0.001$; Fig. 2; Table S3) and *Betula* GLV emission correlated negatively with soil moisture (Table S4).

Emission rates for all compounds, averaged across the measurement period are presented in Table S6.

In *Salix*, the clustering of the samples in the PCA showed that the VOC emission blends of the mid-season measurements were different for the two elevations and from the blends of the late season measurements (Fig. 5c). This was emphasized by a significant elevation $\times$ seasonality interaction on the PC2 scores ($p = 0.008$; Table S3). There was no significant effect of sex on either the PC1 or PC2 scores alone, but the emission blends differed between the *Salix* sexes across the diagonal line of the score plot (Fig. 5c).

In *Betula*, PCA of the VOC emission rates showed that the emission blends differed between the elevations and seasons (Fig. 4f). PC1 clearly separated mid-season and late-season blends ($p < 0.001$), and there was a significant interaction of elevation and seasonality ($p = 0.006$; Table S3), because the low elevation late season blends had the highest scores, while the mid-season blends from the same elevation had the lowest scores. The loading plot showed that higher relative abundances of most VOCs in the late season drove the separation due to seasonality (Fig. S2). PC2 separated elevations, with high elevation having higher and low elevation lower scores along this axis ($p = 0.002$; Table S3).

3.4. Light response curves

The LRCs for net CO₂ assimilation (photosynthesis) had a typical sigmoidal shape, both in *Salix* (Fig. 6a, b) and *Betula* (Fig. 6c). The difference between low and high elevation A_N in *Salix* was increasingly higher at higher light levels (1000 and 2000 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). ANOVA analysis of the constants derived from the photosynthesis LRCs in *Salix* revealed

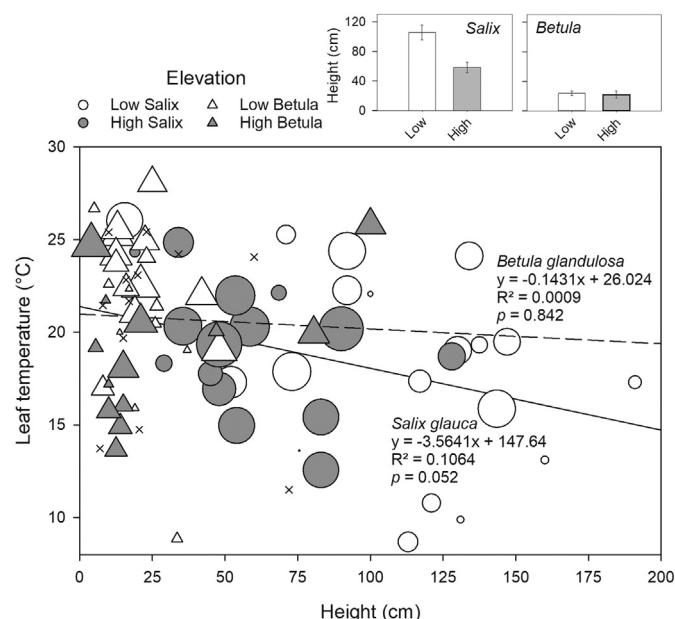


Fig. 3. Scatter plot of the height of the measured plants (cm) and leaf temperature (°C). Symbols show the measurements obtained from the two elevations, (circles, *Salix*, $n = 36$; triangles, *Betula*, $n = 41$) sized according to the ambient PPFD levels (ranging from 76 to 1760 $\mu\text{mol m}^{-2} \text{s}^{-1}$; crosses are the measurements where ambient PPFD data are missing). The regression lines and their corresponding coefficients across all the measurements within a species are shown (solid line, *Salix*; dotted line, *Betula*). Shrub height at low and high elevation is presented in the inset (mean $\pm$ SE).

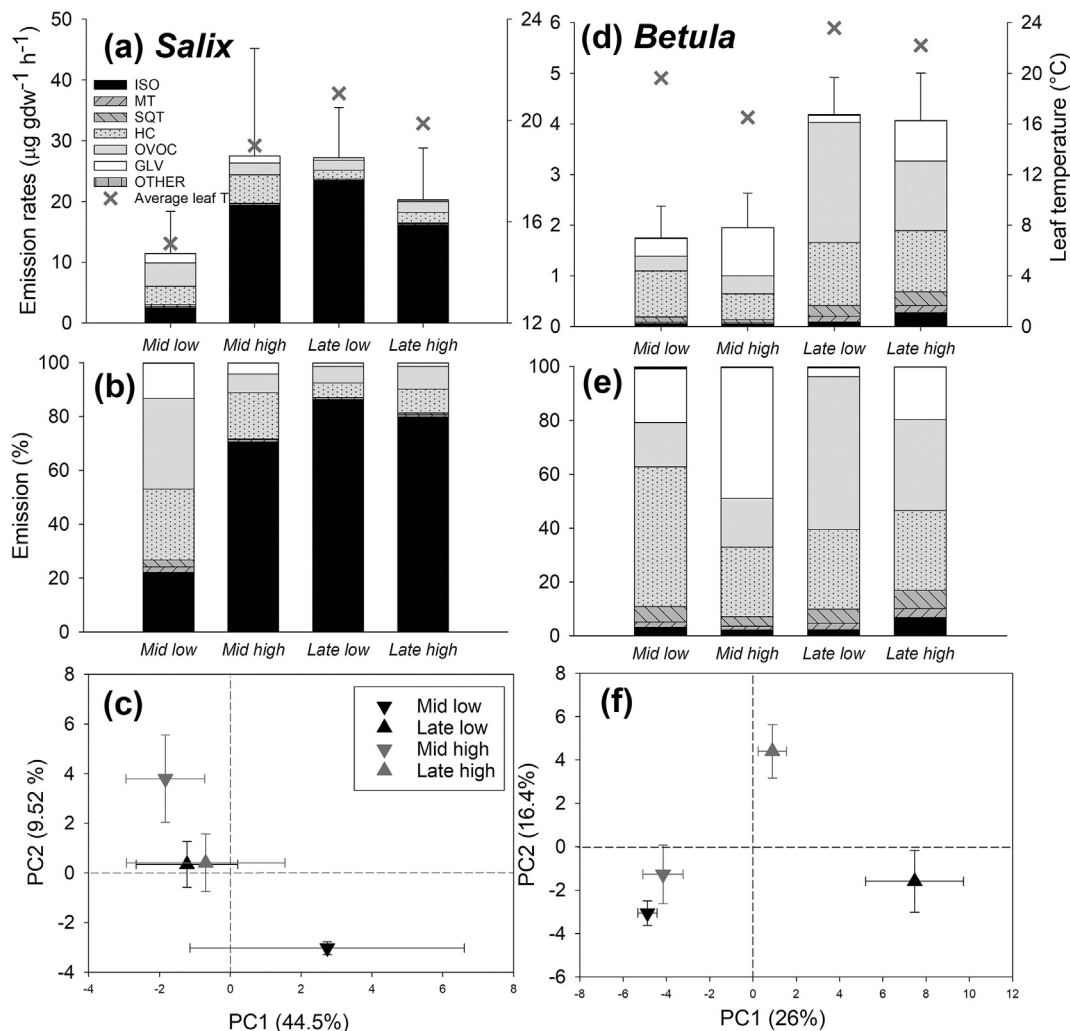


Fig. 4. (a) Total VOC emission rates (mean + SE) stacked by isoprene (ISO), monoterpenes (MT), sesquiterpenes (SQT), hydrocarbons (HC), oxygenated VOCs (OVOC), green leaf volatiles (GLV), and other VOCs in *Salix* for mid-season low elevation (Mid low, $n = 11$), mid-season high elevation (Mid high, $n = 7$), late season low elevation (Late low, $n = 9$), and late season high elevation (Late high, $n = 9$). (b) Emission rates for *Betula* at Mid low ($n = 13$), Mid high ($n = 8$), Late low ($n = 11$), and Late high ($n = 13$). Mean leaf temperatures for each combination of elevation and seasonality are shown. The relative contributions of each compound group to the total VOC emissions in *Salix* and *Betula* are shown in panels (c) and (d), respectively. Principal component (PC) analyses of VOC blends from (e) *Salix* and (f) *Betula*. The variance explained by each PC is shown in the parentheses. The loading variables are presented in Fig. S2.

that the only significant difference between elevations or sexes was in the initial slope of the curve (k ; $p = 0.001$), which is defined as the constant of proportionality and thus, does not reflect any particular natural process (Prado et al., 1994; Prado and Moraes, 1997). There was no significant difference between the photosynthesis constants in *Betula*.

The LRCs for isoprene emissions in *Salix* had a linear shape (Fig. 6d, e) and ranged from $0.2 \mu\text{g g}^{-1} \text{dw h}^{-1}$ to $6.7 \mu\text{g g}^{-1} \text{dw h}^{-1}$ at low elevation and 0.16 to $10.1 \mu\text{g g}^{-1} \text{dw h}^{-1}$ at high elevation (Fig. 6d).

The two *Salix* sexes had very similar isoprene emission rates in response to different light levels (Fig. 6e). Emission rates in *Betula* varied without a clear correlation with the light levels in either GLVs (Fig. 6f) or the other VOC groups (data not shown).

4. Discussion

The main aim of this study was assessing the elevational differences in plant traits and VOC emission blends in two dominant deciduous shrub species during the mid- and late growing season in South Greenland. We found that many of the differences we observed mirrored the local, current environmental conditions at the measurement time more than the elevational differences. We measured higher leaf temperatures in the late season

compared to the mid-season, so the effects of seasonality on plant traits and VOC emissions that we observed could probably be attributed to these temperature differences, in addition to plant phenology. On a similar note, soil moisture was higher in the valley compared to the mountain slope, so the effects of elevation in our models can be partly due to the differences in soil moisture.

4.1. Differences in plant traits and VOC emissions between elevations

The environment at high elevation poses many direct and indirect challenges for plant life, such as lower air temperatures, lower soil moisture content, higher PPFD, and higher air turbulence compared to the environment at lower altitudes (Higgins et al., 2021; Körner, 2021b). Nevertheless, we observed a 37% higher carbon assimilation rate (A_N) at $1000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ in *Salix* at high elevation compared to low elevation. However, the A_N values we report here are similar to the A_N value measured in *S. glauca* in West Greenland in 2014 (Gamm et al., 2018), where values increased as the soil dried, akin to the lower soil moisture we measured at high elevation. Moreover, an alpine willow species, *Salix atropantha*, has been shown to express 70% higher A_N at a high elevation site (3500 m a. s.l.; $14.6 \mu\text{mol CO}_2 \text{m}^{-2} \text{s}^{-1}$) compared to a low-elevation-mountain site

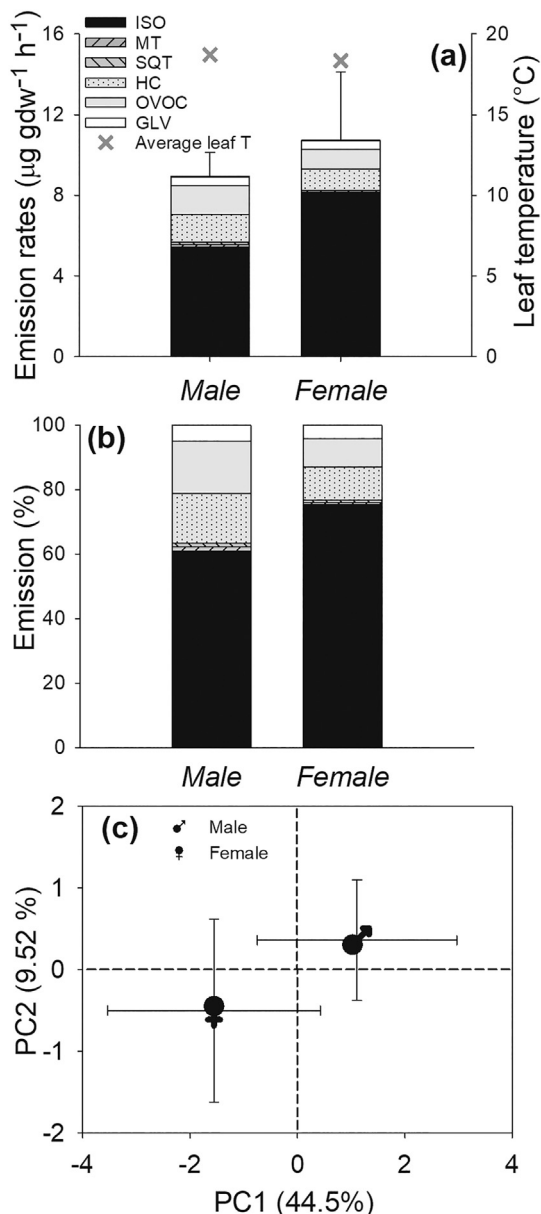


Fig. 5. (a) Total VOC emission rates (mean + SE) stacked by isoprene (ISO), monoterpenes (MT), sesquiterpenes (SQT), hydrocarbons (HC), oxygenated VOCs (OVOC), green leaf volatiles (GLV), and other VOCs for male ($n = 21$) and female ($n = 15$) *Salix*. (b) The relative contributions of each compound group to the total VOC emissions in male and female *Salix*. (c) Principal component (PC) analyses of VOC emission blends obtained from male and female *Salix*. The variance explained by each PC is shown in parentheses. Loading variables are presented in Fig. S2a.

(2500 m a.s.l.; $7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in North-West China (Shi et al., 2015). The isoprene emission rate in *Salix* was higher at high elevation, probably because high-elevation plants exhibited higher A_N and *de-novo* production of isoprene is known to be dependent on and increases with the rate of CO_2 assimilation (Monson et al., 1992; Sharkey et al., 2008; Laothawornkitkul et al., 2009).

For *Betula*, there were no differences in A_N between elevations, with an average of $10.1 \pm 0.7 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ across both elevations and seasons. However, this A_N rate for the *B. glandulosa* that we observed was comparable to the A_N of *B. nana* from our recent study in northern Sweden, $7.9 \pm 0.8 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ (Simin et al., 2021).

The Fv/Fm and PI, traits reflecting the photosynthetic performance, did not differ between elevations in *Salix* leaves similar to A_N , which is in line

with the findings of Mosbacher et al. (2013), who showed no effect of long-term warming on photosynthetic performance indices in *Salix*. However, Fv/Fm and PI were likely affected by *Salix* leaf temperature at the time of measurement, as they correlated positively with leaf temperature and were higher in the late season when the average leaf temperature of *Salix* leaves was 20.5°C , which is closer to its optimal temperature for photosynthesis (between 24 and 31°C in another tundra willow species, *Salix myrsinites*, (Simin et al., 2021)), compared to the average *Salix* leaf temperature of 17°C in the mid-season. Fv/Fm and PI in *Betula* changed in a similar pattern to *Salix*, but not to the same degree.

Salix leaves were greener at high elevation, possibly due to plant phenology differences at the two elevations (Argus, 1965).

Our leaf surface structure results also suggest that soil moisture differences between our two sites may have larger impacts than temperature differences. According to Thitz et al. (2017), glandular trichomes on the bottom side of the leaf have an important, but still unknown role in *Betula* spp. Glandular trichomes are formed in the early stages of leaf development, based on long-term environmental conditions (Valkama et al., 2004). We observed that the glandular trichome density on the bottom side of the *Betula* leaves was 24% higher in the valley, where the soil moisture was higher, compared to the mountain slope. This finding is an accordance with an experimental study by Wang et al. (2016) who found significantly higher glandular trichome density on the bottom side of *B. pendula* leaves in waterlogged soils compared to control soils. Additionally, Schollert et al. (2017) found that glandular trichome density increases in *B. nana* leaves subjected to experimental snow addition compared to controls, suggesting that increased soil moisture indeed leads to higher densities of glandular trichomes.

Furthermore, GLV emissions in *Betula* correlated negatively with soil moisture and were significantly higher at high elevation, where the soil moisture was limited, compared to at low elevation. Minor-to-moderate drought leads to increased GLV emissions, due to fracturing of the internal cell membranes in leaves (Pag et al., 2013; Jardine et al., 2015). GLVs are also known to be released as a reaction to mechanical damage (Rowen and Kaplan, 2016; Ameye et al., 2018), which can result from strong winds on mountain slopes (Heindel et al., 2015). In addition, GLVs can be emitted by heat and cold shocks (Copolovici et al., 2012), with sudden changes in temperature being more common at 450 m compared to 50 m altitude (Beniston et al., 1997; Pepin et al., 2015).

Our results emphasize the importance of local soil moisture conditions, in addition to temperature variation between elevations. Since the soils in the high latitudes depend mostly on the snow meltwater (Reid, 1967), soil moisture conditions are likely to cause small-scale microsite variation and will continue to play a significant role in shaping the expansion of deciduous shrubs in the tundra (Bjorkman et al., 2018; Gamm et al., 2018; Berner et al., 2020; Mekonnen et al., 2021). The effects of low soil moisture were especially noticeable in *Betula*.

4.2. Seasonal effects on plant traits and VOC emissions

The higher rate of stomatal conductance (G_s) in the mid-season compared to the late season in both plant species may be related to higher temperatures (which is supported by the negative correlation of G_s with leaf temperature) and higher VPD in the late season. High VPD causes the stomata to close and thus, lowers the G_s (Marchin et al., 2016). The sharp increase in VPD was the most likely reason for strong increases in WUE in the late season and, to a lesser degree, Tr (Rashid et al., 2018), which is also affected by soil moisture (Madhu and Hatfield, 2014). Higher WUE in the late season, when higher leaf temperatures were measured, means that both species were more conservative in their use of H_2O for fixing CO_2 from air. This might be important for the local carbon dynamics in future as air temperatures in tundra ecosystems are expected to increase worldwide (Berner et al., 2020; IPCC, 2021). WUE is suggested to be more sensitive to temperature in *Salix*, as it correlated positively with leaf temperature, unlike the WUE of *Betula*.

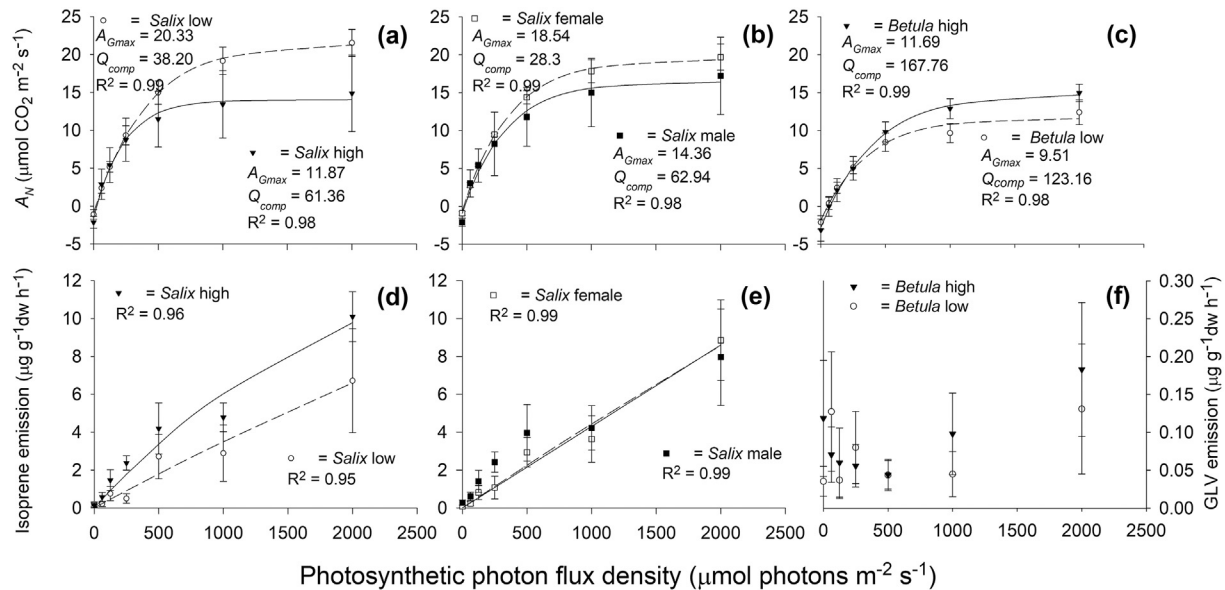


Fig. 6. Light response curves for net CO₂ assimilation (A_N) in *Salix* (a, b), and *Betula* (c), isoprene emissions in *Salix* (d, e), and GLV emissions in *Betula* (f; means $\pm$ SE; $n = 4$ in *Salix*, $n = 5$ in *Betula*, per curve/line). The lines are fitted according to Eq. 2, solid line, high elevation; dashed line, low elevation. Q_{comp} (light compensation point), A_{Gmax} (estimate of maximum gross photosynthetic rate), and R^2 (wellness of fit) are presented for each photosynthesis curve.

The increase in chlorophyll index we recorded from the mid- to late season for both *Salix* and *Betula* is in agreement with an earlier study on two aspen species (Croft et al., 2017). Nitrogen content is, due to strong correlation, often used as a proxy for A_N and chlorophyll content (Sage et al., 1987) and we also observed a fair increase in nitrogen-balance index (NBI) values in *Salix* during the late season, which indicates high productivity (Körner, 2021d (Körner, 2021c).

Higher *Salix* isoprene emission rates in the late season compared to the mid-season are most likely because of the higher leaf temperatures measured in the late season, which is supported by the strong correlation between isoprene emission and leaf temperature in our correlation analysis. Isoprene synthesis and emissions are highly temperature-dependent (Kramshøj et al., 2016; Seco et al., 2020; Simin et al., 2021). Stronger VPD in the late season probably also played a role in regulating the overall VOC emissions in *Salix* (Duan et al., 2019; Simin et al., 2021).

The total VOC emissions in *B. glandulosa* are quantitatively and qualitatively similar to *B. nana* emissions from other studies (Vedel-Petersen et al., 2015; Rieksta et al., 2021; Simin et al., 2021). The majority of the *Betula* VOC emission rates were higher in the late season rather than the mid-season, most likely because of higher leaf temperatures and VPD measured in the late season, as both VOC synthesis and emission processes are strongly temperature-dependent (Laothawornkitkul et al., 2009; Kramshøj et al., 2016; Lindwall et al., 2016; Seco et al., 2020; Simin et al., 2021), as supported by the positive correlation of isoprene, SQT and OVOC emission with leaf temperature in *Betula*. In addition, the VOC emission blends, as analyzed by PCA, clearly differed between the mid- and the late season measurements.

4.3. Differences between male and female *Salix*

We found no evidence for superior photosynthetic performance in male *Salix* plants compared to females, which was expected because of the higher energy investments required of female plants (Cornelissen and Stiling, 2005). The traits in *Salix* that differed clearly between sexes were the greenness (GCC), VOC blends, and OVOC emission rates. The OVOC emission rate was 55% higher in males compared to females. There were many compounds characteristic of the male *Salix* VOC blends, one of which, methyl salicylate, is an airborne signal for activating disease resistance and is the key to plant defense mechanisms (Shulaev et al., 1997; Park et al., 2007).

Phenoxyethanol is a strong microbicide (Saviuc et al., 2017), while methyl α -methylbutanoate is an aromatic compound also found in pineapple fruits (Liu et al., 2011). Herbivorous insects have been reported to prefer male *Salix* plants over female plants (Romero-Pérez et al., 2020; Simon et al., 2021). Stronger OVOC emissions from male plants compared to female plants have previously been reported by Tollsten and Knudsen in *S. caprea* and *S. cinerea* (1992), which leads us to believe that the increased OVOC emissions of male *Salix* shrubs are a measure to offset herbivory pressure.

4.4. Relationship between plant height and leaf temperature

Salix shrubs at 50 m a.s.l. were 45% taller compared to the shrubs at 450 m a.s.l., which is an expected response to climatic differences along elevation. Bjorkman et al. (2018) showed that tundra shrubs are increasing in height worldwide as a result of higher air temperatures. The increase in *Salix* shrub height in the Arctic also follows a latitudinal temperature gradient, as Walker (1987) showed a 115% increase in the height of *S. lanata* between the northern shores of Alaska and 100 km south and inland, where the climate was warmer. Hudson et al. (2011) observed a 28% increase in *Salix* height after 16 years of warming with open top chambers. A recent study in West Greenland also found that deciduous tundra shrubs grow taller in a warmer ecosystem (Higgins et al., 2021).

We expected that taller shrubs would have a lower leaf temperature than low-statured shrubs. Indeed we found an almost significant correlation between *Salix* leaf temperature and shrub height across elevations, a 2 °C decrease with a 50 cm height increase (4 °C m⁻¹). This result suggests that the decoupling of leaf- from air-temperatures, a characteristic of tundra vegetation (Helliker and Richter, 2008; Lindwall et al., 2016; Seco et al., 2020; Körner, 2021e), becomes weaker for the top canopy leaves of tall shrubs, compared to low-statured shrubs, in at least some tundra shrub species. The leaf-to-air temperature difference in taller growing tundra species warrants future research, because it is potentially important for ecosystem carbon exchange in the Arctic, where temperature is the key abiotic factor regulating plant activity (Berry and Bjorkman, 1980). The *B. glandulosa* we measured were of low stature overall and of comparable height at high and low elevation, so we could not assess the relationship between leaf temperature and height in that population, whereas the larger height variation in *S. glauca* allowed us to assess this relationship.

4.5. Light response curves

Our *Salix* A_N LRCs closely resembled the curves from other deciduous plant studies in shape and assimilation rates (Niinemets et al., 2015; Karlsson et al., 2020). The top canopy leaves of the low elevation plants tended to have higher A_{Gmax} and Q_{comp} , and had an increasingly stronger A_N at higher light levels (when PPFD was 1000 or 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$), possibly reflecting the light-competing strategy of tall shrubs. This strategy might be an increasingly important advantage in the tundra (Mekonnen et al., 2021) at low elevation where higher soil moisture availability was not limiting photosynthesis. The rate of A_N of low elevation *Salix* at light level of 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was similar to one of Tora, Wilhelm, and Inger varieties of commercial willows (Karlsson et al., 2020) and higher than in the LRC of a mature hybrid aspen leaf (Niinemets et al., 2015). At high elevation, the A_N LRC was comparable to an Ester willow variety, which is suited to dry climates (Karlsson et al., 2020). However, due to the relatively low number of replicates, differences in A_N from LRCs between the elevations have to be taken with caution.

The isoprene LRCs we observed for *S. glauca*, however, differed in shape from previously studied species (Niinemets et al., 2015; van Meeningen et al., 2017; Karlsson et al., 2020). Using the same standard technique of gradually reducing the PPFD available to the leaf in the chamber, the rate of isoprene emissions in temperate species typically plateau at PPFD levels exceeding 1000 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Monson et al., 1992; Grote and Niinemets, 2008; Niinemets et al., 2015), but we observed that the emissions increased in an almost linear fashion between 0 and 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. The strong increase in isoprene emissions at high irradiance is probably a consequence of adaptations of tundra plants to strong light in open landscapes (Körner, 2021e). The expected increase in cloud cover in the Arctic caused by climate change (IPCC, 2021) has the potential to significantly decrease the isoprene emissions in *Salix* (Sharkey and Yeh, 2001).

In *Betula*, the relationship between VOC emissions and light intensity in our LRCs was not clear. In some major VOC groups, like GLVs, the emission rate increased with light availability and did not decrease when PPFD was 2000 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Our *Betula* A_N LRCs closely resembled the curves from other studies in shape and assimilation rates (Niinemets et al., 2015; Karlsson et al., 2020). The high elevation shrubs tended to have higher A_N , A_{Gmax} , and Q_{comp} values, but the difference was not statistically significant.

5. Conclusions

We assessed the impacts of elevation on plant traits and VOC emissions of two common deciduous tundra shrubs at two elevations in South Greenland. Even though the air temperatures we recorded at low elevation were 10% higher compared to at high elevation, our results suggest that soil moisture differences (40% and 48% higher around low elevation *Salix* and *Betula* shrubs than at high elevation, respectively) were also likely important for the studied plant traits. Moreover, soil moisture in high latitudes tends to decrease during the growing season (Reid, 1967) and this decrease may differ among elevations.

We found some evidence that increasing shrub height will lead to lower leaf temperatures: the temperature of *Salix* leaves decreased by 2 °C with a height increase of 50 cm (4 °C m^{-1}), but the relationship has not been observed in *Betula*. Low-elevation *Salix* shrubs were 45% taller compared to high-elevation shrubs, but despite this, exhibited a 37% lower rate of carbon assimilation, a 63% lower rate of isoprene emission, and were 4% less green compared to the shrubs from high elevation. In contrast, *Betula* shrubs did not vary significantly in height or the rate of carbon assimilation across the elevations. Low-elevation *Betula* stomatal conductance was 40% higher, glandular trichome density 24% higher, and the emission of GLVs 235% lower compared to the high-elevation site.

The expansion of tundra shrubs in the Arctic is stimulated by rising air temperatures, but limited in places with low soil moisture (Hudson et al., 2011; Berner et al., 2020; Higgins et al., 2021; Mekonnen et al., 2021). Based on our results, the tundra plant traits and physiology are also

dependent on temperature, and the local soil moisture and light levels, suggesting that effects of climate warming, alone, poorly predict the actual plant responses.

CRedit authorship contribution statement

Tihomir Simin: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Cleo L. Davie-Martin:** Data curation, Formal analysis, Methodology, Validation, Writing – review & editing. **Julie Petersen:** Data curation, Formal analysis, Writing – review & editing. **Toke T. Høye:** Data curation, Methodology, Validation, Writing – review & editing. **Riikka Rinnan:** Conceptualization, Supervision, Funding acquisition, Project administration, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2022.155783>.

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