

Variations in permafrost environments determine populations and leaf traits of two closely related common shrub species (*Rhododendron subsect. Ledum*) in Interior Alaska

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Abstract

Permafrost considerably influences boreal forest ecosystems by constraining the niche space of woody plants. The permafrost influence on the ecosystems could dramatically change with permafrost thawing owing to recent rapid climate warming. However, it remains unclear how shrub species dominating the understory in boreal forests are associated with the permafrost environments. We investigated two closely related common shrubs, Labrador teas (*Ericaceae*, *Rhododendron* subsect. *Ledum*): *R. groenlandicum* and *R. tomentosum*, which exist sympatrically in the discontinuous permafrost zone of the Interior Alaska. We employed field surveys and trait measurements across permafrost gradients under the same climatic conditions to examine the associations among permafrost environments, populations, and leaf traits of the two species. Contrastive habitats were found between the two species: *R. groenlandicum* is abundant under darker, drier, thicker-active-layer conditions, whereas *R. tomentosum* is common under brighter, wetter, thinner-active-layer conditions. This suggests that habitat segregation between these species occurs in the discontinuous permafrost zone. *Rhododendron tomentosum* dominating permafrost conditions had more conservative leaves compared to *R. groenlandicum*. Moreover, both species had more conservative leaves under permafrost conditions. These intraspecific variations were mainly directly associated with canopy openness in *R. groenlandicum* but with active-layer thickness in *R. tomentosum*. In summary, our study suggests that large environmental variations driven by the inhomogeneous permafrost distributions can lead to the sympatric distributions of closely related shrub species in the discontinuous permafrost zone, and that the conservative leaves can contribute to their adaptation under permafrost conditions.

INTRODUCTION

Permafrost is one of the most crucial environmental factors determining ecosystem dynamics and landscapes of boreal forests (Jin et al., 2021). In the discontinuous permafrost zone of Interior Alaska, north-facing slopes and lowland bogs where the permafrost exists are dominated by open black-spruce (*Picea mariana*) forests. Contrastingly, deciduous broad-leaved or mixed forests are typical on south-facing slopes and beside rivers where permafrost do not form (Viereck et al., 1983). Such permafrost gradients can enable the effective evaluation of permafrost influence on boreal forest ecosystems, thereby expanding our understanding of how permafrost degradations drive long-term boreal forest biome shifts. Moreover, the permafrost influence on the boreal ecosystems would dramatically change with permafrost thawing in circumpolar regions (Biskaborn et al., 2019; Jin et al., 2021) that are warming two-four times as fast as the globe (Box et al., 2019; Rantanen et al., 2022). Thus, plant responses to permafrost gradients in the boreal forests must be investigated to comprehend and project the implications of permafrost change on the ecology and functioning of boreal forest ecosystems.

Shrubs represent a high proportion of the understory in boreal forests and arctic tundra (Viereck et al., 1983). In the open peatland forests underlain by permafrost, the understory shrubs play a substantial role in gas exchanges, energy budgets, and nutrient cycling on an ecosystem scale, owing to their high ground cover and faster leaf turnover rates compared to overstory black spruces (Chapin & Tyron, 1983; Ikawa et

al., 2015). Moreover, responses to changes in permafrost, as well as other associated environmental factors, could differ between understory shrubs and overstory trees (Ruess et al., 2003; Hébert et al., 2010a, 2011; Ikawa et al., 2015; Noguchi et al., 2021). Various understory microenvironmental factors and resource availabilities greatly vary across the permafrost gradients, even under the same climatic conditions, such as active-layer thickness (the thickness of the soil layer above permafrost that thaws during summer), ground temperature, and availability of nutrients, water, and light (Viereck et al., 1983; Rohrs-Richey & Mulder, 2007; Tanaka-Oda *et al.*, 2016; Noguchi et al., 2021). However, few studies have examined the associations between shrub ecological characteristics and such complex environmental variables, including light variations, across the wide range of gradients from dominant- to non-permafrost conditions (Standen & Baltzer, 2021) while some studies have been conducted in tundra ecosystems with no variation in light availability due to the absence of crown trees (e.g., Myers-Smith *et al.*, 2019).

Labrador teas (Ericaceae, *Rhododendron* spp. subsect. *Ledum*) are one of the most common understory evergreen shrubs in circumpolar environments, from boreal forests to tundra in Eurasia, North America, and Greenland (Hultén, 1968; Hébert & Thiffault, 2011; Dampc & Luczkiewicz, 2013; Johnson et al., 2020). The Labrador teas have considerable phenotypic plasticity and trait variation in response to environmental factors such as light intensity and active-layer thickness (Rohrs-Richey & Mulder, 2007; Hébert et al., 2010a, 2011). Thus, they can be ideal taxa for evaluating how shrub species have adapted to permafrost environments and would respond to recent rapid climate change in circumpolar ecosystems. Although the taxa have been used in folk medicine (Dampc & Luczkiewicz, 2013), the Labrador teas are also ecologically significant species for sustaining the ecosystem dynamics of boreal forests. For example, the shrub production in boreal peatlands is contributed primarily by these species (Wieder, 2006). Their presence decreases soil nitrogen availability, resulting in negative interactions with the growth of other plant species (Inderjit & Mallik, 1996; Castells et al., 2003; Hébert et al., 2010b). The Labrador teas are an intermediated host of spruce rust fungi that can suppress the growth of overstory spruces (Hennon, 1987). Recent studies suggest that neighboring birch leaves absorb volatile compounds produced by these shrubs and re-release them, thereby suppressing insect attacks (Himanen et al., 2010; Mofikoya et al., 2018). In tundra, a Labrador-tea species is projected to most greatly expand its distribution range under climate warming (García Criado *et al.*, 2023). However, variations in the populations and leaves of Labrador tea taxa across the wide range of permafrost gradients in boreal forests has not been evaluated because most previous studies exploring the ecology of Labrador teas were conducted in less-permafrost forests or tundra.

In the discontinuous permafrost zone of the Interior Alaska, two Labrador-tea species sympatrically exist at a regional scale: *Rhododendron groenlandicum* (Oeder) Kron & Judd (previously *Ledum groenlandicum*) and a closely related dwarf species *R. tomentosum* subsp. *decumbens* (Aiton) Elven & Murray (previously *L. palustre* subsp. *decumbens*) (Fig. 1; Hultén, 1968; Kron & Judd, 1990; Elven & Murray, 2008; Hart et al., 2017; Johnson et al., 2020). *Rhododendron groenlandicum* tends to be taller than *R. tomentosum*. The leaves of both species have curling at the edges and glandular and non-glandular trichomes on the leaf surface. However, *R. tomentosum* tends to have shorter, narrower, more pointed, and more tightly revolute leaves than *R. groenlandicum*. Furthermore, *R. tomentosum* has an

abrupt bend of the flower pedicels just beneath the apex (Sifton, 1963; Hébert & Thiffault, 2011; Dampc & Luczkiewicz, 2013; Mofikoya et al., 2018). Previous studies using morphological characteristics of many specimens reported that the extreme rarity of *R. groenlandicum* and *R. tomentosum* hybrids regardless of the appreciable geographic and ecological overlaps in the discontinuous permafrost zone (Fig. 1b; Savile, 1969; Lantai & Kihlman, 1995). In contrast, the crossing of *R. groenlandicum* and *R. neoglandulosum* results in *R. columbianum* in the western United States and western Canada (Savile, 1969; Hébert & Thiffault, 2011).

In this study, we examined the associations among populations, leaf traits, and environmental habitats of *R. groenlandicum* and *R. tomentosum* across permafrost gradients in the discontinuous permafrost zone of the Interior Alaska to elucidate how the most common shrub species in the boreal forests respond to permafrost conditions. We address the following questions: (1) Do populations and habitats differ across permafrost gradients between these two Labrador-tea species? (2) Do variations in leaf traits differ between the two species? and (3) How do the leaf traits of each species vary across permafrost gradients?

MATERIALS AND METHODS

Sites

The present study was conducted in ten sites across permafrost gradients in the discontinuous permafrost zone of the Interior Alaska (Fig. 1d): seven sites in or near a lowland bog (Bog sites; B1–7) in the Poker Flat Research Range (PFRR) and three sites on a north-facing slope (Slope sites; S1–3) in the Caribou Poker Creek Research Watershed (CPCRW) of the University of Alaska Fairbanks. As the CPCRW sites are near the PFRR sites (approximately 3 km), all sites were considered to be under almost the same climatic conditions: mean annual temperature and precipitation are -2.8°C and 241.5 mm y^{-1} , respectively (in the PFRR; Nakai et al., 2013). The information on site locations is listed in Table 1.

Table 1

Site locations and environmental variables (Mean $\pm$ SE; N = 16 for ALT, N = 4 for SWC and CO). The B7 site is under a non-permafrost condition. When ALT > 100 cm, ALT = 100 cm was used expediently. Small letters indicate the results of Steel-Dwass test (significance level = 0.10). ALT: active-layer thickness, SWC: volumetric soil water content, CO: canopy openness.

Sites	Altitude (m)* ¹	Longitude	Latitude	ALT (cm)	SWC (cm ³ cm ⁻³)	CO (%)
B1	212	65°07'13"N	147°29'19"W	53 $\pm$ 2 ^a	17.0 $\pm$ 1.7 ^a	68.5 $\pm$ 0.9 ^a
B2	211	65°07'15"N	147°29'26"W	49 $\pm$ 3 ^a	30.7 $\pm$ 5.5 ^{abc}	70.0 $\pm$ 2.2 ^{abc}
B3	209	65°07'16"N	147°29'34"W	53 $\pm$ 3 ^a	18.6 $\pm$ 1.8 ^a	51.0 $\pm$ 3.5 ^d
B4	208	65°07'16"N	147°29'41"W	46 $\pm$ 1 ^b	26.7 $\pm$ 1.7 ^c	76.9 $\pm$ 1.3 ^e
B5	207	65°07'17"N	147°29'44"W	49 $\pm$ 2 ^a	24.8 $\pm$ 1.3 ^c	74.0 $\pm$ 1.9 ^{be}
B6	207	65°07'17"N	147°29'48"W	85 $\pm$ 9 ^c	21.0 $\pm$ 3.4 ^{abc}	38.9 $\pm$ 2.7 ^{df}
B7	205	65°07'19"N	147°29'57"W	100 $\pm$ 0 ^d	17.4 $\pm$ 1.4 ^a	27.9 $\pm$ 5.0 ^{fg}
S1	253	65°09'05"N	147°29'29"W	48 $\pm$ 2 ^a	18.2 $\pm$ 1.8 ^a	62.0 $\pm$ 2.5 ^c
S2	317	65°08'56"N	147°29'54"W	61 $\pm$ 9 ^{ac}	14.7 $\pm$ 1.8 ^a	45.3 $\pm$ 1.2 ^d
S3	343	65°08'51"N	147°30'03"W	57 $\pm$ 3 ^a	15.4 $\pm$ 4.1 ^a	27.7 $\pm$ 0.7 ^g

*¹: Altitude was determined using Google Earth based on longitude and latitude coordinates.

The sites with a smaller site number tend to be under thinner-active-layer conditions across each gradient (Fig. 1d; Viereck et al., 1983). Among the B1–7 sites in the PFRR, B1–5 were in sparse black-spruce forests on permafrost, B6 was in a denser black-spruce forest in the boundary zone between permafrost and non-permafrost forests, and B7 was in a non-permafrost dense conifer-broadleaf mixed forest (borehole surveys by Iwahana unpublished). Among the B1–5 sites, B3 was in a slightly denser forest than the others, probably due to a difference in sediment conditions or slightly less permafrost influence. Among the S1–3 sites in the CPRW, S1 was in a sparse black-spruce forest on permafrost with a thinner active layer, and the S2–3 sites were in a larger, denser black-spruce forest on permafrost with a thicker active layer (Noguchi et al., 2021). The photographs of the sites are shown in Fig. S1.

Populations and sizes

Four quadrats (1 m $\times$ 1 m) were established in each site to investigate the abundances of *R. groenlandicum* and *R. tomentosum*. As it is hard to distinguish individuals (genets), owing to their great rhizomes, the numbers of all ramets of each species were counted in each quadrat in August 2022. Each quadrat was split into four square sections (0.5 m $\times$ 0.5 m). Next, four ramets of each species that were

closest to the centers of each split were selected in each quadrat. Then, their heights and ground diameters were measured with a ruler and a vernier caliper, respectively. The flowering periods were monitored in the B3–4 and B7 sites (Appendix S1). Here, we identified *R. groenlandicum* and *R. tomentosum* based on the morphology and trichome color of flower pedicels: *R. tomentosum* has an abrupt bend of the flower pedicels and reddish trichomes, whereas *R. groenlandicum* has a loose bend of the flower pedicels and whitish trichomes (Sifton, 1963; Hébert & Thiffault, 2011; Dampc & Luczkiewicz, 2013; Johnson et al., 2020).

Traits

Ten ramets of each species were randomly selected in each site to measure the morphological and physiological characteristics of leaves. Enough distances between the ramets were obtained (> 3 m) to collect the ramets from different individuals. One shoot per ramet, including leaves and branches of the current and previous years, was collected in August 2022, when the current leaves matured.

Leaf area (LA), aspect ratio (AR), specific leaf area (SLA), leaf dry matter content (LDMC), and sapwood-to-leaf area ratios (Huber value, HV; Huber, 1928) were measured for each shoot. The areas of the current and previous leaves were measured from the scanned leaf images (LIDE400, Canon, Tokyo, Japan) using Image J software (National Institutes of Health, Maryland, USA). The representative values of LA and AR were used as those of the largest leaf of each shoot. The weights of these leaves were measured using a digital scale (0.001 g precision; GH202, A&D, Tokyo, Japan) before and after drying in an oven at 70°C for three days. SLA was calculated as the total leaf area per total dry mass of current-year leaves. LDMC was calculated as the dry mass per fresh mass of current-year leaves. The sapwood area was calculated based on the diameter of the previous-year-stem base, assuming that sapwood occupies the entire cross-section of the stem (Amada et al., 2017). HV was calculated as the sapwood area per total area of current- and previous-year leaves.

Nitrogen content, C/N ratio, and carbon and nitrogen stable isotope ratio ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) were measured using the dry samples of the current-year leaves. The dry leaf samples were ground into fine powders with a pestle and mortar. Nitrogen and carbon concentrations (N_{mass} and C_{mass}), $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ were obtained using continuous-flow isotope ratio mass spectrometry (Flash 2000 elemental analyzer and ConFlo IV interfaced with a DeltaV Plus Mass Spectrometer, Thermo Scientific, USA). The analyses were performed by the Alaska Stable Isotope Facility at the University of Alaska Fairbanks's Water & Environmental Research Center. Nitrogen content per unit leaf area (N_{area}) was calculated as N_{mass} per SLA. The C/N ratio was calculated as C_{mass} per N_{mass} .

Environmental conditions

To examine variations in the environmental conditions across the permafrost gradients, three environmental factors were measured in each quadrat: active layer thickness (ALT), soil water content (SWC), and canopy openness (CO). Measurements of soil nitrogen availability could also be effective for

the ecological strategy of Labrador tea species in boreal ecosystems. However, it is hard to examine the soil nitrogen availability in the sites because boreal shrub species highly depend on both organic (amino acids) and inorganic nitrogen (McKane et al., 2002). In addition, the fine roots of Labrador teas often exist in thick moss layers where it is challenging to distinguish *soils* from live mosses. Therefore, we measured ALT, which could be somewhat related to soil nitrogen availability (Rohrs-Richey & Mulder, 2007; Tanaka-Oda *et al.*, 2016; Jasinski et al., 2022).

ALT can be associated with ground temperature and plant nutrient use. At the end of August 2022, a metal rod (6 mm diameter, 1 m length) was used to probe for the bottom of the active layer at four points in each quadrat. When ALT > 1 m, ALT = 1 m was used expediently for all subsequent analyses.

SWC can be associated with plant water use. One soil core where roots existed (up to 30 cm depth; 3.6 cm diameter; only organic layer) in each quadrat was collected after two consecutive sunny days in September 2022 to obtain a broader range of SWC. The weights of these soils were measured using a digital scale (0.01 g precision) before and after drying in an oven at 100°C for 24 h. Finally, SWC was calculated as volumetric soil water content (unit: $\text{cm}^3 \text{ cm}^{-3}$).

CO can be associated with plant light use. One hemispherical photograph was taken at 1 m above the ground surface (higher than the heights of Labrador teas) in each quadrat at the beginning of August 2022 when leaves of deciduous crown trees remained (THETA SC2, RICOH, Tokyo, Japan). CO was calculated as a ratio of the sky in the upper hemisphere using an R program (Honjo et al. 2019).

Statistical analyses

All analyses were conducted with the R statistical package (v.4.2.1; R Foundation for Statistical Computing, Vienna, Austria).

The Steel-Dwass test was used to examine the variations in environmental factors (ALT, SWC, and CO) across the permafrost gradients. Spearman correlation analyses were also conducted to examine the relationships between each environmental factor.

The Kruskal-Wallis test was conducted to examine the differences in the numbers, heights, or ground diameters of ramets or each measured leaf trait among the populations.

Moreover, principal component analysis (PCA) was conducted to examine intraspecific and interspecific variations in leaf traits of the two species. We visualized the results using biplots to show the relationships between the leaf traits and principal components (Fig. 7). Scores and variables resulting from the PCA were scaled for visual depiction of data.

Path analyses were performed to examine how population characteristics or leaf traits associate with environmental variables. A full model and working hypotheses are shown in Fig. 2. Here, the average values of environmental variables in each quadrat or in each site (i.e., the four quadrats) were used for the population or leaf-trait analyses, respectively. Akaike's information criterion (AIC) was used to

determine which associations were significant for each population characteristic or leaf trait. The ranges of environmental factors were different between the two species because *R. tomentosum* was not found in the B7 and S2–3 sites (Fig. 3a). Thus, to distinguish the effects of different environmental ranges from the species responses in the analyses, we analyzed the associations in *R. groenlandicum* with samples from the limited sites where *R. tomentosum* existed (RG_{limit}) in addition to ones with all samples (RG_{all}) (Fig. 7, S4–6, and Table S3).

As non-linear distributions in response to environmental conditions (e.g., unimodal distributions suggesting a limited range of habitat preference) cannot be examined using the path analyses, a generalized linear mixed model (GLMM) was also performed to evaluate the associations between the number of ramets and each environmental habitat. Both linear and quadratic terms were assumed for the environmental factors in the model because these species may have a unimodal distribution across environmental gradients. A significant negative quadratic term can be selected when the species have a unimodal distribution across the environmental gradient (Amada et al., 2020). Similar to the path analyses, ALT, SWC, and CO values in each quadrat were employed as explanatory variables. We examined the estimated effects of ALT, ALT^2 , SWC, SWC^2 , CO, and CO^2 as fixed effects and the site as a random effect on the numbers of ramets in each species. We assumed that the number of ramets followed the Poisson distribution. Finally, AIC was used to select the best-fit model.

RESULTS

Environmental conditions

ALT, SWC, and CO remarkably varied across the permafrost gradients (Table 1). Among the Bog sites, ALT was smallest in B4 (46 cm), followed by B1–3 and B5 (approximately 50 cm), and B6 (85 cm). However, it was largest in B7 (> 100 cm) where no permafrost exists. In B6, ALT was approximately 62 cm in two quadrates but > 100 cm in the others. SWC was largest in S2, followed by B4–5, B6, B1, B3, and B7. CO was largest in the B4 site, followed by B5, B1–2, B3, and B6–7. Among the Slope sites, ALT and its variation tended to be larger in S2–3 than in S1. However, no significant difference was found in SWC. CO was the largest in the S1 site, followed by S2 and S3.

ALT was significantly negatively correlated with CO and weakly negatively correlated with SWC. In contrast, CO was significantly positively correlated with SWC (Table 2).

Table 2
Correlation coefficients
between environmental
variables. ALT: active-layer
thickness, SWC: volumetric
soil water content, CO:
canopy openness. ***: $P <$
0.001.

	CO	SWC
ALT	-0.62***	-0.30***
CO	—	0.55***

Populations

The number of ramets greatly varied in each species among the sites: the mean at each site was 2–113 ramets m^{-2} in *R. groenlandicum* and 0–252 ramets m^{-2} in *R. tomentosum*. However, the trends were contrastive between the two species. *Rhododendron groenlandicum* was abundant in the B7 and S2–3 sites with greater ALT, whereas the ramets were found at all sites (Fig. 3a,b). However, *R. tomentosum* was common under permafrost conditions such as the B1–5 and S1 sites. Its abundance was greatest in the B4 site with the smallest ALT, and no ramets of *R. tomentosum* were found in the B7 and S2–3 sites with greater ALT (Fig. 3a,b).

The heights and ground diameters also varied among the sites. Within each site where two species were found (i.e., B1–6 and S1), *R. groenlandicum* tended to have taller, thicker stems than *R. tomentosum* except for the B4–5 sites, where these values were similar between the two species (Fig. 3c,d).

Figure 4 shows the best-suited models for explaining the associations among environmental factors and the number, height, or ground diameter of ramets in each species based on the full model illustrated in Fig. 2. The subsequently selected models with $\Delta\text{AIC} < 2.00$ are also shown in Table S1 and Fig. S2.

In both species, the number of ramets was directly associated with CO and SWC and indirectly with ALT through SWC and CO. Moreover, the associations were stronger with CO than with SWC (Fig. 4a,b). However, the trends were contrastive. *Rhododendron groenlandicum* was dominant under darker, drier conditions with a thicker active layer. In contrast, *R. tomentosum* was dominant under brighter wetter conditions with a thinner active layer (Fig. 4a,b). The number of ramets in *R. groenlandicum* was negatively directly associated with ALT but also positively indirectly with ALT through SWC and CO (Fig. 4a).

The results of GLMM analyses evaluating associations between the number of ramets and SWC or CO were mostly constant with those of path analyses, but the unimodal distributions across the CO gradient were found in both species (Fig. 5; Table S2). The peaks of the number of ramets were found at CO = 40% and 68% in *R. groenlandicum* and *R. tomentosum*, respectively. This demonstrates that the number of *R.*

groenlandicum ramets also tended to decrease in much denser forests and that of *R. tomentosum* tended to decrease in much sparser forests, although its variance also increased.

Associations of the heights or ground diameters of ramets with SWC or CO were similar between the species. The ramets of both species were taller under darker, drier conditions and had thicker stems under brighter, wetter conditions (Fig. 4c–f). However, the direct associations with ALT were contrastive. *Rhododendron groenlandicum* had taller, thicker stems whereas *R. tomentosum* was smaller under greater ALT conditions.

Traits

All measured morphological and physiological characteristics considerably varied among the populations (Fig. 6). In the sites with greater ALT (i.e., with a larger site number), leaves and shoots of both species tended to have larger LA, greater SLA, smaller LDMC, smaller HV, greater N_{mass} , and higher $\delta^{15}\text{N}$. When comparing the two species within a site where both were found, *R. groenlandicum* tended to have larger LA, smaller AR (i.e., broader leaves), smaller HV, greater N_{area} , and larger $\delta^{13}\text{C}$.

Figure 7 shows the results of PCA using morphological and physiological characteristics. The PC1, PC2, and PC3 axes explain 72.7% of leaf-trait variations. PC1 scores were positively correlated with LA, SLA, N_{mass} , and $\delta^{15}\text{N}$ and negatively correlated with LDMC, C/N ratio, and HV. Such trends indicate that the PC1 axis was related to the leaf economic spectrum (Wright et al., 2004). Leaves with smaller PC1 scores had more conservative leaf economic traits whereas those with greater PC1 scores were more acquisitive (Thomas et al., 2020). PC2 scores were positively correlated with N_{area} and $\delta^{13}\text{C}$, which are strongly associated with drought tolerance (Amada et al., 2017). PC3 scores were correlated with AR; leaves with greater PC3 scores were more elongated.

Although there was some overlap in the leaf-trait variations between the two species, their trends were different (Fig. 7). Intraspecific variations in leaf traits were greater in *R. groenlandicum* than in *R. tomentosum*. As for interspecific variations, *R. groenlandicum* tended to have larger PC1–2 and smaller PC3 scores compared to *R. tomentosum*, that is, more acquisitive broader leaves with higher drought tolerance. The PC scores in each site are shown in Fig. S3.

The path analyses were performed for the PC1, PC2, or PC3 scores to demonstrate which environmental variable influences the intraspecific leaf-trait variations of each species across the permafrost gradients. Figure 8 shows the best-suited models for explaining the associations among environmental factors and PC scores in each species based on the full model illustrated in Fig. 2. The subsequently selected models with $\Delta\text{AIC} < 2.00$ are shown in Table S3 and Fig. S4–6.

The PC1 scores were directly negatively associated with CO, slightly positively with SWC, and indirectly positively with ALT through CO in *R. groenlandicum* (Fig. 8a). These results demonstrate that *R. groenlandicum* had more conservative leaves under brighter, drier, and thinner-active-layer conditions. Contrastingly, PC1 scores were directly associated with ALT, and somewhat with CO in *R. tomentosum*

(Fig. 8c). This demonstrates that *R. tomentosum* had more conservative leaves under brighter, thinner-active-layer conditions. As the trends were similar between RG_{limit} and *R. tomentosum* (Fig. 8b,c), the difference between the two species was primarily due to the different ranges of habitat environments.

PC2 scores showed similar associations with environmental variables in both species. The PC2 scores were directly positively associated with CO, negatively with SWC, and indirectly negatively with ALT through CO (Fig. 8d–f). This means that both species had leaves with higher drought tolerance under brighter, drier, and thinner-active-layer conditions.

The associations of the PC3 scores with the measured environmental variables were small compared to those of the PC1 and PC2 scores (Fig. 8g–i). The PC3 scores were slightly negatively associated with SWC and CO, and indirectly positively with ALT through CO in *R. groenlandicum* (Fig. 8g). *Rhododendron groenlandicum* had more elongated leaves under darker, drier conditions with a thicker active layer. The PC3 scores were directly positively associated with ALT, slightly with CO, and indirectly negatively with ALT through CO in *R. tomentosum* (Fig. 8i). As the coefficients were greater for a direct pathway than they were an indirect pathway through CO (Fig. 7i), the positive influence of ALT on the variations in the PC3 scores was dominant. This indicates that *R. tomentosum* had more elongated leaves under thicker-active-layer conditions.

DISCUSSION

This study demonstrates the variations in environmental habitats and leaf-trait variations of *R. groenlandicum* and *R. tomentosum*, across permafrost gradients under similar climatic conditions. We found contrastive trends in their habitats across the permafrost gradients: *R. groenlandicum* is abundant in sites with greater ALT, whereas *R. tomentosum* is common under permafrost conditions. *Rhododendron tomentosum* had more conservative leaves compared to *R. groenlandicum*. Evaluation of their intraspecific variations revealed more conservative leaves under permafrost conditions in both species. We describe the associations among the populations, leaf traits, and environmental habitats to elucidate the Labrador tea ecology and project the influence of climate change through permafrost degradation on understory vegetation.

Population and Permafrost

Several specimens and detailed morphological identifications previously indicated that *R. groenlandicum* and *R. tomentosum* distributions largely overlap from interior Alaska to Labrador and Greenland (Fig. 1b; Hultén, 1968; Savile, 1969). However, whether the environmental habitats are different between the two species in the sympatric regions and why the hybrids of the closely-related species are very rare remain unclear (Savile, 1969; Lantai & Kihlman, 1995).

This study quantitatively demonstrates the contrastive trends in habitats across the permafrost gradients in the sympatric region. *Rhododendron groenlandicum* is abundant under darker, drier conditions with a thicker active layer, whereas *R. tomentosum* is common under brighter, wetter conditions with a thinner

active layer (Fig. 3–4). These differences suggest that habitat segregation via the variation in permafrost conditions occurs in the discontinuous permafrost zone.

The contrastive distributions are primarily associated with the variations in ALT through changing CO and SWC (Fig. 4a,b). Thinner ALT causes greater CO by suppressing the crown-tree growths, owing to lower nutrient availability and limited unfrozen soil volume for rooting (Tanaka-Oda et al., 2016). Further it leads to higher SWC through lower evaporation, owing to lower ground temperature and thinner water table (Dearborn et al., 2021). This is consistent with our results (Table 2). Although both species typically dominate damp ecosystems with acidic nutrient-poor soils (Hébert & Thiffault, 2011; Dampc & Luczkiewicz, 2013), *R. tomentosum* inhabits wetter, more open peatland forests underlain by permafrost compared to *R. groenlandicum*. Although direct negative association with ALT was also found in *R. groenlandicum* (Fig. 4a), this was because a greater moss layer under thinner-ALT conditions covered the branching bases, resulting in a greater number of ramets.

The abundances were more greatly associated with CO than SWC in both species (Fig. 4a,b). *Rhododendron groenlandicum* is classified as a shade-intolerant species (Klinka et al., 1989; Humbert et al., 2007) with a lower abundance under a relatively closed canopy (La Roi, 1967; Telfer, 1972). However, the species also inhabit closed forest floors rather than open tundra and can tolerate darker conditions (Gucker, 2006; Johnson et al., 2020). Karlin and Bliss (1983), the only previous study comparing the ecologies between the two species, shows that *R. groenlandicum* germination markedly decreases in far-red-rich light regimes but not that of *R. tomentosum*. This suggests that *R. groenlandicum* sensitively responds to the crown-tree shading and requires light strength to some extent in dense forests, such as gap conditions (La Roi, 1967). These findings are consistent with the unimodal distribution of abundance across the CO gradient in *R. groenlandicum* here (peak: 40% of CO; Fig. 5).

Much fewer studies describe the association between light conditions and *R. tomentosum*. Additionally, no sensitivity of its germination to far-red richness is reported (Karlin & Bliss, 1983) because *R. tomentosum* can inhabit only harsh environments where almost no shading is maintained by large crown trees. Moreover, it cannot survive greater light competition between understory species in more nutrient-rich forests, partly due to the smaller size and conservative strategy (Fig. 3–5, 7). However, a slight decrease in the abundance of *R. tomentosum*, even under the greatest CO conditions (> 68% of CO; Fig. 5), was found potentially due to the influence of *Sphagnum* mosses. *Sphagnum* mosses tightly covered the ground in the B4 site with the greatest CO. The light and nutrient competition with *Sphagnum* mosses may suppress the growth and survival of shrubs (Rydin et al., 2006). Vascular plants can access available nitrogen and phosphorus only after mineralization of the mosses (Malmer et al., 2003). Moreover, the seedlings need to grow faster than the *Sphagnum* shoots under the nutrient-poor conditions to avoid being overtopped and buried in the *Sphagnum* carpet (Rydin et al., 2006), although Labrador tea seeds have very short longevity and fewer nutrient stocks due to their tiny size (Karlin & Bliss, 1983) and slow seedling growth (Hébert & Thiffault, 2011).

Our results of soil moisture are inconsistent with a description by Savile (1969) that *R. tomentosum* penetrates only the drier parts of bogs compared to *R. groenlandicum*, whereas their habitats slightly overlap. The greater ALT sometimes increases the surface moisture, owing to thermokarst subsidence (Yoshikawa & Hinzman, 2003). Thus, their distributions might be directly determined by nutrient limitations due to thinner ALT rather than SWC.

Our surveys reveal obvious habitat differences between *R. groenlandicum* and *R. tomentosum* in the sympatric regions in the Interior Alaska. However, this is insufficient to explain the extremely rare hybridization (Savile, 1969). Although the habitat segregation and different flowering peaks could partly suppress the hybridization, the overlaps in their habitats and flowering periods indicate incomplete ecological and phenological isolations (Fig. 3a, S7; Appendix S1). Similarly, habitats and flowering periods overlap between the Labrador tea species tetraploid *R. diversipilosum* and diploid *R. subarcticum* (a synonym of *R. tomentosum*; Elven & Murray, 2008) in Japanese alpine areas, and germination rates of hybrid seeds are considerably low due to the difference in chromosome number (Shiotani & Kudo, 2023). In North America, *R. groenlandicum* has a chromosome number of $2n = 26$ (Wanscher, 1934; Löve, 1982), whereas *R. tomentosum* has that of $2n = 26$ in northwest Alaska (Johnson & Packer, 1968) but that of $2n = 52$ in Manitoba, Canada where diploid *R. groenlandicum* also exists (Löve, 1982). Although phylogenetic studies have been recently conducted with limited samples (Hart et al., 2017), a few hybridizations and gene introgressions speculated using morphological surveys (Savile 1969) between the two species have not yet been confirmed through genetic analyses. Therefore, additional chromosome surveys and phylogeographic and population genetic studies are needed to elucidate the mechanisms underlying reproductive isolation between the species in the regions.

Leaf-trait variations and Permafrost

Leaf traits of Labrador teas greatly varied across the permafrost gradients, even under similar climatic conditions. *Rhododendron groenlandicum* had a greater variation in leaf traits compared to *R. tomentosum* (Fig. 6), which can lead to a wider range of *R. groenlandicum* habitats across the gradients (Fig. 3a,b). However, our data cannot clarify whether such leaf-trait variations are attributable to adaptation or acclimation to permafrost conditions. Furthermore, a few introgressions from *R. tomentosum* (Savile 1969) could partly contribute to the large variation of *R. groenlandicum*. Here, the leaf economic traits were similar between the two species within each site (Fig. S3).

Rhododendron tomentosum dominating permafrost conditions often has more conservative leaves than *R. groenlandicum*, and both species have more conservative leaves under thinner-active-layer conditions. These intraspecific and interspecific variations suggest that the conservative leaves contribute to their adaptation under permafrost conditions. Low nitrogen availability and strong irradiance can substantially restrict the assimilation, growth, and survival of understory shrubs despite limited water stress and small light competition in the sparse peatland forests underlain by permafrost. This is consistent with our results demonstrating that the intraspecific leaf economic trends are primarily associated with CO and ALT and that $\delta^{15}\text{N}$, an indicator of soil nitrogen availability (Mayor et al., 2012), is lower under permafrost

conditions (Fig. 6i, 8a–c). Under such stressful conditions, the low SLA, high LDMC, low N_{mass} , and high C/N ratio (Fig. 6, 7) can contribute to greater leaf toughness with limited nitrogen resources. This reduces the loss of leaves caused by fungal and insect attacks (Onoda et al., 2011). The smaller, narrower leaves with a thinner boundary layer on the leaf surface effectively avoid heat stresses under strong-irradiance conditions (shortwave irradiance in summer daytime is $> 700 \text{ W m}^{-2}$ in PFRR; Kobayashi et al., 2023) through greater sensible-heat conductance (Parkhurst & Loucks, 1972; Amada et al., 2023). Higher nitrogen availability leads to greater light competition among understory plants in the dense forest under thicker-active-layer conditions. Thus, a more acquisitive strategy with larger nutrient-rich leaves and taller heights can be advantageous. Although this study has compared the associations of ALT, SWC, and CO with the traits of shrubs across a wide range of permafrost gradients, such leaf economic trends in response to the permafrost are consistent with previous studies conducted across permafrost gradients focusing on shrub species (Rohrs-Richey & Mulder, 2007), tree species (Tanaka-Oda *et al.*, 2016; Anderson et al., 2020), and community-level traits (Standen & Baltzer, 2021), and with warming experiments (Iturrate-Garcia *et al.*, 2020; Wei et al., 2023).

The environmental factors associated with the leaf economic spectrum also depended on the range of permafrost gradients rather than species. Thus, the result of RG_{limit} was similar to that of RT rather than RG_{all} (Fig. 8a–c). This may reflect changes in the environmental variables restricting shrub growth across the permafrost gradients. The belowground restriction of low ground temperature and nutrient availability can be relaxed from the sparse permafrost forests to the dense less-permafrost forests, and the light restriction can be gradually dominant. Crown trees change their SLA in response to ALT (Anderson et al., 2020). Additionally, community-level SLA and N_{mass} across permafrost gradients within the open peatlands are predominantly associated with ALT and slightly with CO (Standen & Baltzer, 2021). Furthermore, the rich light resources could lead to higher fine-root productivity of shrubs under thinner-active-layer conditions (Noguchi et al., 2021), potentially contributing to greater nutrient uptake in low-nutrient environments. Such changes in resource restrictions across the permafrost gradients would be important for projecting the response of plant functional traits to the long-term biome shifts caused by permafrost degradation under climate warming.

Drought tolerance was associated with both SWC and CO to a similar extent, whereas the associations were consistent between species and between different ranges of permafrost gradients (Fig. 8d–f). Both species have leaves with higher N_{area} and $\delta^{13}\text{C}$ under drier or brighter and thinner-ALT conditions. The drought stress can be greater under lower soil-water-availability and strong-irradiance conditions where water losses from leaves tend to be larger because of high leaf temperature and leaf-to-air vapor pressure (Parkhurst & Loucks, 1972; Amada et al., 2023). Conservative stomatal behaviors and higher photosynthetic enzyme contents, suggested by higher $\delta^{13}\text{C}$ and N_{area} , lead to greater water-use efficiency (equal assimilation/transpiration), contributing to their adaptations under the drought-stress conditions (Amada et al., 2017, 2023).

Roles of Labrador teas under climate change

While community-level functional traits could strongly influence the biogeochemical cycling on an ecosystem scale (Roos et al., 2019; Standen & Baltzer, 2021; Wei et al., 2023), the Labrador tea taxa is likely to have a great influence on the community-level traits in circumpolar regions. The variations in community-level traits under permafrost degradation could be largely attributed to species turnover driven by changing ALT (Standen & Baltzer, 2021). However, both the turnover of contrasting species from *R. tomentosum* to *R. groenlandicum* and the large intraspecific variations of the Labrador teas in response to permafrost degradation can affect the community-level traits, owing to their wide distribution range (Hultén, 1968), high dominance, and large productivity (Gucker, 2006; Wieder, 2006). Wei et al. (2023) show that within-species phenotypic plasticity of dominant understory species, rather than species turnover, alters the community-level traits in a warming experiment. Moreover, the Labrador tea taxa considerably interact with the crown trees and other plants by decreasing soil nitrogen availability (Inderjit & Mallik, 1996; Castells et al., 2003; Hébert et al., 2010b), fungal disease (Hennon, 1987), and volatile compounds (Himanen et al., 2010; Mofikoya et al., 2018). As *R. tomentosum* is projected to most greatly expand its distribution range in tundra under climate warming (García Criado *et al.*, 2023), such positive and negative interactions may also affect the community-level traits by altering the abundance and leaf traits of the other species.

Permafrost degradation can alter resource restrictions on understory shrubs through long-term biome shifts in boreal forests. In the earlier stage of permafrost degradation, understory shrubs can respond to the changes in ALT, influencing nutrient availability, ground temperature, and unfrozen soil volume for rooting (Rohrs-Richey & Mulder, 2007), similar to crown trees (Anderson et al., 2020). Denser, larger forests can gradually develop, owing to fewer resource restrictions on the crown trees in the later stage, (Dearborn et al., 2021). Moreover, the closed canopies and deeper thaw depths lead to darker, drier understory conditions, wherein the light restrictions on shrubs may be stronger than the nutrient restrictions. Our results suggest that both the abundances and trait variations of *R. groenlandicum* and *R. tomentosum* are sensitive to the changes in resource restrictions through the biome shifts of boreal forests. Although *R. tomentosum* can greatly expand its distribution range in tundra regions under climate warming (García Criado *et al.*, 2023), the habitats of acquisitive *R. groenlandicum* rather than conservative *R. tomentosum* may increase with the permafrost degradation in boreal-forest regions.

CONCLUSIONS

Our investigations across permafrost gradients show an apparent habitat difference between the two species in the sympatric regions in the Interior Alaska: *R. groenlandicum* is abundant under darker, drier conditions with a thicker active layer whereas *R. tomentosum* is common under brighter, wetter conditions with a thinner active layer. This suggests that habitat segregation via inhomogeneous permafrost environments occurs in the discontinuous permafrost zone. Moreover, *R. tomentosum* dominating permafrost conditions has more conservative leaves compared to *R. groenlandicum*, and both species have more conservative leaves under permafrost conditions. These intraspecific and interspecific variations suggest that the conservative leaves can contribute to their adaptation under permafrost conditions. Considering their wide distribution ranges, large leaf-trait variations, and strong

interactions with the neighboring plants, the ecological characteristics of Labrador teas could aid in understanding the evolutionary and ecological responses of shrub species and the boreal ecosystem dynamics under climate change.

Declarations

Competing Interests

None declared.

Author Contributions

GA framed the research questions and conducted all fieldwork and analyses; GA, GI, KN, YM, and HK selected and established the study sites; YK, BYL, and HK supported the chemical analyses; GA took the lead in writing the manuscript with contributions from all other authors.

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Data Availability Statement

The data is available in Supporting Information.

Online Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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Figures

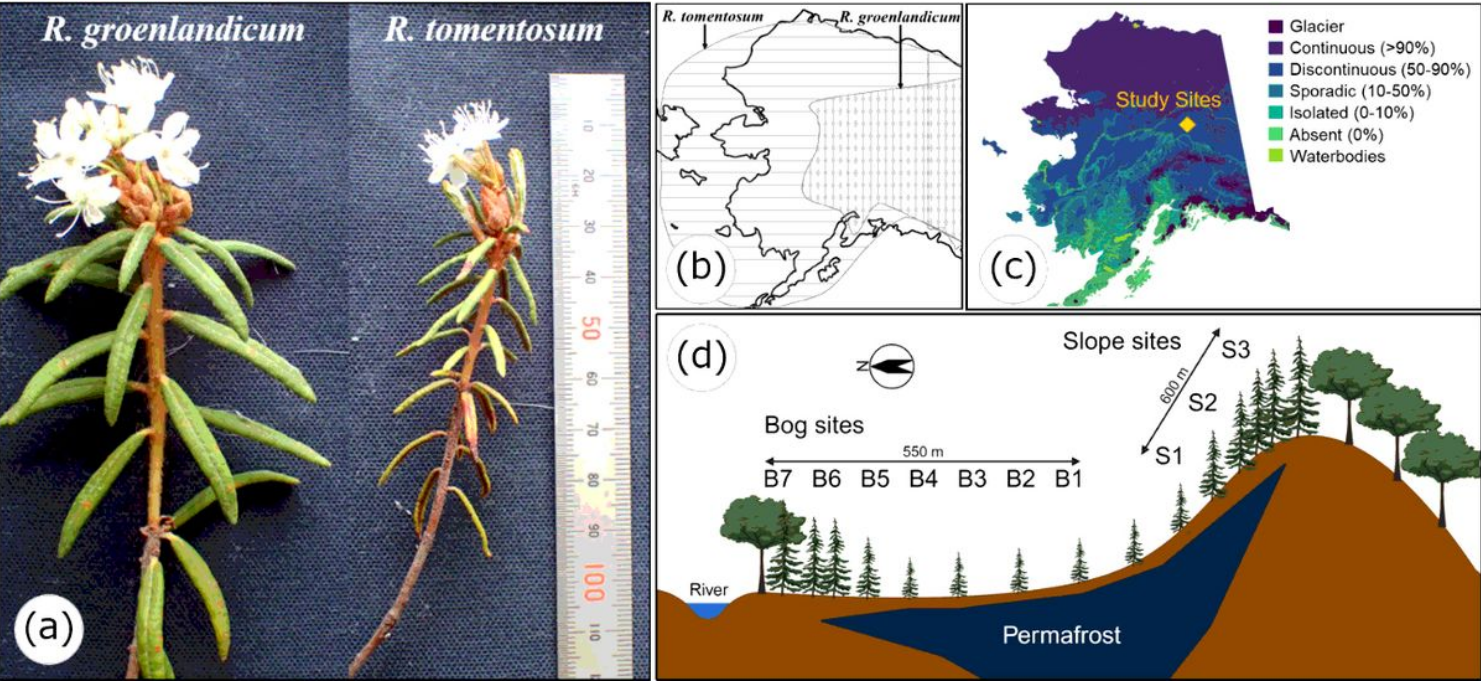


Figure 1

Typical shoots of *Rhododendron groenlandicum* and *R. tomentosum*(a), their distributions in Alaska (b; based on Hultén 1968), permafrost distribution in Alaska (c; based on Jorgenson *et al.* 2008), and a conceptual image of site locations across permafrost gradients (d; based on Viereck *et al.* 1983; created with BioRender.com). In c, a rhombus symbol indicates the location of study sites.

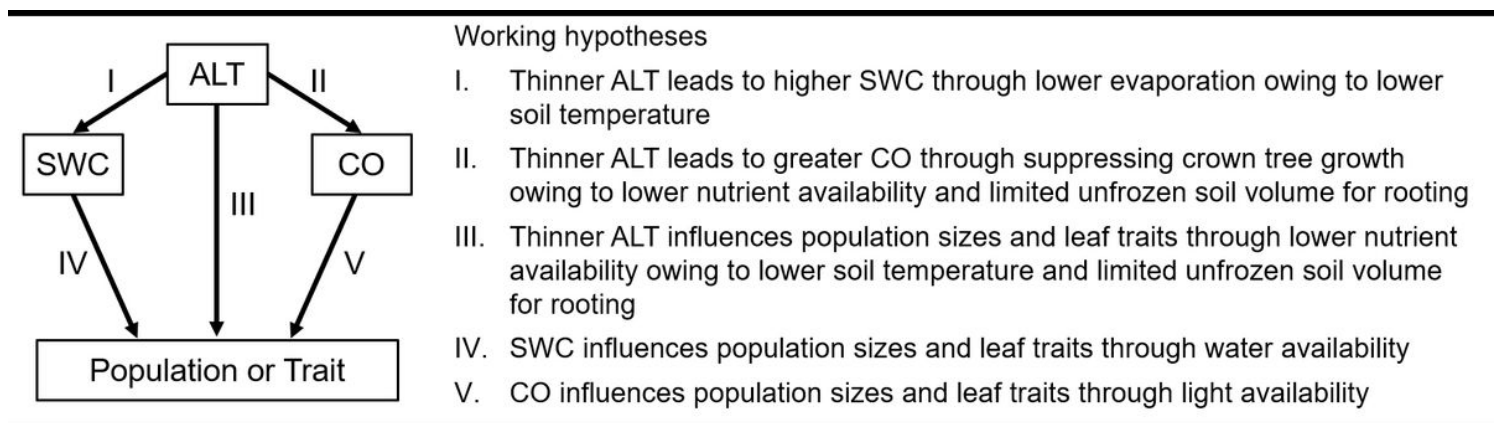


Figure 2

Conceptual full model of hypothesized associations between each variable, and working hypotheses. ALT: active layer thickness; SWC: volumetric soil water content; and CO: canopy openness.

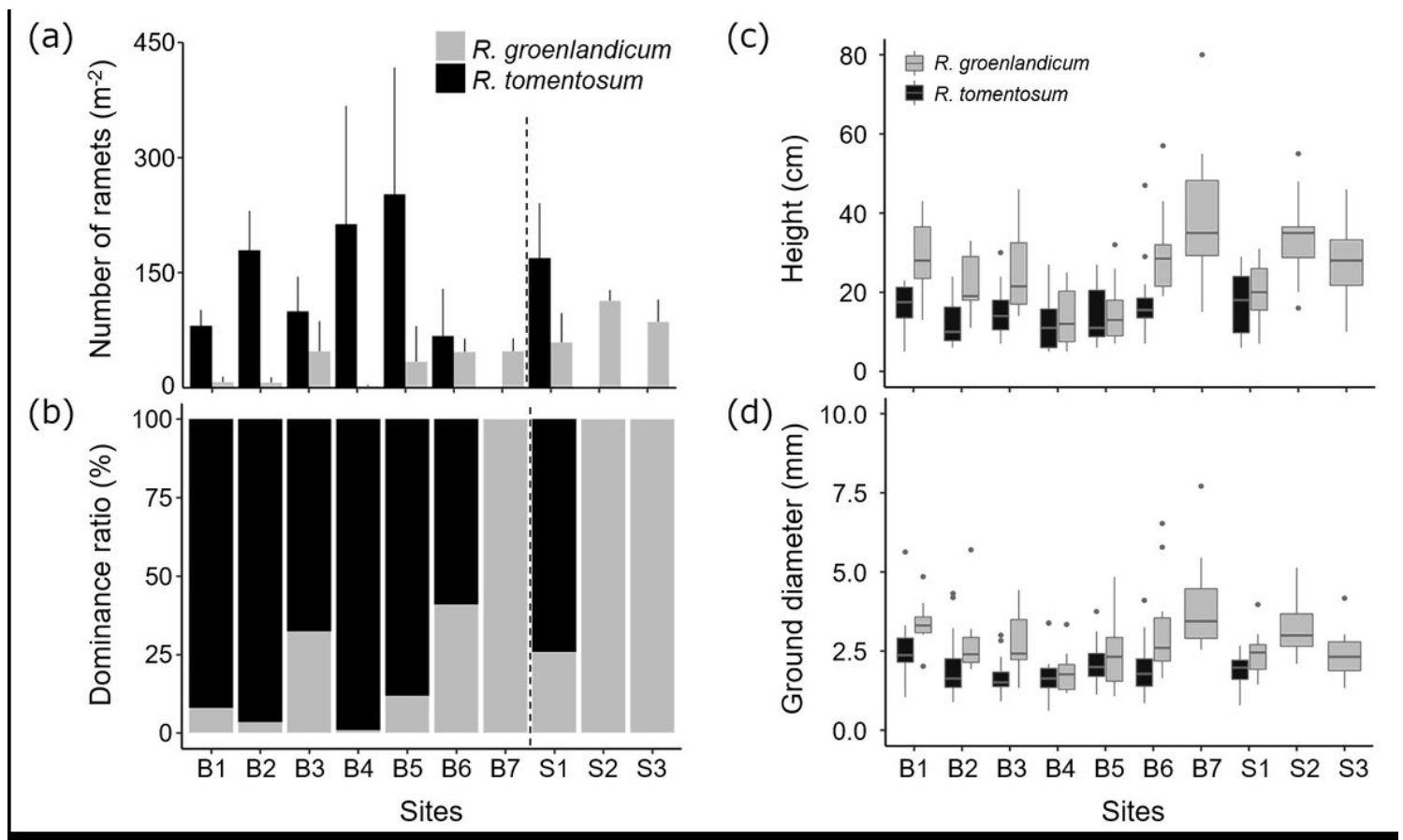


Figure 3

The numbers, dominance ratio, heights, and ground diameters of ramets in *R. groenlandicum* and *R. tomentosum* in each study site. In a, the error bars indicate standard deviation. The numbers, heights, and ground diameters of ramets significantly varied among the populations ($P < 0.001$).

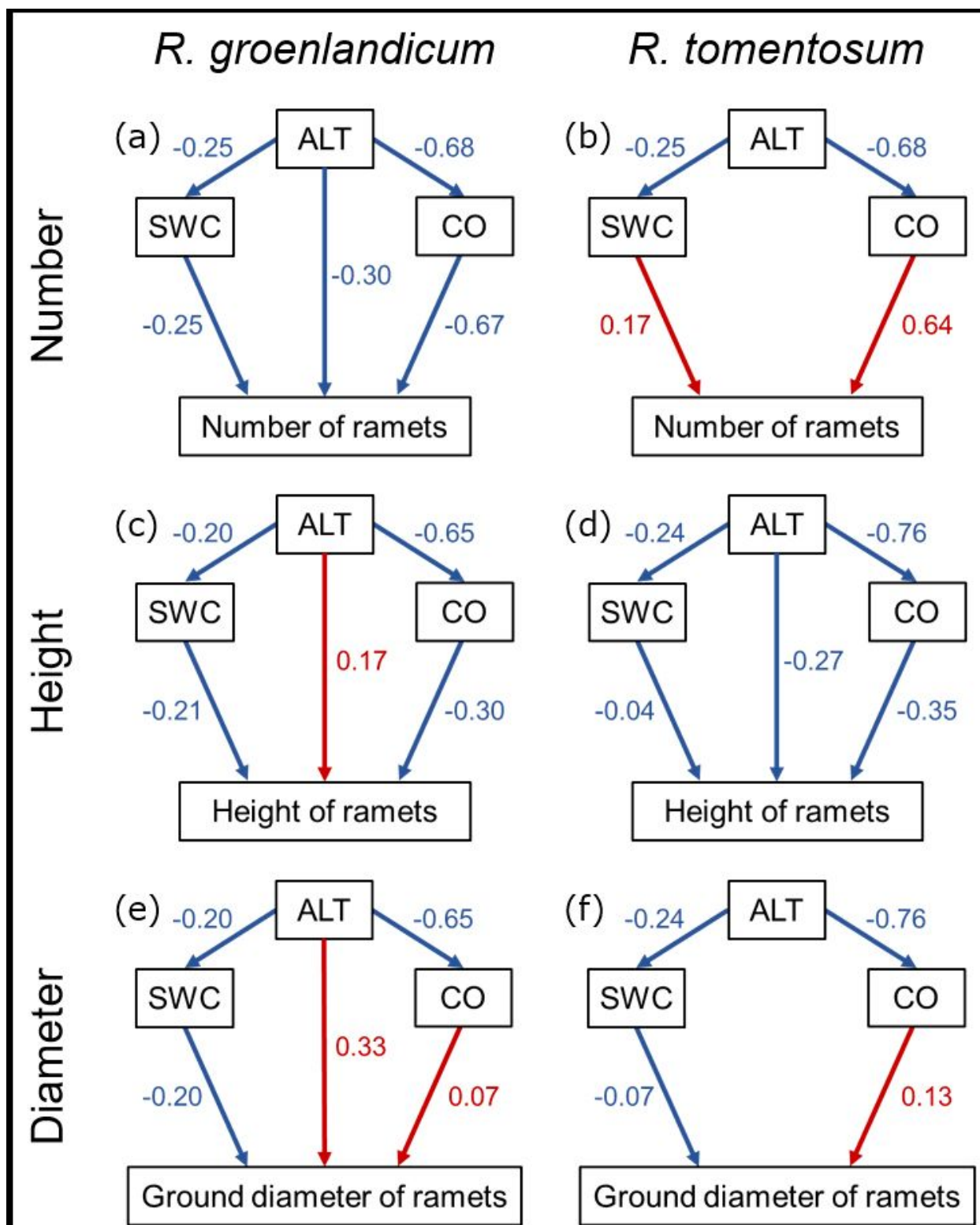


Figure 4

Associations of environmental variables with the numbers, heights, or ground diameters of ramets in each species. These are the best-fit models selected based on AIC (grey line in Table S1). The subsequently selected models with $\Delta AIC < 2.00$ are also shown in Table S1 and Figure S2. Red and blue arrows indicate positive and negative associations, respectively, and the numbers close to the arrows indicate standardized coefficients of each association. Note: the coefficients between ALT and SWC or CO

were slightly different among ab, ce, and df. This is because either species was not found in some quadrats and the quadrats used for the analyses were different among them.

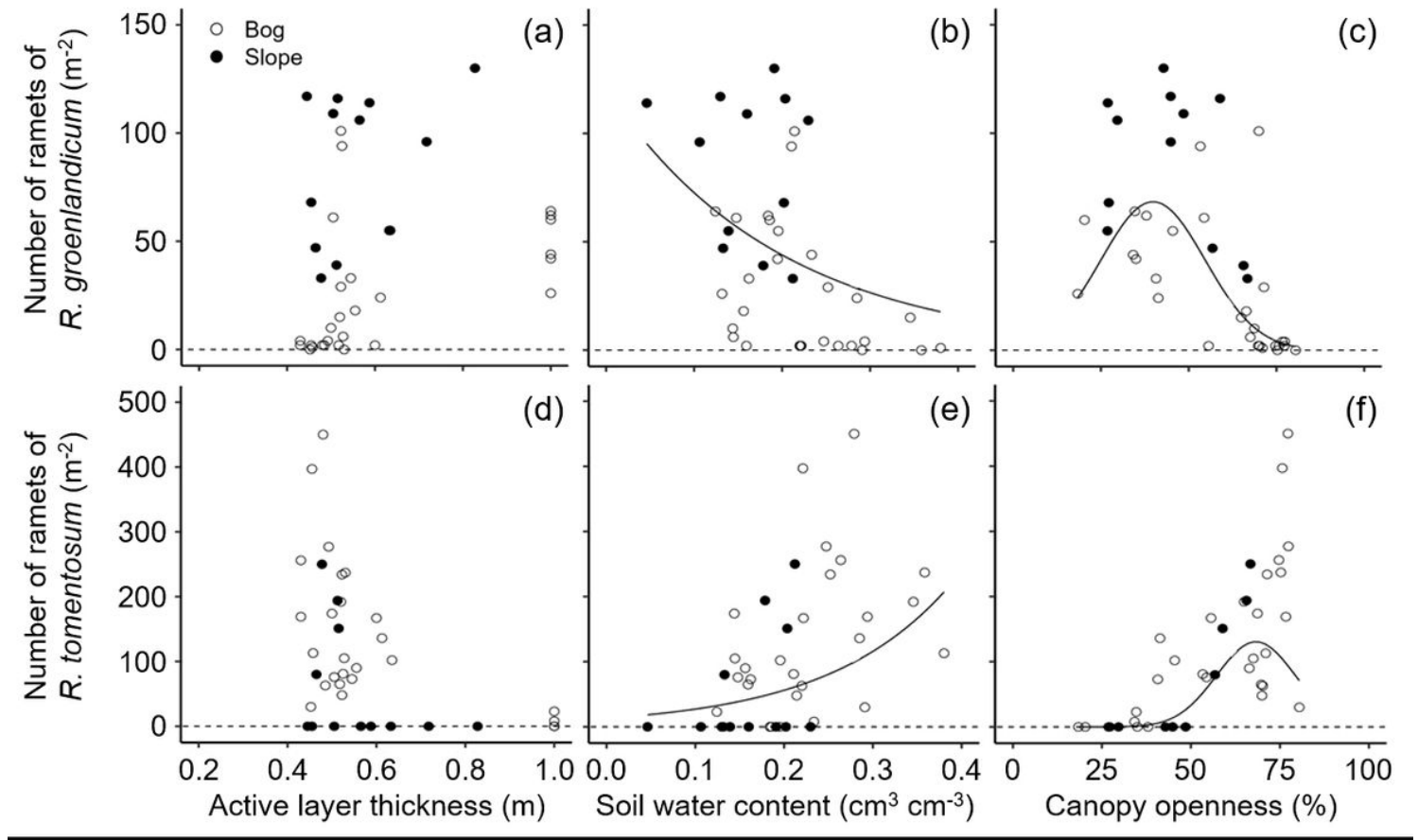


Figure 5

Associations of environmental variables with the numbers of ramets in each species based on GLMM analyses. These are the best-fit model selected based on AIC (grey line in Table S2). The subsequently selected models with $\Delta AIC < 2.0$ are also shown in Table S2. Open and filled circles indicate the data from the Bog or Slope sites, respectively.

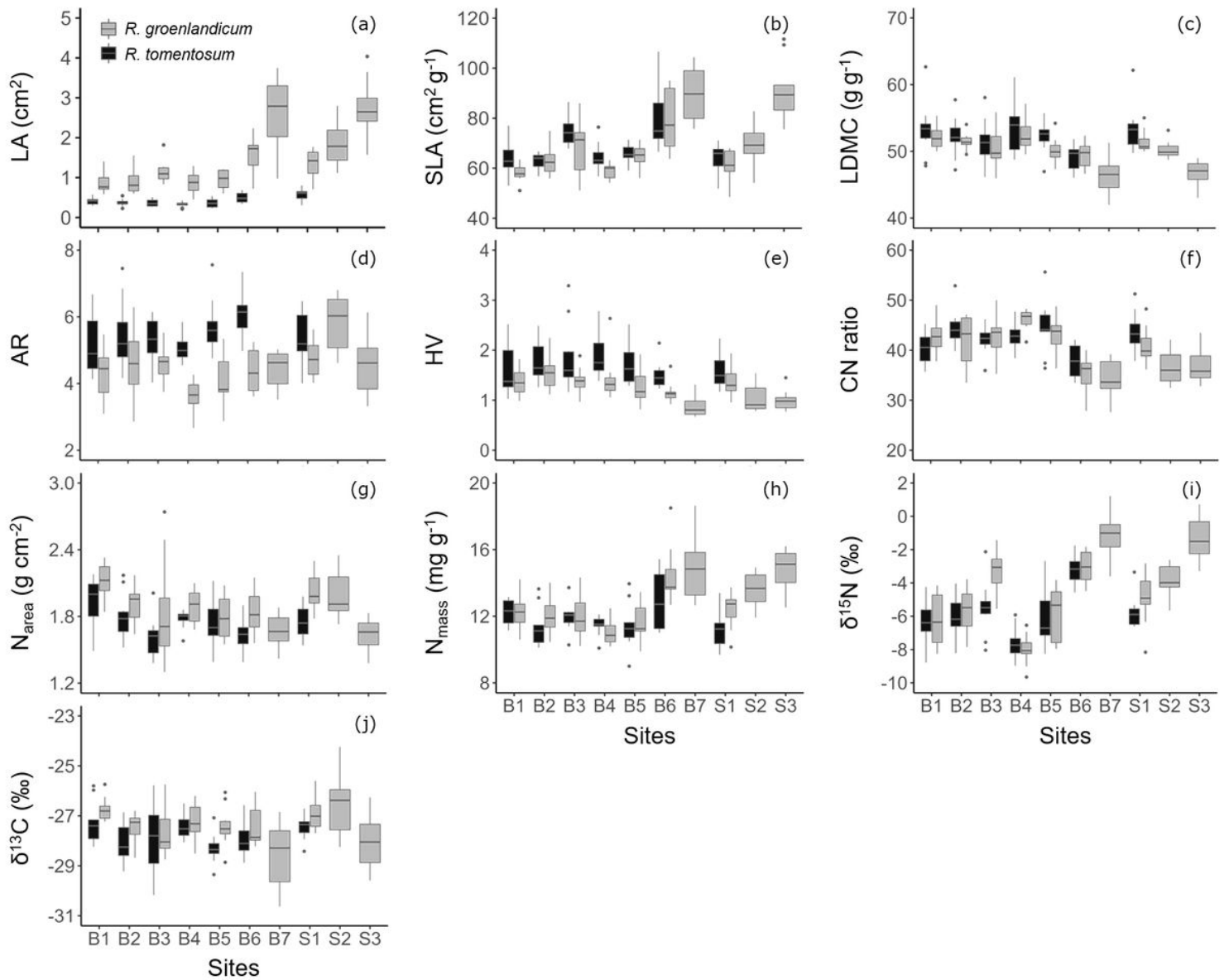


Figure 6

Leaf traits of *R. groenlandicum* and *R. tomentosum* across the permafrost gradients. Each trait significantly varied among the populations ($P < 0.001$). LA: leaf area; SLA: specific leaf area; LDMC: leaf dry matter content; AR: aspect ratio; HV: Huber value (sapwood-to-leaf area ratio); N_{area}: nitrogen content per unit leaf area; N_{mass}: nitrogen concentration per unit leaf dry mass; δ¹³C: carbon stable isotope ratio; δ¹⁵N: nitrogen stable isotope ratio.

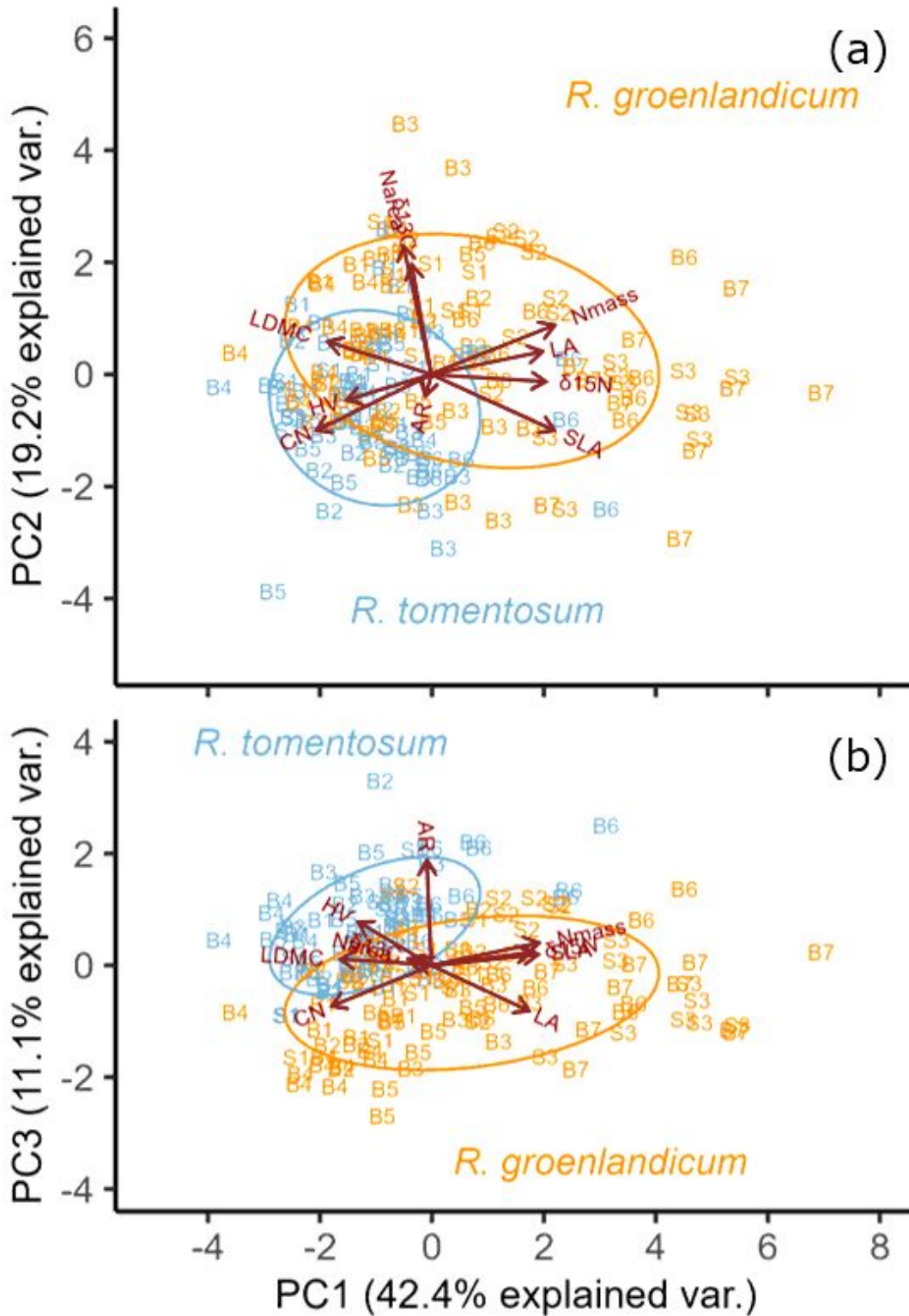


Figure 7

Relationships among leaf traits and PC scores. Orange and blue symbols indicate the PC scores of each measured ramet of *R. groenlandicum* and *R. tomentosum* in each site, respectively. Red arrows indicate the relationships among each leaf trait and PC axes. The PC scores in each site were shown in Figure S3. LA: leaf area; SLA: specific leaf area; LDMC: leaf dry matter content; AR: aspect ratio; HV: Huber value

(sapwood-to-leaf area ratio); CN: C/N ratio; N_{area} : nitrogen content per unit leaf area; N_{mass} : nitrogen concentration per unit leaf dry mass.

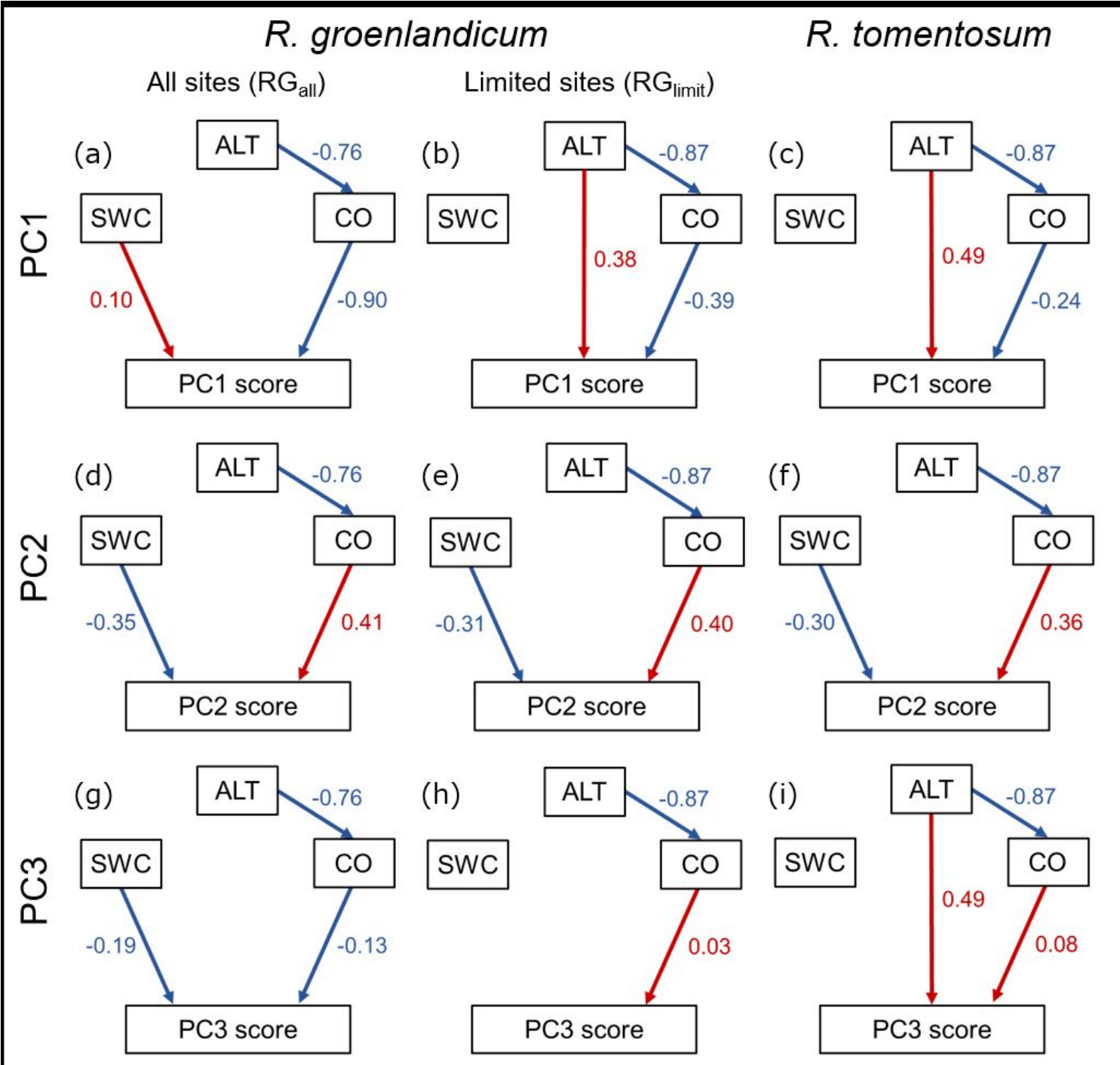


Figure 8

Associations of environmental variables with the PC scores of leaf traits in each species. These are the best-fit model selected based on AIC (grey line in Table S3). The subsequently selected models with $\Delta AIC < 2.0$ are also shown in Table S3 and Figure S4-6. RG_{limit} indicate the results in *R. groenlandicum* when the same sites were used as *R. tomentosum* (i.e., B1-6 and S1; Figure 3). Red and blue arrows indicate

positive and negative associations, respectively, and the numbers close to the arrows indicate standardized coefficients of each association.

Supplementary Files

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