Differences in photosynthetic and water use characteristics of four co-existing tree species on the treeline of the Japanese Alps

Mai Kamakura (m.kamakura1192@gmail.com)
Kyoto University Graduate School of Agriculture Faculty of Agriculture: Kyoto Daigaku Nogaku Kenkyuka Nogakubu https://orcid.org/0000-0001-7215-4322

Wakana A Azuma
Kobe University Faculty of Agriculture Graduate School of Agricultural Science: Kobe Daigaku Daigakuin Nogaku Kenkyuka Nogakubu

Hikari Yahara
Shinshu University Graduate School of Science and Technology: Shinshu Daigaku Daigakuin Sogo Rikogaku Kenkyuka

Koichi Takahashi
Shinshu University Graduate School of Science and Technology: Shinshu Daigaku Daigakuin Sogo Rikogaku Kenkyuka

Naoki Makita
Shinshu University Graduate School of Science and Technology: Shinshu Daigaku Daigakuin Sogo Rikogaku Kenkyuka

Research Article

Keywords: alpine tree, hydraulic capacitance, hydraulic conductance, osmotic adjustment, treeline, water use efficiency

Posted Date: April 19th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2811034/v1

Abstract

In this study, we analyzed photosynthetic and water use characteristics of two deciduous broad-leaved (*Sorbus matsumurana* and *Betula ermanii*) and two evergreen coniferous (*Abies mariesii*and *Pinus pumila*) tree species on the treeline ¾i.e., the line connecting the highest patches of forest composed of trees at least three meters high¾of Mt. Norikura, central Japan, to explore the physiological adaptations of trees to alpine environments. We monitored diurnal changes in leaf gas exchange rates and leaf and soil water potentials in each tree species during the growing season, as well as bulk leaf water relations based on pressure–volume curves and whole-tree hydraulic structures. The four species under study relied on different physiological characteristics at both leaf and plant levels for adaptation to their natural habitat. In *S. matsumurana*, photoassimilate acquisition occured during the short growing season, and reducing transpirational water loss was associated with osmotic adjustment. In contrast, *B. ermanii* showed higher photosynthetic rates, while maintaining higher hydraulic conductance and transpirational water loss in the absence of severe stomatal restrictions. In turn, *A. mariesii* showed higher leaf succulence, and a leaf water storage capacity that was favorable for growth, while reducing water loss. Meanwhile, *Pinus pumila* compensated for water loss through higher leaf hydraulic capacitance and cell-wall elasticity, and exhibitedthe longest daily transpiration period, while maintaining high stomatal conductance, even as soil water potential decreased. Our observations demonstrates that tree species rely on specific physiological characteristics to adapt to the alpine treeline environment.

Introduction

With increasing mountain elevation, environmental conditions, such as cold, drought, low nutrient availability, water logging, and stormy winds, determine the upper limit of tree growth and distribution (Wieser and Tausz 2007). The transition zone between two distinct vegetation communities from forest to barren, treeless alpine areas is known as the treeline ecotone (Körner and Paulsen 2004), where the treeline connects the highest patches of forest composed of trees at least three meters in height and represents one of the most obvious vegetation boundaries (Körner 2012). In East Asia, climate change is predicted to cause severe long-term effects on forest ecosystems, thereby shifting vegetation distribution towards higher latitudes and elevations, and changeig species composition with increasing annual mean temperatures (Tsunekawa et al. 1996). Treeline obsrvations are therefore critical to improve ourunderstanding of biodiversity distribution and to assess the vulnerability of alpine forest ecosystems to environmental stress factors.

Previous studies along elevation gradients have focused interspecific and intraspecific differences in adaptations of trees by comparing leaf morphological and physiological characteristics. Thus, for example, lower photosynthetic rates and leaf nitrogen content (N_{mass}), a proxy for assimilation capacity, and higher leaf mass per unit area (LMA) with increasing elevation (Hikosaka et al. 2002; Zhang et al. 2005), have suggested a slow return on nutrient investment and leaf dry mass (Wright et al. 2004). Conversely, high-elevation plants have been reported to exhibit higher photosynthetic rates and leaf nitrogen content than those of low-elevation plants (e.g., *Metrosideros polymorpha* in Cordell et al. 1999;

herbaceous plants of the Austrian Alps in Körner and Diemer 1987). These results suggest that optimal strategies in alpine environments may differ among species and plant functional types. Therefore, the question arises as to whether tree species coexisting in the same alpine region developed similar physiological adaptation strategies. Particularly, tree species growing on the alpine treeline have demonstrated the ability to survive under environmental stress conditions and to influence carbon and water fluxes in ecosystems (Körner 2021). Therefore, understanding how trees grow and adapt to their habitat to successfully acquire the resources they need (i.e., light, water, and nutrients) on the alpine treeline will help us distinguish among tree species distribution and growth patterns through the analysis of their resource acquisition and utilization characteristics.

In general, tree growth at the alpine tree line in temperate regions is controlled by temperature (Körner and Paulsen 2004), whereas, at latitude lower than those of temperate regions, it can be controlled by severe water deficit or strong moisture seasonality (Morales et al. 2004). For instance, stomatal closure and reduced minimum daily water potential during the dry season in mature *Pinus canariensis* trees growing on the alpine treeline in subtropical regions, suggested potential seasonal effects from edaphic water deficit and possibly climatic drought (Gieger and Leuschner 2004). However, *P. canariensis* growth was not reduced, implying a physiological adaptation at play to increase water use efficiency (WUE). On the other hands, productivity or resource use efficiency of deciduous *Fagus crenata* on the alpine treeline in temperate regions was greater at higher elevations than that of evergreen *Abies mariesii* conifers (Hikosaka et al. 2021). In terms of annual productivity of the alpine treeline during the short growing season, optimal strategies to increase carbon gain while reducing water loss are vital for deciduous trees. Additionally, other differences in water use between deciduous and evergreen tree species coexisting in the alpine treeline, have been reported. For example, the deciduous conifer *Larix decidua* reportedly accumulates solutes within cells, decreasing the osmotic component of water potential, thereby allowing the maintenance of higher stomatal conductance during daytime, whereas *Pinus cembra*, an evergreen conifer, showed a water-saving strategy based on immediate stomatal closure upon water deficit initiation (Anfodillo et al. 1998; Badalotti et al. 2000). Osmotic adjustment can maintain leaf turgor under water deficit, which is important for seedling establishment at higher elevations, in addition to promoting soil water uptake and transport to the leaves. However, whether water relations differ between deciduous and evergreen trees coexisting in the alpine treeline remains poorly understood.

In this study, we evaluated photosynthetic and water use characteristics of four co-existing tree species on the alpine treeline in a temperate region during the growing season, in central Japan. We studied two deciduous broad-leaved (*Sorbus matsumurana* and *Betula ermanii*) and two evergreen coniferous (*Abies mariesii* and *Pinus pumila*) tree species. These four tree species not only co-exist in the alpine treeline of Mt. Norikura (Miyajima et al. 2007), but are generally dominant in the alpine treelines of Japan (Gansert et al. 2004). First, we compared bulk leaf water relations among tree species based on the corresponding pressure–volume (*P*– *V*) curves. We hypothesized that, if leaf cells regulate osmotic pressure, tissue elasticity, and/or capacitive discharge of stored water, they will maintain positive turgor pressures and higher water contents, as proposed by Bartlett et al. (2012) and Scholz et al. (2011). Subsequently, we

measured diurnal changes in leaf gas exchange rates and leaf water potentials (Ψ_{leaf}) to investigate differences in leaf water use among species. The carbon gain ratio of photosynthesis to water consumption by evapotranspiration was calculated from leaf gas exchange data and leaf water stress was evaluated from Ψ_{leaf}, and the values subsequently compared to determine differences in water use characteristics among the species under study. Water potential gradients within trees known as the “soil–plant–atmosphere continuum (SPAC)”, occur predominantly during daytime in the summer (Mayr et al. 2003; Zimmermann 1978). Therefore, we analysed whether an increasing water potential gradient that may be affected by leaf osmotic adjustment, or an increased soil-to-leaf hydraulic conductivity contributed to maintaining the SPAC and consequently, leaf transpiration rate. With respect to this, we hypothesized that deciduous trees exhibit osmotic adjustment, whereas evergreen trees exhibit water-saving strategies in response to moderate water deficits during the growing season.

Materials And Methods

Study site and tree selection

This study was conducted at the treeline (2,500 m asl) on the eastern slope of Mount Norikura (36°06' N, 137°33' E, 3026 m asl), in Nagano Prefecture, Japan (Fig. 1a, b). At the Nagawa weather station (1,068 m asl, Japan Meteorological Agency), which is closest to the study site, mean air temperature and total annual precipitation from 2009 to 2019 were 8.4°C and 1961 mm, respectively. Two deciduous broad-leaved tree species, namely, *Sorbus matsumurana* (Makino) Koehne. (Fig. 1c) and *Betula ermanii* Cham. (Fig. 1d), and two evergreen conifers, namely, *Abies mariesii* Mast. (Fig. 1e) and *Pinus pumila* (Pall.) Regel. (Fig. 1f) are dominant tree species in this area. The elevation at the study site is the upper limit for *B. ermanii* and *A. mariesii* and the lower limit for *P. pumila*.

We established a 20 × 20 m^2 plot and selected three individual trees from each species. Average values of vertical height, stem length, and stem diameter were recorded at a height of 50 cm for each selected trees as shown in Table 1. *S. matsumurana* and *P. pumila* are small shrubs that grow by creeping on the ground, whereas *B. ermanii* and *A. mariesii* are timber species that can grow much taller (Takahashi 2003). Based on daily observations conducted by using a time-lapse camera, we determined that leaf flush occurred in late June for deciduous species and between late June and mid-July for evergreen species, after the snow had melted in mid-June. Leaves of the two deciduous species changed color in late September and fell in mid-October, indicating a growing season of approximately three months in a year. Some leaves of the two evergreen conifers changed color and fell in mid-September while the remaining leaves overwintered. Both P-V curve measurements and the field observations described below were conducted for the same individuals during the growing season. In addition, there were no significant differences in environmental conditions during the growing season between 2017, when P-V curves were measured, and 2018 and 2019, when field measurements were recorded: Precipitation during the growing season (from June to October) in 2017, 2018, and 2019 were 204, 243, 240 mm, respectively; meanwhile, mean temperatures were 16, 17, and 18°C, respectively (Japan Meteorological Agency)..

Table 1
Mean values (± SE) of vertical height (cm), stem length (cm), and stem diameter at 50 cm height (cm) for *Sorbus matsumurana*, *Betula ermanii*, *Abies mariesii*, and *Pinus pumila*.

Tree species	Vertical height (cm)	Stem length (cm)	Stem diameter at 50 cm height (cm)
Sorbus matsumurana	214 ± 10	323 ± 28	5.2 ± 0.5
Betula ermanii	407 ± 54	435 ± 32	9.4 ± 0.8
Abies mariesii	419 ± 51	419 ± 51	22.5 ± 3.5
Pinus pumila	142 ± 25	240 ± 28	7.6 ± 1.9

Leaf water relations based on P–V curves

To compare the bulk leaf water relations among the selected tree species, we collected small branches with attached foliage (30–50 cm length) from three individuals of each species, which were immediately re-cut under water on 29 July 2017. In each tree, we selected only unshaded branches that received sufficient light were selected. The leaves on these branches were covered with plastic bags in the laboratory overnight. After full rehydration, we measured fresh weight at saturation (FW_{leaf}, g), and obtained four to six *P*–*V* curves for each tree species using the bench-drying approach (Schulte and Hinckley 1985; Tyree and Hammel 1972). For evergreen species *A. mariesii* and *P. pumila*, shoots comprising second- and current-year internodes were used. We repeatedly measured Ψ_{leaf} using a pressure chamber (Model 600, PMS Inc., Corvallis, OR, USA) and the fresh weight of the sample leaves or shoots prior to water potential measurements, based on the methods of Hinckley et al. (1980). Following each *P*–*V* curve, total leaf surface area (*LA*, m^2) of the sample leaves and shoots was measured, them, leaves were oven-dried to measure dry weight (DW_{leaf}, g), and relative water content (RWC) was calculated. Subsequently, we calculated osmotic potential at saturation (Ψ_{sat}, MPa), osmotic potential at turgor loss (Ψ_{tlp}, MPa), relative water content at turgor loss (RWC_{tlp}, MPa), cell wall elasticity modulus (ε, MPa), leaf succulence (S_{leaf}, g H_2O m^{-2}) and leaf hydraulic capacitance (C_{leaf}, mol m^{-2} MPa^{-1}) from the *P*–*V* curves constructed (Schulte and Hinckley 1985).

Osmotic potential at turgor loss (Ψ_{tlp}) is an indicator often used to compare the ability to maintain leaf turgor within and among species, both spatially and temporally (Bartlett et al. 2012; Dawson 1990). In turn, RWC_{tlp} represents the amount of water leaf cells lose before losing turgor. Meanwhile, Ψ_{sat} can be used as an indicator of the extent of osmotic adjustment achieved by increasing cellular solute concentration to prevent turgor loss. Further, the positive component of water potential caused by turgor pressure against the cell walls is known as pressure potential (Ψ_p, MPa), and is expressed as the value of water potential before the leaf wilting point and calculated as follows: $\Psi_p = (\Psi_{leaf} - \Psi_{sat})$. Cell wall elasticity ($\varepsilon$, MPa) was calculated after Schulte and Hinckley (1985), using the following formula: $\varepsilon = \Delta\Psi_p / \Delta RWC$. Additionaly, S_{leaf} represents the maximum amount of stored water relative to leaf

transpiration area (Azuma et al. 2016; Bacelar et al. 2004), and can be calculated as follows: S_{leaf} = (FW_{leaf} − DW_{leaf})/LA. Lastly, C_{leaf} is the amount of water released per change in Ψ_{leaf} per LA; thus, it corresponds to the product of S_{leaf} and ε (Azuma et al. 2019; Scholz et al. 2011), and can be calculated as follows: C_{leaf} = ΔRWC/$\Delta\Psi_{leaf}$ (DW_{leaf}/LA) ((FW_{leaf}-DW_{leaf})/DW_{leaf}) /M, where M is the molecular weight of water.

Field observation of leaf gas exchange and leaf and soil water potential

To compare the characteristics of daytime leaf gas exchange among species, we measured diurnal changes in net assimilation rate (A, μmol m^{-2} s^{-1}) and stomatal conductance for water vapor (g_s, mol m^{-2} s^{-1}), transpiration rate (E, mmol m^{-2} s^{-1}), and dark respiration rate (R_d, μmol m^{-2} s^{-1}), all under ambient environmental conditions, using an LI-6400 (Li-Cor Inc., Lincoln, NE, USA) gas-exchange measurement system fitted with a 6 cm^2 clear-top leaf chamber for broad leaves, or a 10 cm^2 clear-top leaf chamber for coniferous leaves. One intact leaf from three individuals of each tree species were used for these measurements. For each individual, only unshaded shoots receiving sufficient light were selected. Each leaf selected for measurement was clamped such as hold it fixed it in its natural position and direction. Incident photosynthetic photon flux density (PPFD) beside the leaf chamber was measured using a photon sensor (LI-190SA, Li-Cor Inc.). In addition, air CO_2 concentration, temperature, and relative humidity in the chamber were recorded and adjusted to ambient levels. Leaf-to-air vapor pressure deficit (VPD, kPa) was calculated from air temperature and relative humidity in the chamber. Air entered the chamber at a flow rate of 500 μmol s^{-1}. For the two coniferous species, leaf gas exchange rate was recalculated by calculating the leaf area receiving light after the gas exchange measurement was completed. In turn, R_d was measured in the dark, following the measurement of diurnal variation of leaf gas exchange. Using pressure chamber, bulk leaf water potential (Ψ_{leaf}, MPa) was measured simultaneously with leaf gas exchange in leaves that were in the same position (adjacent to the branch) as that used for leaf gas exchange measurement. Leaf gas exchange and Ψ_{leaf} measurements were conducted at 1.5–2.0-h intervals during the daytime from30 to 31 July 2018, from 29 to 30 July 2019, and from 12 to 13 September 2019. Soil water potential (Ψ_{soil}, MPa) at a depth of 10 cm was recorded 20-min intervals during the measurement period using tensiometer (DIK-3210, Daiki Rika Kogyo Co. Ltd., Saitama, Japan).

Maximum net assimilation rates (A_{max}) and maximum stomatal conductance (g_{smax}) of each tree species were calculated from leaf gas exchange data under light saturation conditions. Instantaneous water use efficiency (WUE, i.e., the A to E ratio) and intrinsic water use efficiency (WUE_i, i.e., the A to g_s ratio) for each tree species were calculated from all leaf gas exchange data. Minimum Ψ_{leaf} (Ψ_{leaf_min}) for each tree species was calculated using leaf water potential minimum values on each measurement day.

Water relations in SPAC

To compare whole-tree water relations among tree species, we calculated hydraulic conductance from the soil to the leaves (K, mmol m^{-2} s^{-1} MPa^{-1}) of three individuals of each species using field observation data. The following equation was used: $K = E/\Delta\Psi_{soil-leaf}$, where E is the transpiration rate per unit leaf area (mmol m^{-2} s^{-1}) and $\Delta\Psi_{soil-leaf}$ (water potential difference from soil to leaf, MPa) is the component of water potential driving sap flow. Calculations for E and $\Delta\Psi_{soil-leaf}$ were performed using leaf gas exchange and leaf and soil water potential data for the entire field observation period, respectively.

Statistical analysis

To examine interspecific differences in leaf gas exchange characteristics and leaf water relations among four tree species, we performed one-way analysis of variance (ANOVA) and Tukey's HSD tests using JMP14 software (SAS Institute, Cary, NC, USA).

Results

Leaf water relations on the P–V curve

Comparisons of bulk leaf water relations among the tree species revealed that Ψ_{tlp} was lowest in *S. matsumurana*, followed by *P. pumila*, *A. mariesii*, and *B. ermanii*, whereas RWC_{tlp} was significantly lower in *P. pumila* than that in any other species (Table 2). Concomitantly, the osmoregulatory ability of the cells (Ψ_{sat}) was lowest in *S. matsumurana*, followed by *A. mariesii*, *B. ermanii*, and *P. pumila*. In contrast, ε (degree of cell wall elasticity) exhibited the opposite order (Table 2; Fig. 2). In turn, S_{leaf} was highest in *A. mariesii*, followed by *P. pumila*, *B. ermanii*, and *S. matsumurana*. Lastly, C_{leaf} (integrated result of S_{leaf} and ε) was highest in *P. pumila*, followed by *A. mariesii*, *B. ermanii*, and *S. matsumurana* (Table 2).

Table 2

Bulk leaf parameters of water relations (mean ± SE, n = 4–6) of each tree species calculated from pressure-volume (*P*– *V*) curves. Osmotic potential at turgor loss (Ψ_{tlp}, MPa); osmotic potential at saturation (Ψ_{sat}, MPa); relative water content at turgor loss point (RWC_{tlp}, MPa); cell wall elasticity (ε, MPa); leaf succulence (S_{leaf}, g H_2O m^{-2}); and leaf hydraulic capacitance (C_{leaf}, mol m^{-2} MPa^{-1}). Different lowercase letters within columns indicate significant differences among tree species as per Tukey's HSD test ($p < 0.05$)

Tree species	Ψ_{tlp} (MPa)	Ψ_{sat} (MPa)	ε (MPa)	RWC_{tlp} (MPa)	$S_{leaf,s}$ (g H_2O m^{-2})	C_{leaf} (mol m^{-2} MPa^{-1})
Sorbus matsumurana	−2.15 ± 0.08 b	−1.53 ± 0.11 b	11.70 ± 1.62 a	0.86 ± 0.01 a	137 ± 5 c	0.47 ± 0.04 c
Betula ermanii	−1.59 ± 0.04 a	−1.16 ± 0.04 ab	5.88 ± 0.44 bc	0.76 ± 0.02 a	157 ± 3 c	1.10 ± 0.09 bc
Abies mariesii	−1.70 ± 0.09 ab	−1.33 ± 0.08 ab	7.86 ± 0.59 b	0.81 ± 0.01 a	319 ± 19 a	1.80 ± 0.15 b
Pinus pumila	−1.96 ± 0.29 ab	−1.03 ± 0.20 a	2.56 ± 0.44 c	0.57 ± 0.05 b	217 ± 12 b	3.10 ± 0.45 a

Leaf gas exchange rates and Ψ_{leaf}

Measurements of leaf gas exchange rates and Ψ_{leaf} revealed the diurnal patterns for each tree species (Fig. S1), and the interspecific differences for each parameter measured (Table 3). The values of A_{max} and g_{smax} were significantly higher in *B. ermanii* than those in the other species. Conversely, WUE and WUE_i were significantly higher in *A.mariesii* and lower in *B. ermanii* than in the other species. Between deciduous broad-leaved trees, *S. matsumurana* showed significantly higher values for WUE and WUE_i than those of *B. ermanii*, and between evergreen conifers, *A. mariesii* showed higher values for WUE and WUE_i than those of *P. pumila*.

Table 3
Maximum net assimilation rates (A_{max}), maximum stomatal conductance (g_{smax}), dark respiration rate (R_d), instantaneous water use efficiency (WUE = A/E) and intrinsic water use efficiency ($WUE_i = A/g_s$), and minimum water potential (Ψ_{leaf_min}) in leaves of each tree species (means ± SE). A_{max} and g_{smax} were calculated from leaf gas exchange data under light saturation (n = 15). R_d was obtained from leaf gas exchange data under darkness (n = 18). WUE and WUE_i were calculated from all leaf gas exchange data (n = 99). Different lowercase letters within columns indicate significant differences among tree species as per Tukey's HSD test ($p < 0.05$)

Tree species	A_{max} ($\mu mol\ m^{-2}\ s^{-1}$)	g_{smax} ($mol\ m^{-2}\ s^{-1}$)	R_d ($\mu mol\ m^{-2}\ s^{-1}$)	WUE (A/E) ($\mu mol\ mmol^{-1}$)	WUE_i (A/g_s) ($\mu mol\ mol^{-1}$)	Ψ_{leaf_min} (MPa)
Sorbus matsumurana	8.97 ± 0.19 c	0.174 ± 0.008 c	-0.86 ± 0.04 a	3.18 ± 0.09 b	44.76 ± 1.98 b	-1.65 ± 0.05 b
Betula ermanii	12.42 ± 0.52 a	0.343 ± 0.007 a	-1.24 ± 0.04 c	2.60 ± 0.10 c	29.66 ± 1.58 c	-1.20 ± 0.05 a
Abies mariesii	10.58 ± 0.21 bc	0.183 ± 0.005 c	-1.10 ± 0.03 b	4.30 ± 0.18 a	65.75 ± 2.54 a	-1.34 ± 0.07 a
Pinus pumila	10.01 ± 0.13 c	0.282 ± 0.009 b	-0.98 ± 0.02 b	3.10 ± 0.07 b	34.53 ± 1.49 c	-1.49 ± 0.06 ab

Water relations in SPAC

Figure 3 shows the relationship between K, $\Delta\Psi_{soil-leaf}$, and E along SPAC for each tree species, based on diurnal leaf gas exchange and Ψ_{leaf} and Ψ_{soil} data measured during the entire field observation period. In *S. matsumurana*, $\Delta\Psi_{soil-leaf}$ showed significantly variation during the daytime;however, K remained relatively low compared with the corresponding values for the other species. In contrast, diurnal variation in $\Delta\Psi_{soil-leaf}$ in *B. ermanii* was small but K varied significantly, thus, contributing to the maintenance of a high E. Meanwhile, for *A. mariesii* and *P. pumila*, diurnal variations in $\Delta\Psi$ and K did not show any characteristic trend, although E increased at a higher $\Delta\Psi_{soil-leaf}$ in *P. pumila* than it did in *A. mariesii*.

Discussion

In this study, we analyzed the physiological responses of four co-existing tree species to moderate water deficit during the growing season on the treeline of Mt. Norikura, Japan. We hypothesized that the two deciduous species under would adapt by controllig osmotic pressure, hence maintaining high turgor and evapotranspiration rates, whereas evergreen species rely on some water-saving strategy. Instead, and contrary to our prediction, our data indicated that the physiological responses of the four tree species did not depend on growth habit. Further, each tree species relied on species-specific physiological strategies to adapt to the alpine environment at both leaf and plant levels, despite growing in close proximity, in the same treeline environment.

The results of *P–V* curve analysis showed differences in leaf water relations among species. Maintaining positive leaf turgor and high RWC in the foliage may be achieved by managing intracellular osmotic concentration, tissue elasticity, and capacitive discharge of stored water (Bartlett et al. 2012; Scholz et al. 2011). Thus, for example, *S. matsumurana* registered the lowest Ψ_{tlp} values among all four species, which was subject to osmotic adjustment (low Ψ_{sat}). Furthermore, although *B. ermanii* registered the highest Ψ_{tlp} values, it did not exhibit any characteristic particular trait at the leaf level. Coniferous species showed higher water-storage capacity than that of broadleaf species, partly explained by the fact that terminal shoots were used for measurements, which contained more apoplastic water in the axial xylem. *A. mariesii* and *P. pumila* showed the highest S_{leaf} and C_{leaf} values, respectively. Overall, the leaves of *S. matsumurana* and *A. mariesii* showed a higher capacity for osmotic adjustment, whereas *B. ermanii* and *P. pumila* leaves showed a higher capacity to maintain turgor pressure in response to decreasing leaf RWC (Fig. 2). Thus, our findings showed that the differences in adaptive strategies involving water relations among species, reflect fundamental trade-offs in osmotic concentration, tissue elasticity, and capacitive discharge of stored water in the alpine environment of the study site. Osmotic adjustment is a form of biochemical acclimation that requires additional metabolic energy, whereas tissue elasticity is a morphological form of acclimation that can be achieved by adjusting resource partitioning. In addition, many processes involved in ATP supply and tissue formation are limited by low temperature on the alpine treeline (Körner 2021). Thus, it would be reasonable to consider this as the optimal strategy, as *B. ermanii* and *p. pumila* trees were found to be more resource-consumptive, whereas *S. matsumurana* and *A. mariesii* behaved in a more resource-conservative manner.

Field measurements of leaf gas exchange showed that, among the four species under study, *B. ermanii* and *p. pumila* characteristically exhibited high g_{smax} and low WUE and/or WUE_i values (Table 3). Consistently, LMA of *B. ermanii* trees growing at Mt. Norikura increased at higher elevations to increase mechanical stiffness and the resulting CO_2 deficit was compensated by an increase in g_s, which caused an increase in leaf N_{mass}; hence, a positive carbon balance was maintained(Takahashi and Otsubo 2017). Additionally, we found that alpine dwarf needle-species *P. pumila* sustained high *A* rates via higher g_s, a response similar to that observed in deciduous *B. ermanii* trees. Due to reduced stomatal restriction to transpirational water loss, dwarf shrubs growing at high elevations have shown little diurnal variation in g_s throughout most of the growing season (Körner and Mayr 1981). Therefore, trees of *B. ermanii* and *P. pumila* may have exhibited typical stomatal responses of plants growing at higher elevations. On the other hand, the deciduous dwarf species *S. matsumurana* and the evergreen coniferous *A. mariesii* showed the higher values for WUE and WUE_i among all four tree species (Table 2). Minimum leaf water potential (Ψ_{min}) during the study measurement period was consistently higher than osmotic potential at turgor loss (Ψ_{tlp}, Tables 2 and 3) with values near the critical level for normal physiological functioning, at > − 2 MPa (Anfodillo et al. 1998; Körner and Cochrane 1985; Lindsay 1971). However, *S. matsumurana* and *P. pumila* limited water consumption by regulating stomatal opening and maintained stable Ψ_{leaf} during the daytime, despite absence of severe water stress.

Based on the leaf physiological characteristics and soil water potential recorded during our field observations, we compared whole-tree water relations among the four tree species using a SPAC model (Fig. 3). The model showed that *S. matsumurana* attained a high $\Delta\Psi_{soil-leaf}$, whrease *B. ermanii* sustained a high *E* value via a high *K* value. These results were consistent with our observation that *S. matsumuran*a showed a particularly high capacity for leaf osmotic adjustment among the four tree species (the lowest Ψ_{sat} in Table 2) and *B. ermanii* showed a constant *A* rate concomitantly with the highest g_{smax} (Table 3 and Fig S1). Meanwhile, *A. mariesii* and *P. pumila* maintained both $\Delta\Psi_{soil-leaf}$ and *K* values unaltered. As reported by Ishida et al. (1992), there is a carbon trade-off between leaf osmotic adjustment that requires energy for metabolic reactions, and *K*, which must be invested in root and stem growth. Based on this interpretation, our findings suggest that the four tree species studied invest photoassimilate differently. Previous research has suggested that tree distribution at treeline elevations are limited by processes related to assimilate partitioning rather than production (Körner 2021).

In conclusion, photoassimilate acquired by *S. matsumurana* whrease reducing water loss during the short growing season was seemingly invested in metabolic reactions leading to osmotic adjustment. In contrast, *B. ermanii* showed higher photosynthetic rate, concomitant with higher hydraulic conductance and water consumption in the absence of water severe stomatal restrictions, which explains how it can exist as a tall tree in this habitat. However, this species is not expected to be able to grow at elevations above the treeline due to the close association between the shortened growing season and the prevailing lower temperatures. Evergreen conifer *A. mariesii*, showed higher leaf succulence and a water storage capacity favorable for growth, while reducing water loss. Conversely, evergreen coniferous species *P. pumila* compensated for water loss with a higher leaf hydraulic capacity and cell wall elasticity, thereby maintaining higher stomatal conductance without suppressing transpiration. This indicated that drought tolerance in *P. pumila* maintained high hydraulic conductance and photosynthetic rates through its capacity to resist water storageIn addition, *P. pumila* can grow at high elevations beyond the treeline and grow adaptively on the treeline in a water-consuming manner due to the evergreen and dwarf growth habit.

Our findings highlight the fact that not all alpine trees share the same pattern of physiological responses, despite growing at the same elevation. Instead, they show unique species-specific characteristics. Thus, further investigation of the physiological characteristics of alpine plant species is needed to improve our understanding of these interspecific variations in alpine environments.

Declarations

Acknowledgements

All authors gratefully acknowledge Mr. Masaya Takagi of Kyoto University and Mr. Mizuki Okamoto, Ms. Maiko Akatsuki, Ms. Natsuko Tanikawa, and Mr. Nobuto Oshima of Shinshu University, for their support in the field investigation at Mt. Norikura. In addition, we are grateful to Dr. Yoshiko Kosugi for her valuable comments on this study.

Funding

This study was partly supported by the Japan Society for the Promotion of Science (JSPS) Grant-in Aid for Scientific Research (17J05154, 18J40014, 20K06147) and the Coca-Cola Foundation.

Competing Interests

The authors declare they have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the conception and design of the study. Data collection and analysis were performed by Azuma AW, Kamakura M, Yahara H and Makita N. The first draft of the manuscript was written by Azuma AW and Kamakura M. All authors commented on previous versions of the manuscript, in addition to reading and approving the final manuscript for its publication.

Data Availability

The datasets generated and/or analyzed during the execution of this study are available from the corresponding author upon reasonable request.

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Figures

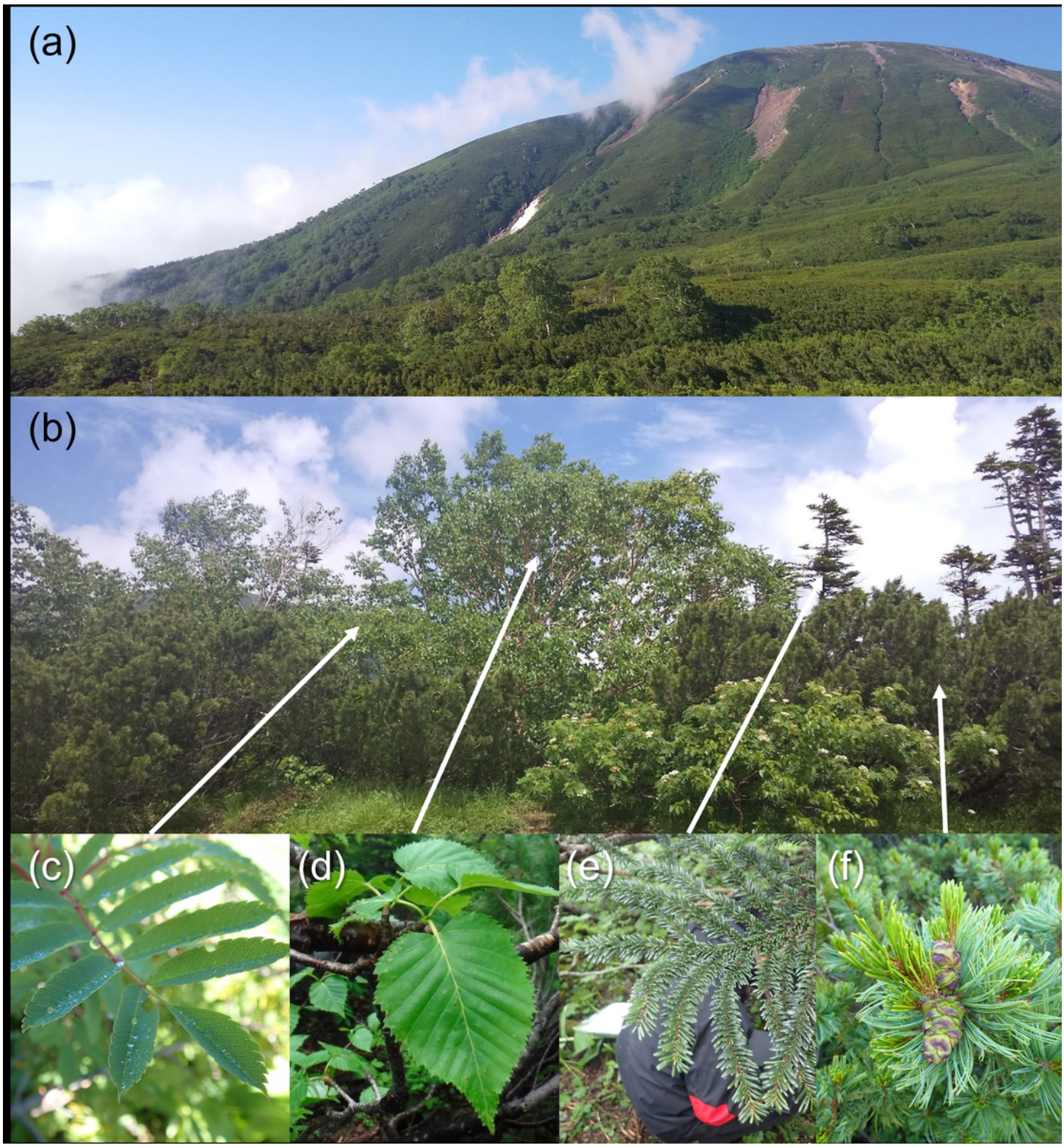

Figure 1

(a) Distant view of the study site. The photograph shows the alpine tree line (2500 m asl) in proximity to our study site (bottom) and dwarf shrubs of *Pinus pumila* (Pall.) Regel (top). (b) Front of the study plot. (c) *Sorbus matsumurana* (Makino) Koehne. (d) *Betula ermanii* Cham. (e) *Abies mariesii* Mast. (f) *P. pumila* (Pall.) Regel.

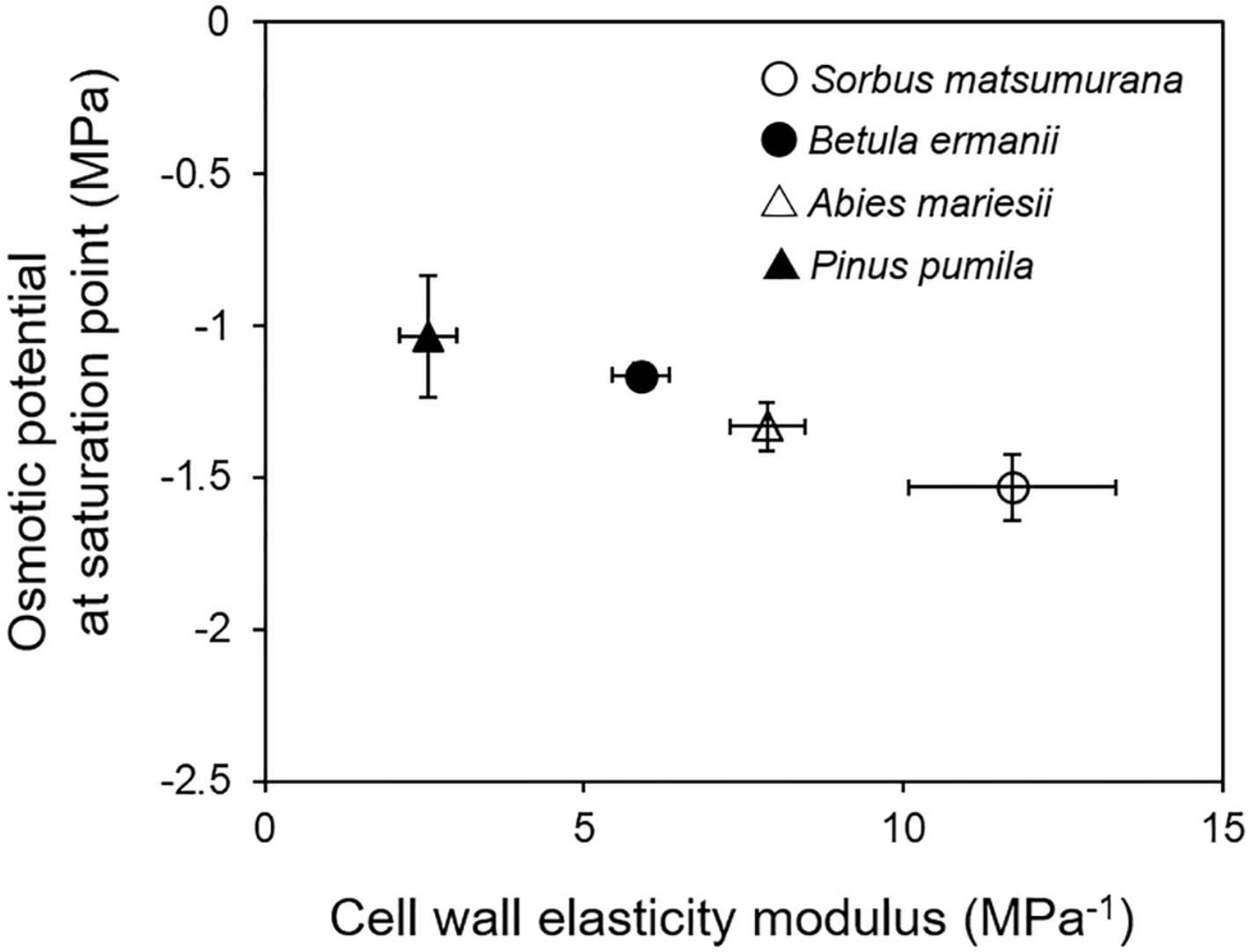

Figure 2

Mean values of osmotic potential at saturation (Ψ_{osat}) and cell-wall elasticity estimated from four to six pressure–volume (*P*– *V*) curves of each tree species. Bars indicate standard errors (SE).

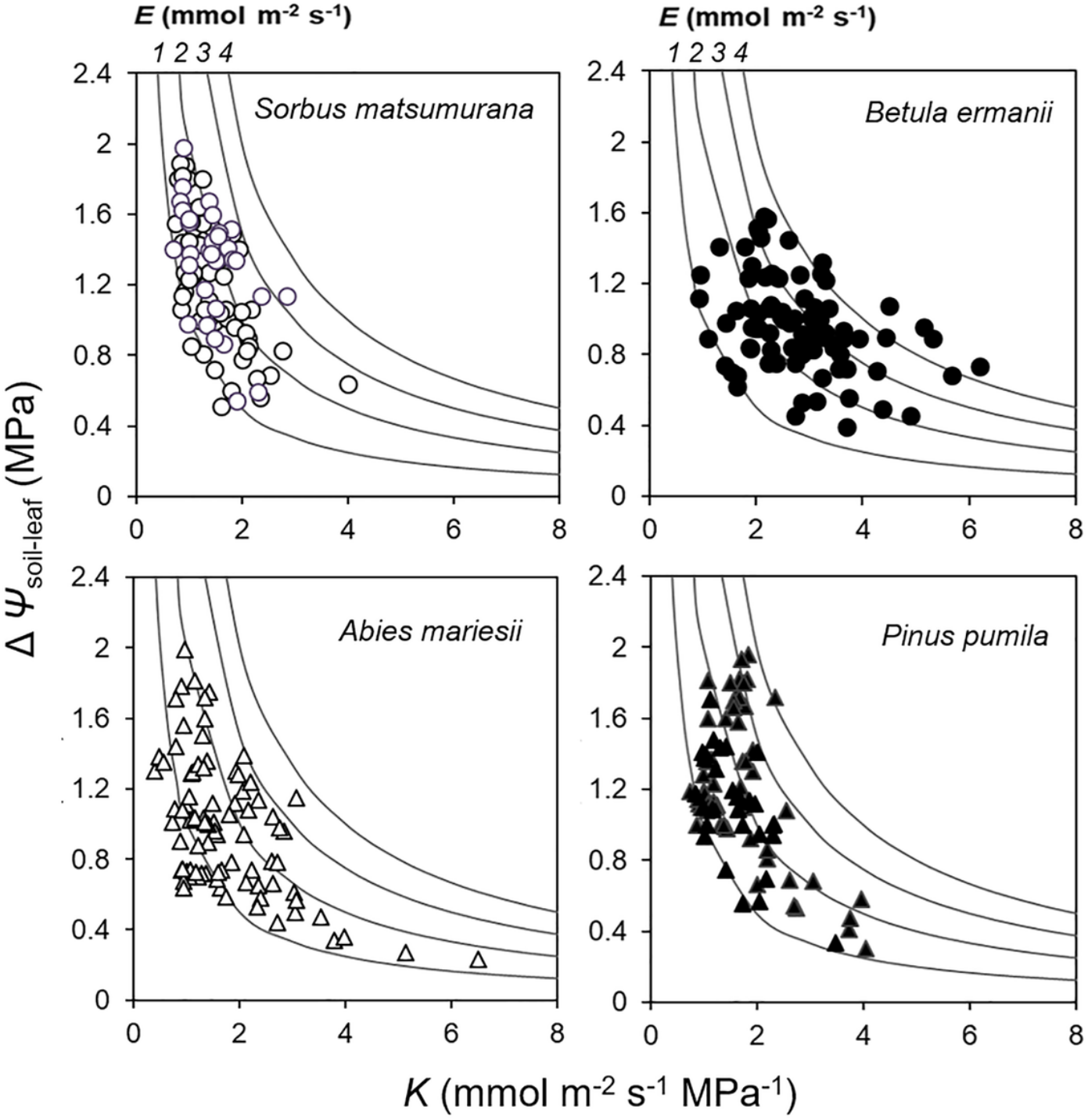

Figure 3

Relationships between hydraulic conductance (*K*), water potential difference from soil to leaf ($\Delta\Psi_{soil\text{-}leaf}$), and transpiration rate per unit leaf area (*E*, lines in each box) in the soil–plant–atmosphere continuum (SPAC), observed from diurnal leaf gas exchange and leaf water potential data in each tree species for 30 to 31 July 2018; 29 to 30 July 2019; and 12 to 13 September 2019.

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