Subalpine dwarf shrubs differ in vulnerability to xylem cavitation: An innovative staining approach enables new insights

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Abstract
Dwarf shrubs are a key functional group of the high-elevation vegetation belt. Despite their ecological relevance and high sensitivity to environmental changes, the hydraulic adaptations and species-specific variations in drought tolerance within this growth form are mostly unknown. Here, we assess the xylem vulnerability to cavitation of important character species of the Alpine dwarf shrub heaths in Central Europe. Due to the high percentage of nonfunctional xylem areas in these species, vulnerability curves were gained by an innovative staining approach with safranin, validated by hydraulic and xylem anatomical measurements. The loss of 50% conducting xylem area appeared in the range of \(-1.78\) to \(-2.91\) MPa. Midday plant water potential during an intense summer drought remained above these critical thresholds but was correlated with cavitation resistance. No trade-off between hydraulic safety and efficiency was detected across analyzed species. We conclude that the characteristic clustered occurrence of species in the heterogeneous mountain terrain (mainly interpreted as a consequence of varying snow cover dynamics) is also reflected in species-specific adjustments in xylem hydraulics. The interspecific variety in vulnerability thresholds and plant water potentials during summer drought indicates different hydraulic strategies and adjustments in water relations between these co-occurring shrubs.

1 | INTRODUCTION

Dwarf shrubs are an important functional group of high mountain vegetation, and several essential character species of subalpine and alpine plant communities belong to this specific growth form (Körner, 2003). Due to climate and land-use changes, an expansion of dwarf shrubs was reported for several mountain areas (e.g., Rixen et al., 2010; Tasser & Tappeiner, 2002), with multiple impacts on ecosystem structure and functioning (Maestre et al., 2016). In the European Alps, many sociophysical and ecophysiological aspects of dwarf shrub heaths were intensively studied due to a long tradition in environmental and botanical investigations (Grabherr, 1980; Tranquillini, 1976). The ecological relevance of this vegetation type and its high sensitivity to environmental changes were demonstrated (e.g., Körner, 2003; Larcher, 2003). However, important hydraulic traits of these plant communities, such as water regulation strategies, xylem water transport, xylem anatomical characteristics, and species-specific drought resistance, have rarely been investigated. A few studies dealt with stomata regulation and cuticular transpiration (Ganthaler & Mayr, 2015a, 2015b; Pisek & Winkler, 1953); root traits and belowground biomass (Larcher, 1977), in situ water potentials during summer (Anadon-Rosell et al., 2017; Körner & Mayr, 1981), and xylem...
vulnerability and efficiency (Ganthaler & Mayr, 2015a, 2015b), but they were mostly limited to single species.

At high altitudes, plant life is commonly less constrained by moisture limitation than at low altitudes (Roe, 2005); however, local water shortages can periodically occur. These water shortages are especially prevalent in skeletal soils with thin humus layers and can amplify the effects of the overall extreme physical environment on mountain plants (Körner, 2003). These harsh environmental conditions include low temperature, persisting snow cover, intensive radiation and high wind load, and also apply to the characteristic dwarf shrub heaths in the Central Alps (Cernusca, 1976). Dominant species of these plant communities mainly belong to the family Ericaceae, which show clonal reproduction and form extensive networks of subterranean rhizomes. However, the species differ in leaf habit (deciduous or evergreen), leaf characteristics (i.e., leaf area, cuticula thickness, stomata density), soil requirement (silicate or calcareous), rooting depth, and growth size (creepers to upright plants of 60 cm height; Larcher, 1977), and thus also very likely differ in their water relations.

Although individual species frequently coexist on Alpine sites, their distribution is strongly shaped by the topography, which was shown to be linked with snow cover height and duration during winter (Carrer et al., 2019; Neuner, 2014). There is evidence that individual dwarf shrub species suffer from drought stress if the snow melts earlier in the season (Rixen et al., 2010) and that varying snowpack dynamics affect the vegetation composition. In the specific case of subalpine dwarf shrub heaths, a strong snow persistence gradient was detected from Vaccinium myrtillus stands with on average 6-month snow cover to wind-exposed Kalmia procumbens carpets where snow persists only 1 month (Körner, 2003). Importantly, the topography of mountain terrains also leads to an uneven distribution of precipitation and water melt during the growing season, for instance, between ridges and depressions. It should also be noted that Alpine plants create their own microclimate based on the plant stature (such as cushions or dense carpets), with large effects on species-specific evaporative losses (Cernusca, 1976).

The relevance of summer drought and drought resistance in Alpine dwarf shrubs is largely unknown. Few studies dealt with Alpine shrubs, such as Rhododendron ferrugineum, Rhododendron hirsutum, and Juniperus communis (Beikircher & Mayr, 2008; Mayr et al., 2010; Unterholzner et al., 2020). The paucity of published data on both shrubs and dwarf shrubs is likely due to the scarce economic interest in shrubs compared to trees, concerning all habitat types, but also due to methodological challenges. The small dimensions of the twigs, the dense branching pattern, and a high proportion of nonfunctional xylem areas in dwarf shrubs (see more details below) make some hydraulic measurements (e.g., centrifuge technique; Cochard et al., 2013) unfeasible, and most other classical hydraulic methods (compare Sergent et al., 2020) are not easily applied or require specific adaptations.

The lower growth height of dwarf shrubs may imply different requirements for a safe and efficient water transport compared to trees (see, e.g., Tyree & Ewers, 1991, Patiño et al., 1995, Beikircher & Mayr, 2008), and hydraulic studies on this growth form may provide new insights into the physiology of plant water transport by presenting aberrations from widely analyzed model species. Moreover, dwarf shrubs are adapted to extraordinary climatic and environmental conditions at high elevation. Shallow and rocky soils coupled with strong exposure to wind and radiation on these sites reduce the soil water retention capacity and increase transpiration rates. Thus, the mosaic-like distribution of dwarf shrub species may also be related to the small-scale variation in water availability and in evaporative losses between ridges and depressions. Importantly, winter stress may require further morphological and anatomical adaptations on the whole plant level, which affect the water balance during both winter and summer and lead to specific xylem hydraulic adjustments, including the resistance to cavitation. The relevance of winter stress for plant hydraulics at higher elevation has been shown for several tree and shrub species (e.g., Mayr et al., 2003, 2019).

Preceding studies on Vaccinium species revealed a rather low resis-
tance to cavitation compared to co-occurring trees (Ganthaler & Mayr, 2015a, 2015b) and a surprisingly high proportion of nonfunctional xylem areas in several dwarf shrub species (A. Ganthaler et al., unpublished). The latter can cause methodical problems, as non-functional conduits may not be permanently blocked, and pressures used in classical hydraulic measurements (e.g., Sperry method; Sperry et al., 1988) can reactivate nonfunctional conduits and lead to artificially increased hydraulic conductivities. Staining approaches, based on perfu-
sion of stem segments with a dye and subsequent determination of the stained, therefore conductive, cross-sectional xylem area, have already been shown to be a reliable alternative method (Hietz et al., 2008, Nolf et al., 2016; A. Ganthaler et al., unpublished) to quantify the percent loss of conductivity (PLC) with progressive dehydration. Although the approach is comparably time-consuming, it has further advantages as it enables insights into distribution patterns of cavitation events.

In the present study, we investigated (1) whether stem hydraulic vulnerability of dwarf shrubs species can be accurately determined by staining conductive xylem elements with safranin and (2) to what extent the vulnerability to drought-induced cavitation varies across co-occurring species in Alpine dwarf shrub communities. We hypothe-
sized that (3) xylem vulnerability is related to the species' moisture indicator values and/or (4) to the experienced water potential ($\Psi$) during summer drought periods ($\Psi_{drought}$).

Therefore, six dwarf shrub species characteristic of the subalpine zone of the Central European Alps (Arctostaphylos uva-ursi (L.) Spreng., K. procumbens (L.) Gift & Kron, Calluna vulgaris (L.) Hull, Erica carnea L. [all evergreen], and V. myrtillus L., and Vaccinium gaultherioides Bigelow [deciduous]) were selected and analyzed by hydraulic measurements, staining experiments, and $\Psi$ measurements during a persistent summer drought.

2 | MATERIALS AND METHODS

2.1 | Study sites and plant material

Two sites near Innsbruck, Austria (Figure 1) were selected for field measurements and sampling of plant material: the first (Mt Patscherkofel,
1883 m; 47°22'N, 11°47'E) represents a characteristic subalpine dwarf shrub heath above the treeline on silicate soil (Grabherr, 1980; Larcher, 1977). It is characterized by large populations of *A. uva-ursi*, *C. vulgaris*, *K. procumbens*, *V. gaultherioides*, and *V. myrtillus*, co-occurring with *R. ferrugineum* shrubs. The second site in a calcareous area (Höttinger Bild, 980 m; 47°17'N; 11°22'E) was selected to include also the calcicole species *E. carnea*. A detailed characterization of analyzed species, including indicator values for moisture, light, temperature, continentality, and nutrients, is given in Table S1.

Measurements were performed between June and September 2016. For hydraulic analyses, whole plants, including major roots, were collected on the field sites and transported with their roots in water and covered with a dark plastic bag immediately to the laboratory, where they were rehydrated overnight. Measurements were made on healthy, several-year-old plants with a stem diameter between 2.5 and 4.5 mm.

### 2.2 Vulnerability curves

Saturated plants of *A. uva-ursi*, *C. vulgaris*, *E. carnea*, and *K. procumbens* (*n* = 14–21 per species) were dehydrated on the bench (Cochard et al., 2013; Sperry et al., 1988). At increasing levels of dehydration, whole plants were equilibrated in dark plastic bags and plant Ψ was determined on one side branch, up to 10 cm long, with a pressure chamber (model 1505D pressure chamber; PMS Instrument). One stem section per plant, 3–5 cm long and at least 3 cm distant from the basal end was excised.
under water. Therefore, samples were decorticated, and the ends were recut about 5 mm for several times (in total at least 1.5–2 cm) with a sharp wood carving knife to gradually release tension and remove microbubbles (Venturas et al., 2015). It was previously shown (Ganthaler & Mayr, 2015a, 2015b) that this sample preparation protocol causes no cutting or rehydration artifacts (see Trifili et al., 2014; Wheeler et al., 2013) in Alpine dwarf shrubs, which are characterized by few mm long vessels. Prepared samples were then connected to the safranin or water reservoir as described in the following sections.

2.2.1 Staining conductive xylem with safranin

Samples were sealed in a hydraulic system (modified after Sperry et al., 1988) connected with a reservoir filled with 0.1% (wt/vol) filtered (0.22 μm) safranin. The pressure was set to 5 kPa, and samples remained connected until the outflow was deeply red stained. After a drying period of 30 min at room temperature, cross sections were made from the middle part of the stained stem sections with a slide microtome (Schlittenmikrotom G.S.L. 1, Schenking Dapples) and analyzed with a light microscope (Olympus BX41; Olympus Austria) interfaced with a digital camera (ProgRes CT3, Jenoptik). Pictures were evaluated with the software ImageJ (ImageJ 1.45; public domain, National Institutes of Health) using the included tool “color thresholds” to determine the stained xylem area (A_s) and total xylem area (A_t).

As analyzed species have a high proportion of functionally inactive (permanently blocked or air-filled) xylem elements (A. Ganthaler et al., unpublished), these nonfunctional areas (A_nf) had to be excluded from the analysis in order to exactly determine the loss of conductive area with progressive dehydration. This included mainly the innermost year-rings for A. uva-ursi, K. procumbens, E. camea, and the latewood of each year-ring in C. vulgaris, but also parenchyma rays (mainly E. camea) and areas with clear wound reaction and deposit of secondary compounds (all species; see also Figures 2 and 3; see also Results section). All these xylem parts never stained, also in saturated samples, and did not contribute to conductivity. As the pattern and extent of these areas vary between species, they had to be excluded manually, based on the visible staining pattern and extensive experience with the determination of conductive xylem in saturated samples of these dwarf shrub species by staining and x-ray microtomography (microCT) scans (A. Ganthaler et al., unpublished). The percent loss of conductive area (PLA) was finally calculated as:

\[
PLA = \left(1 - \frac{A_s}{A_t - A_{nf}}\right) \times 100.
\]

2.2.2 Hydraulic and wood anatomical control measurements

To validate the staining method, a reference vulnerability curve was measured by hydraulic flow measurements for E. camea, as in this species, nonfunctional xylem areas were shown to be permanently blocked and not removable by high pressure flushing (A. Ganthaler et al., unpublished). Samples were sealed in a hydraulic system (modified Sperry apparatus; Sperry et al., 1988, Losso et al., 2018) equipped with a mini-flow meter (mini Cori-Flow100 g h⁻¹, Bronkhorst High Tech) and a reservoir filled with distilled, filtered (0.22 μm) and degassed water containing 0.005% (vol/vol) Micropur (Katadyn Products) to prevent microbial growth.

The initial hydraulic conductance (K_i) was measured at 5 kPa. Samples were then flushed for 20 min at 70 kPa (Ganthaler & Mayr, 2015a) to remove embolism, and the hydraulic conductivity was measured again. Flushing was repeated until measurements showed no further increase in conductivity to obtain final hydraulic conductance (K_f). The PLC was then calculated as follows:

\[
PLC = \left(1 - \frac{K_i}{K_f}\right) \times 100.
\]

Furthermore, xylem anatomical measurements were performed to assess the comparability of PLA and PLC and to validate vulnerability curves. Mean conduit diameter (d), mean hydraulic diameter (d_h; calculated according to Kolb & Sperry, 1999), and diameter size...
distribution were determined for each species and separately for stained and unstained xylem areas on three samples per species with a PLA of about 50%. Measurements were performed according to Ganthaler and Mayr (2015a, 2015b) on 641–985 conduits per species using the software ImageJ.

### 2.3 Additional data

In order to get a comprehensive overview of plant vulnerability to xylem cavitation in the analyzed dwarf shrub communities and to improve the significance of correlation analyses with Ψ following a drought period (Ψ<sub>drought</sub>) and specific hydraulic conductivity (k<sub>s</sub>), a data set comprising both data gained in this and previous studies was used. Additional data were extracted from Ganthaler and Mayr (2015b) on the hydraulic vulnerability of *V. gaultherioides* and Ganthaler and Mayr (2015a) on the hydraulic vulnerability of *V. myrtillus*. Both studies were based on plant material sampled on the same study site (Mt Patscherkofel) and were gained by hydraulic measurements as described for *E. carnea* above (also in *Vaccinium* species nonfunctional xylem areas are permanently blocked; A. Ganthaler et al., unpublished). To improve comparability, raw data were reevaluated and fitted with a Weibull regression curve like described for data gained in the present study (see below).

In addition, k<sub>s</sub> values (specific hydraulic conductivity related to the entire xylem cross-sectional area) of all six species (from A. Ganthaler et al., unpublished) were used for correlation analyses, including also k<sub>s</sub><sup>c</sup> (corrected specific hydraulic conductivity, related to the functional xylem area). Also, these measurements were based on plant material sampled from the same populations (Höttinger Bild for *E. carnea*, Mt. Patscherkofel for all other species).

### 2.4 Measurements following a drought period

On five plants per species, Ψ was measured following a period of 7 days of rain-free weather with hot temperatures and minimal precipitation in the preceding 2 weeks (~23 mm within the period August 10–28, 2016; Zentralanstalt für Meteorologie und Geodynamik [ZAMG], 2020). These conditions represent a typical summer drought period in the study area, and only in extreme years like 2018 even longer periods without significant precipitation occur (ZAMG, 2020). Side branches, about 10 cm long, of randomly selected plants within a defined 5 × 5 m plot (on both study sites) were cut, immediately wrapped in cling film and transported in a tight plastic bag to the laboratory, where Ψ was measured with the Scholander apparatus (Model 1000 Pressure Chamber, PMS Instrument Co.).

At the time of Ψ measurements, the volumetric soil water content (SWC<sub>drought</sub>) was measured with a Hydrosense II Soil Moisture Measurement System (Campbell Scientific) on 10 spots within the selected plot and related to the SWC measured 24 h after strong rainfall (SWC<sub>field capacity</sub>). The relative SWC after drought (SWC<sub>rel</sub>) was then calculated as:

\[
SWC_{rel} = \left( \frac{\text{SWC}_{drought}}{\text{mean SWC}_{field capacity}} \right) \times 100.
\]

### 2.5 Statistics

For vulnerability analyses, PLC/PLA was plotted versus the corresponding Ψ (data pooled per species) and a Weibull regression curve was fitted to each vulnerability curve (R package FIT-PLC, R i386 3.2.5; Duursma & Choat, 2017). Then Ψ at 12%, 50%, and 88% PLC (Ψ<sub>12</sub>, Ψ<sub>50</sub>, Ψ<sub>88</sub>) was calculated. Differences between methods (hydraulic analyses versus staining conductive xylem, for *E. carnea*) and between
species were assessed using 95% confidence intervals obtained via bootstrap resampling (performed in R Studio). Student’s t-tests and correlation analysis (Ψ50 vs. Ψdrought and Ψ50 vs. k and ksc) were performed using SPSS v.24.0 (SPSS Inc.) at a probability level of 5%.

3 | RESULTS

3.1 | Vulnerability to xylem cavitation

Vulnerability curves of E. carnea obtained with the presented staining approach did not significantly differ from control measurements gained with the classical hydraulic method (Table 1, Figure 2). Although the vulnerability curve obtained from staining conductive xylem was slightly steeper, the differences in Ψ50 were negligible (0.14 MPa). Figure 3 illustrates the determined areas of conductive, embolized, and nonfunctional xylem in E. carnea stems with progressive dehydration. Patches of embolized conduits were scattered over the entire functional xylem area and no trend for earlier embolization of older or larger conduits could be identified. Furthermore, xylem anatomical measurements demonstrated similar mean conduit size (d and dsc) and size distribution for all analyzed species (Table S2) and no significant differences between conductive and embolized xylem areas (Figure S1).

Accordingly, also for the further three analyzed shrub species, staining of conductive xylem elements revealed characteristic sigmoidal vulnerability curves (Figure 4). Differences in hydraulic thresholds were small and not significant between C. vulgaris, E. carnea, and K. procumbens (Ψ50 – 1.78, – 2.11, and – 1.89 MPa; Table 1), but a significantly lower Ψ50 was observed for A. uva-ursi (Ψ50 – 2.91 MPa). The latter species also exhibited lower Ψ12 (–1.66 MPa) and Ψwsc values (–4.22 MPa; Table 1) and a clear trend for earlier conduit embolization in older compared to youngest year rings (not shown).

Vulnerability thresholds of Vaccinium species (taken from Ganthaler & Mayr, 2015a, 2015b) were in the range of the other dwarf shrubs in this plant community. V. gaultherioides was slightly more vulnerable to cavitation (Ψ50 – 2.70 MPa) than the most resistant species, A. uva-ursi, and vulnerability of V. myrtillus (Ψ50 – 1.86 MPa) was between K. procumbens and C. vulgaris (Table 1 and Figure 5). Notably, the vulnerability curve for K. procumbens was characterized by a distinctly steeper slope compared to all other species, resulting in a very narrow Ψ12–Ψ50 range (1.09 MPa vs. 1.96–2.57 MPa in remaining species; Figure 5).

3.2 | Correlation with plant water potential during drought periods

After the described summer drought period, determined Ψdrought of analyzed dwarf shrubs was –1.03 ± 0.03 MPa (V. myrtillus), –1.16 ± 0.06 MPa (K. procumbens), –1.18 ± 0.02 MPa (C. vulgaris), –1.19 ± 0.05 MPa (E. carnea), –1.31 ± 0.04 MPa (A. uva-ursi), and –1.37 ± 0.05 MPa (V. gaultherioides). Ψdrought was significantly correlated with the species-specific Ψ12 and Ψ50 thresholds (Figure 6), whereby more cavitation-resistant species had lower Ψdrought. While Ψdrought never reached values inducing 50% embolization, Ψ12 thresholds were exceeded in C. vulgaris, E. carnea, and V. myrtillus (Figure 6).

Absolute SWCdrained at the time of Ψdrought measurements equaled 5.4% and 4.8%, corresponding to a SWCf of 22.2% at the silicate and 23.9% at the calcareous site in relation to the conditions at field capacity (field capacity of the two soil types was 24.1% and 35.2%, respectively).

3.3 | Correlation with hydraulic conductivity

Within the analyzed group of species, no correlation between the vulnerability to xylem cavitation and the specific hydraulic conductivity (ksc, related to the whole xylem cross-sectional area) or the corrected specific hydraulic conductivity (ksc, related to the functional xylem

| Table 1 | Vulnerability to xylem cavitation of analyzed dwarf shrub species obtained by hydraulic measurements (flowmeter) and staining conductive xylem (staining) in the present study and by Ganthaler and Mayr (2015a, 2015b) |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Method | Ψ12 | CI2.5% | CI97.5% | Ψ50 | CI2.5% | CI97.5% | Ψ88 | CI2.5% | CI97.5% | n |
| Ec | Flowmeter | −0.88 | −0.56 | −1.25 | −2.25 | −1.97 | −2.52 | −4.17 | −3.39 | −5.21 | 26 |
| | Staining | −1.04 | −0.63 | −1.30 | −2.11 | −1.83 | −2.34 | −3.44 | −2.87 | −3.90 | 21 |
| Au | Staining | −1.66 | −1.36 | −1.95 | −2.91 | −2.72 | −3.10 | −4.22 | −3.63 | −4.63 | 15 |
| Cv | Staining | −0.71 | −0.47 | −0.98 | −1.78 | −1.53 | −2.10 | −3.28 | −2.73 | −4.01 | 17 |
| Kp | Staining | −1.31 | −0.84 | −1.49 | −1.89 | −1.64 | −2.13 | −2.40 | −2.25 | −3.31 | 14 |
| Vg | Flowmeter | −1.70 | −1.29 | −2.53 | −2.70 | −2.37 | −3.05 | −3.66 | −3.13 | −4.32 | 27 |
| Vm | Flowmeter | −0.87 | −0.66 | −1.14 | −1.86 | −1.67 | −2.10 | −3.09 | −2.36 | −3.73 | 30 |

Note: Parameters Ψ12, Ψ50 and Ψ88 correspond to the water potential at 12%, 50%, and 88% loss of conductivity/loss of conductive area, respectively, and CI 2.5% and CI 97.5% indicate the confidence interval for each parameter; n is the number of pooled samples per vulnerability curve. Letters indicate statistically significant differences in the respective parameter between the species Erica carnea (Ec), Arctostaphylos uva-ursi (Au), Calluna vulgaris (Cv), Kalmia procumbens (Kp), Vaccinium gaultherioides (Vg), and Vaccinium myrtillus (Vm).

aPresent study.

bGanthaler and Mayr (2015a, 2015b).
area) was detected (Figure 7). Notably, *A. uva-ursi*, the species with an exceptional high proportion of non-functional xylem areas, showed comparably high $k_{sc}$.

## DISCUSSION

The present data set demonstrates a range in vulnerability thresholds of about 1 MPa across studied dwarf shrubs, whereby vulnerabilities correlated with midday $\Psi_{dmax}$ but not with $k_i$ or $k_{sc}$. A direct comparison of the vulnerability curve based on the proportion of stained xylem with the curve gained by classical hydraulic measurements (performed for *E. carnea*; Figure 2) proved that the staining method revealed reliable results. The validity of the method for all analyzed species was underlined by the small species-specific differences in $d$ and conduit size distribution, and by consistent $d_i$ in conductive and embolized xylem areas (Figure S1; Table S2). The latter gives evidence of equivalent conductive capacity in stained and unstained xylem areas and thus good agreement between PLA and PLC. The approach thus seems like a valuable alternative or complementary method to measure xylem vulnerability (as previously shown for conifers; Mayr & Cochard, 2003, Hietz et al., 2008) also for angiosperm species with their complex anatomical wood structure. The slightly diverging curve
**Figure 5** Comparative analysis of the hydraulic vulnerability for all six Alpine dwarf shrub species. (A) Fitted vulnerability curves with solid vertical lines indicating 50% loss of conductivity/loss of conductive area (Ψ₅₀) and (B) water potential range between 12% and 88% loss of conductivity/loss of conductive area (Ψ₁₂⁻Ψ₈₈) with black vertical bars indicating Ψ₅₀.

**Figure 6** Vulnerability to xylem cavitation (water potential at 12% and 50% loss of conductivity/cond. area, Ψ₁₂ and Ψ₅₀) versus the water potential measured in situ after a drought period (Ψ_drought). Shown are mean ± SE for each species (Au, Arctostaphylos uva-ursi; Cv, Calluna vulgaris; Ec, Erica carnea; Kp, Kalmia procumbens; Vg, Vaccinium gaultherioides; Vm, Vaccinium myrtillus) and the linear regression line. Areas below the 1:1 line are shed in yellow.
Vulnerability to xylem cavitation (water potential at 50% loss of conductivity/cond. area, Ψ50) versus specific hydraulic conductivity (ksc, related to the whole xylem cross-sectional area; filled symbols) and corrected specific hydraulic conductivity (ksc, related to the functional xylem area; open symbols). Each data point represents mean ± SE of one species (Au, Arctostaphylos uva-ursi; Cv, Calluna vulgaris; Ec, Erica carnea; Kp, Kalmia procumbens; Vg, Vaccinium gaultherioides; Vm, Vaccinium myrtillus).

On one hand, this characteristic may be related to their growth stature, as the reduced growth height leads to comparatively low cumulative hydraulic resistance along the pathway. Thus, compared to trees, smaller Ψ gradients along the axis (Tyree & Ewers, 1991) and, consequently, less negative Ψ minima appear. It is not uncommon that plants with shorter stature exhibit low resistance to cavitation, often combined with low hydraulic efficiency (Gleason et al., 2016). Such a combination with lacking trade-off between xylem hydraulic efficiency and safety also applied to the analyzed dwarf shrubs (Figure 7). On the other hand, Alpine dwarf shrubs exhibit a large set of specific ecophysiological adaptations regarding nonxylem traits affecting their water relations. For instance, they are characterized by a 2–3 times higher belowground than aboveground biomass (Larcher, 1977). The extensive network of long and deep roots improves the water uptake and counterbalances the limited water retention capacitance of mountain soils and the modest annual precipitation on the study sites (about 800 mm; ZAMG, 2020), even for species growing on exposed, shallow-grounded places such as K. procumbens. Deeper rooting makes dwarf shrubs less dependent on the water content of upper soil layers and less vulnerable to drought conditions than, for example, herbaceous species (Anadon-Rosell et al., 2017). Dwarf shrubs are also characterized by a lower leaf area index and growth rate compared to trees (Körner, 2003), which may facilitate a water saving strategy.

This overall favorable hydraulic situation, as also demonstrated by only moderate Ψ even in a dry period (see Section 3), points to a rather unproblematic water regime in these plant communities, at least during summer. Similarly, Larcher (1977), Körner and Mayr (1981), and Anadon-Rosell et al. (2017) reported that Ψ of these species rarely drops below −1.5 to −1.8 MPa on sunny summer days, even after long periods without rain. Thus, plants do not reach critical Ψ for cavitation, despite the relatively high xylem vulnerability, but are operating with rather narrow hydraulic safety margins (~0.6–1.2 MPa) against critical levels of drought stress, comparable to several forest species across the world (compare Choat et al., 2012). Notably, A. uva-ursi and V. gaultherioides exhibited a significantly wider safety margin between Ψ50 and Ψdrought compared to the other species. Within the analyzed species group, the vulnerability to drought-induced cavitation was correlated with Ψdrought (Figure 6), indicating that species-specific xylem characteristics are adjusted to (a) water availability and/or (b) plant water use strategies, which both affect plant Ψdrought. We found no evidence for the former, as Ψ50 was not related to the moisture ecological indicator values (Table S1) according to Landolt (2010). For instance, K. procumbens, the characteristic species on exposed ridges with a low indicator value for soil moisture, exhibited a rather high Ψ50, while V. gaultherioides, mainly growing in depressions and more humid sites, was significantly more cavitation resistant (compare Table 1).

In contrast, several relationships with plant traits affecting transpirational control and water losses became apparent. Several studies indicate that Alpine plants respond sensitively to the evaporative demand and species start closing their stomata with increasing vapor pressure deficit even before a relevant drop in plant Ψ (Bowman et al., 1995; Johnson & Caldwell, 1975). Moreover, according to gas exchange measurements of Larcher (1977) on four of the analyzed species, CO2 uptake during dehydration is first limited in K. procumbens (at ~18% relative water saturation deficiency) and C. vulgaris and V. myrtillus (at ~27%), while V.
gaultherioides keeps stomata open up to ~42% saturation deficiency. This corresponds to the distinct higher resistance to cavitation of V. gaultherioides compared to the other three species (compare Figure 5).

Moreover, a comparison of the two Vaccinium species by Anadon-Rosell et al. (2017) revealed higher stomatal conductance on sunny days around noon for V. gaultherioides (362 mmol m⁻² s⁻¹) than for V. myrtillus (179 mmol m⁻² s⁻¹). These findings were further supported by lower midday Ψ in V. gaultherioides both under natural conditions and artificial rain exclusion (−1.81/−2.04 MPa) compared to −1.57/−1.68 MPa in the less resistant species V. myrtillus. Moreover, analyses by Pisek and Winkler (1953) showed that the cuticular transpiration of Alpine plants can vary substantially, for instance, between 5 mg h⁻¹ g⁻¹ fresh weight in A. uva-ursi to 24 mg h⁻¹ g⁻¹ fresh weight in V. myrtillus. It has to be considered that plant water losses are not only affected by leaf and stomata characteristics but are driven by the vapor pressure deficit, which in turn is strongly affected by the growth stature and overall aerodynamic canopy resistance (Körner, 2003). K. procumbens, for example, although it tolerates strong wind exposure on windswept ridges, forms low stature prostrate mats, which enable to maintain a high air humidity within the cushion (Cernusca, 1976). Without this protection, however, it develops quick and severe drought damage (Larcher, 2003), which is in accordance with the comparably high ΨSO and steep vulnerability curve found in our study.

No indication was found that deciduous dwarf shrub species operate closer to hydraulic limits compared to evergreen species (risking defoliation under stress), at least by analyzing the safety margin between ΨSO and Ψdrought (compare Figure 6). Vulnerability thresholds (Table 1) also could not be related to the phylogenetic classification of the species (Kron et al., 2002), as no consistent differences between the clades Arbutoideae (A. uva-ursi), Ericoideae (C. vulgaris, E. carnea, K. procumbens) and Vaccinioideae (V. gaultherioides, V. myrtillus) were detected. Thus, in Alpine dwarf shrub species, various hydraulic and morphological patterns seem to be balanced in different ways to cope with the available soil water and microclimate. From a wider perspective, ecophysiological adaptation of plants in this extreme environment has to match multiple trade-offs between various abiotic and biotic stress factors during summer and winter. Water availability during summer obviously is not a strong influencing factor for species-specific xylem resistance to cavitation, but xylem safety is coordinated with other hydraulically relevant plant functional traits. Nevertheless, species-specific variation in xylem hydraulic characteristics within these plant communities can have wide ecological implications under changing environmental conditions, such as reduced competitiveness of individual species, with potential negative effects on ecosystems services and stability.

5 | CONCLUSIONS

The comparably low xylem resistance to cavitation in combination with several root and leaf functional traits optimized for water acquisition and saving indicate that the hydraulic strategy of Alpine dwarf shrubs is oriented toward avoidance of low Ψ rather than withstanding low Ψ. Although several physiological traits require further analysis (e.g., rooting depth, stomata regulation, and recovery from cavitation-induced embolism) this strategy may be critical under changing climatic conditions. Pronounced interspecific differences in xylem vulnerability are probably coordinated with occurring xylem tensions in the field, which in turn are strongly influenced by growth stature, leaf characteristics, and stomata regulation. Remarkably, the high variety of ecophysiological adaptations to the environment reported for these co-occurring species also applies to their water use strategy and coordination of individual hydraulic traits.

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AUTHOR CONTRIBUTIONS

Stefan Mayr and Andrea Ganthaler designed the experiments, Andrea Ganthaler performed the measurements, analyzed the data, and wrote the manuscript. Stefan Mayr reviewed and complemented the manuscript.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as all new created data is already contained within this article.

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