Assessing biomechanical durability of alpine and subalpine leaves via measuring bend and fracture attributes

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Abstract

Environmental factors, such as dynamic forces and herbivory, place pressure on plants to develop adaptive features in their leaves for resilience against external mechanical forces. Biomechanical traits enable resilience to such pressures. Biomechanical resilience is the physical robustness of a tissue, due to its structure and composition, which affords resilience to mechanical stress. Plants with a low specific leaf area (SLA) are often long-lived, and in low-nutrient environments it can cost more to replace a leaf than to protect it with biomechanical resilience. The alpine and sub-alpine environments are nutrient limited environments, which places stress on plants, limiting their growth. We hypothesised that SLA would be negatively correlated with measures of plant toughness, specifically bend and fracture moduli, in alpine and subalpine leaves. Additionally, we expected leaf thickness to be positively correlated with puncture resilience. We found no relationship between bend modulus and SLA ($R^2 = 0.140$), nor for fracture modulus and SLA ($R^2 = 0.243$). We found no relationship between leaf thickness and fracture modulus; however, the mid-vein fracture modulus was significantly higher than that of the lamina ($p < 0.001$), which may indicate greater resource investment into vascular tissue via constituent material toughness. Assessing leaf economic strategies of durability and longevity versus less investment and short lifespan can reveal community functional features that occur as a product of environmental pressures. Understanding plant traits that contribute to durability and, by extension, plant longevity, may help to predict how climate change will alter vegetation composition.

Key Words

Biomechanics, ecology, leaf structure, plant communities

Introduction

The alpine zone is a complex mosaic with small-scale variation in plant community composition. This mosaic is a product of the spatial deviation in snow distribution and duration, which is itself a result of the variable aspect and slope of the alpine terrain (Körner 1999). Australian alpine and subalpine plant species display remarkable variation and adaptation to the mosaic of niches that characterise the Australian Alps (Green 2002). As such, the structural and compositional properties of leaves in each niche of this mosaic vary to reflect the variable conditions across the mosaic. One such variable property is leaf biomechanics. These are the structural components of leaf tissue responsible for physical robustness, and therefore durability. Currently, literature on leaf biomechanics of alpine and subalpine plants is limited. Remedying this scarcity will assist in adaptive management of the Australian Alps, particularly in light of the predicted impacts of climate change on alpine and subalpine communities (Pickering *et al.* 2008; Venn *et al.* 2011). In particular, reduction in the extent of summer snowdrift is predicted to lead to substantial reductions in landscape vegetation diversity in the Australian Alps (Edmonds *et al.* 2006). In order to better understand alpine floral adaptations, investigations into functional traits present in these ecosystems would assist in holistically understanding alpine community interactions, and the presence of ecosystem services (de Bello *et al.* 2010). Plant ecologists have placed emphasis on understanding the basis of variation in leaf biomechanical structures because of the role of leaf tissue in driving ecosystems via plant–animal interactions, nutrient cycling and carbon-budgets (Díaz *et al.* 2004; Peeters *et al.* 2007; Méndez-Alonzo *et al.* 2013; Mason and Donovan 2015).

Durability represents physical robustness and is a form of resilience which enables plants to resist damage from perturbations and disturbance. Durability enables the plant to persist and perform in the

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environment and is therefore linked to success in adverse conditions, such as those present in the alpine and subalpine zones. Biomechanical resilience is the physical robustness of a tissue, due to its structure and composition, which affords the organism resilience to mechanical stress. The biomechanical traits that contribute to durability include fracture toughness, flexibility, torsion resilience and tissue density (Kitajima and Poorter 2010). These features are important in leaves due to the critical role of leaves in acquiring energy. Fracture toughness and tissue density of the lamina and vascular tissue in leaves improves the lifespan and survival of a plant (Kitajima and Poorter 2010). In particular, thicker and more fracture-resistant tissues are resilient against herbivory (Peeters *et al.* 2007). Likewise, bend modulus (i.e. flexibility) improves leaf resilience against stresses including dynamic force, such as strong winds, because elasticity reduces the chance of rupturing tissues (Gardiner *et al.* 2016). A more flexible leaf reduces drag in strong winds, reducing the chance of rupturing. Conversely, rigidity prevents leaves shearing from the plant in strong winds. As such, a balance needs to be made between elasticity and rigidity according to the typical wind conditions of the environment. However, durable structural features have a high energy investment cost for the plant and use energy and resources that would otherwise have been allocated into maximising photosynthetic capacity (Méndez-Alonzo *et al.* 2013). As such, the biomass allocation into both durability and photosynthetic capacity is a fine balance dictated by environmental pressures (Mason and Donovan 2015).

We aimed to determine whether alpine and subalpine plant species in the Australian Alps protect their leaves using biomechanical features of durability. To test this, we used specific leaf area (SLA) as a proxy for biomass allocation, and fracture and bend moduli as a measure of durability and structural resilience. SLA is the leaf area per unit leaf mass and is a key functional trait associated with environmental tolerance (Hulshof *et al.* 2013). SLA is often used to predict biomass allocation to the leaves or potential growth rate under different environment conditions, such as water stress, elevation and light availability (Chen 1997; Marron *et al.* 2003; Liu and Stützel 2004; Hulshof *et al.* 2013). Our first hypothesis was that SLA would be negatively correlated with fracture and bend moduli. Secondly, we aimed to investigate the fracture and bend toughness of different parts of the leaf. Our second hypothesis was that the mid-vein and lamina fracture moduli will be correlated with tissue thickness.

Methods

Field site and sample collection

Leaves were collected in the Kosciuszko National Park, Australia, on two days in December 2016, at three locations: Charlotte Pass valley (1,837 m above sea level (asl)), Mount Stillwell lower slopes (1,840 m asl), and Kosciuszko Road (1,633 m asl). The leaves were stored in sealed plastic bags containing wet paper towel to add humidity, in order to maintain freshness before testing. Leaves from 13 species were collected, from 38 individuals (Table 1).

The species were clustered into functional groups according to their physical and phenological characteristics. These groups were: shrub, herb and tree. Functional groups are based on life-form classifications presented by Walker and Hopkins (1990). Shrub species were defined as a small- to medium-height woody plant. Tree species were defined as a perennial plant with an elongated trunk. Herb species were defined as having no persistent woody stem.

Table 1: Leaf samples collected. Charlotte Pass valley (CPV), Mount Stillwell lower slopes (MS), and Kosciuszko road (KR). * indicates non-native. Idenification only possible to genus level in some cases.

| Species name	Number of individuals sampled	Functional form	Location
Acetosella vulgaris*	3	Herb	MS
Astelia psychrocharis	3	Herb	CPV
Brachyscome sp.	3	Herb	CPV
Celmisia sp.	3	Herb	CPV
Craspedia sp.	3	Herb	CPV
Craspedia sp.	3	Herb	MS
Plantago sp.	3	Herb	KR
Taraxacum officinale*	3	Herb	CPV
Hovea montana	3	Shrub	MS
Orites lancifolia	2	Shrub	CPV
Prostanthera cuneata	3	Shrub	CPV
Tasmannia xerophila	3	Shrub	KR
Eucalyptus pauciflora	3	Tree	CPV

Leaf measurements

All of the leaf measurements were made on three leaves per species, with each leaf from a different individual.

The leaves were weighed fresh, for their wet mass, and after drying overnight in a low temperature oven, for their dry mass. Petioles were removed and not included in the mass.

To measure the area of the leaf, fresh leaves were scanned, along with a measure scale, using a flatbed scanner. Area was determined using the software ImageJ to isolate the leaf and measure the pixels within that area, which were converted by the software into mm^2. The length from petiole edge to tip and the width across the widest part of the leaves were measured in ImageJ.

The thickness of the leaves was measured using a micrometre gauge, at seven locations per leaf (Figure 1): the midrib near the petiole, the midrib in the middle of the leaf, the midrib at the tip of the leaf, the lamina as close as possible to the petiole of the leaf, the middle of leaf, the lamina at the tip of the leaf and the lamina on the edge of the leaf in the middle of the leaf. Measurements were made to the nearest half millimetre.
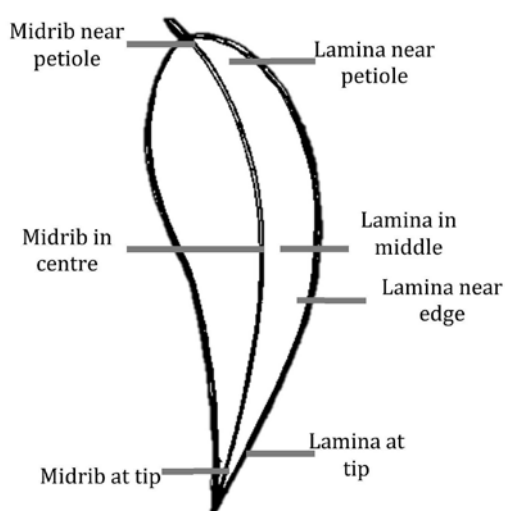
Figure 1: Diagram showing sites for measuring leaf thickness and puncture modulus.

A force gauge was used to measure the fracture and bend moduli. The bend modulus was calculated from the maximum amount of force applied during bending of the leaf. A flat metal probe of 0.50 mm external diameter was pressed against the centre of the leaf to apply force. Twelve samples could not be bent because their length was insufficient for testing in our bend apparatus; these were excluded from bend modulus analysis.

The fracture modulus was calculated from the maximum amount of force applied during puncturing the leaf. A sharp needle probe was pressed into the leaf at seven locations to puncture the leaf. These seven locations were the same as for the thickness measurements, previously described. The distance travelled by the probe was recorded for both fracture and bend tests, for use in the calculation of moduli.

Mathematical formulae

Specific leaf area:

$$\frac{Leaf\ Area\ (mm^2)}{Dry\ leaf\ weight\ (mg)} \qquad (1)$$

Bending modulus:

$$E_{bending} = \frac{l^3 F}{4Wh^3\gamma} \qquad (2)$$

Where *l*: length from platform, *F*: force in newtons, *W*: width of sample, *h*: length of sample, and γ: fixed measurement of deflection.

Fracture modulus:

$$F_T = \frac{F_{max}}{(l\ x\ c)^{-2}} \qquad (3)$$

Where F_{max}: maximum force in Newtons exerted, *l*: thickness of punch point, and *c*: circumference of needle.

Statistical analysis

Microsoft Excel was used to create scatter plots and generate R^2 values to compare various fracture moduli and bend moduli with the SLA of each species, and thickness against fracture moduli. One regression was also run in JMP software, for the bend modulus against SLA for the tree functional group.

T-tests were run in R-studio to compare the lamina and mid-vein fracture moduli. An ANOVA was conducted in JMP to compare the SLA, fracture moduli, and bend moduli among the three functional groups. The significance level for all tests was α = 0.05.

Results

There was no relationship between SLA and bend modulus ($R^2 = 0.146$, Figure 2). Bend modulus is a scale between zero and one. High bend modulus indicates high resistance to bending. Three samples had bend moduli ranging between 0.63 and 0.91, which is much greater than the other leaves tested. Two of these leaves belonged to *Orites lancifolia* and one to *Hovea montana*, both of which are alpine/subalpine shrubs.

Fracture modulus ranged from 280.10 to 9817.27 MPa (SD = 2235.5). As SLA increased, fracture modulus appeared to decrease. However, this was not a significant relationship as only 24.6% of the variation in fracture modulus was explained by SLA (Figure 3).

The leaves belonged to three functional groups: shrub, herb and tree. There was no relationship between fracture modulus and SLA for all groups (Table 2). There was no relationship between bend modulus and SLA for herb and shrub species. There was a relationship between bend and SLA for the tree species

($R^2 = 0.991$, Table 2); however, this was non-significant ($p = 0.062$, *t*-test), and lacked sufficient samples to adequately test ($n = 3$ leaves).
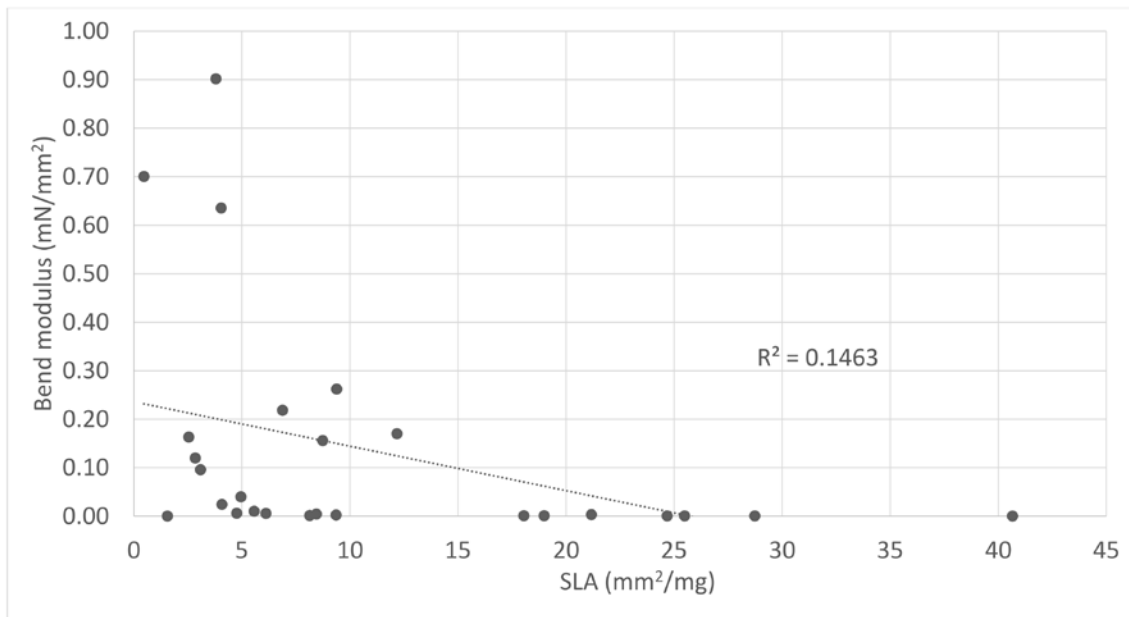

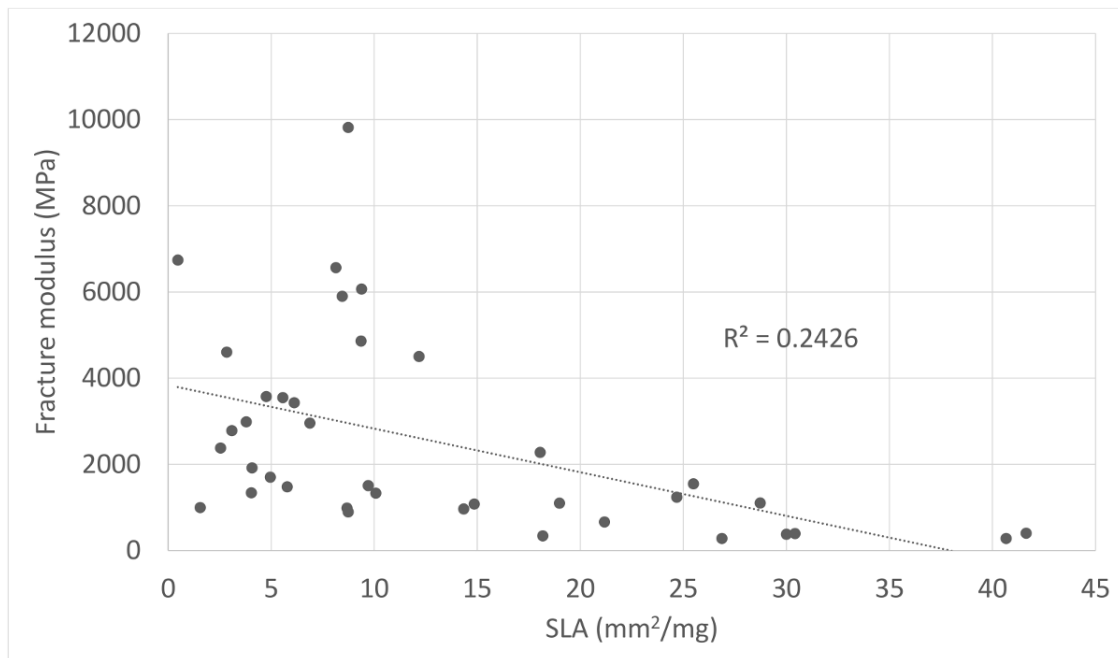**Figure 2: Bend modulus and specific leaf area (SLA) of each leaf measured (n = 26). Linear trendline is shown.**

Figure 3: Average fracture modulus and specific leaf area (SLA) of each leaf measured (n = 38). Average fracture modulus is the mean value of all seven fracture measures taken for each leaf. Linear trendline is shown.

Table 2: Summary of fracture and bend moduli against specific leaf area (SLA) for the leaves tested. Regression analysis of fracture modulus against SLA, and bending modulus and SLA was conducted. R^2 values from linear regression analysis are reported.

Functional group	Sample (n)	Fracture and SLA	Bend and SLA
Herb	8	0.282	0.047
Shrub	4	0.340	0.227
Tree	1	0.056	0.991

There was no significant difference between functional groups for fracture modulus ($F_{2,35} = 0.607$, $p = 0.550$). There was no significant difference between functional groups for SLA ($F_{2,35} = 2.147$, $p = 0.132$). There was a significant difference between functional groups for bend modulus ($F_{2,35} = 4.274$, p=0.022). The tree functional group had a significantly higher bend modulus than the herb functional group ($p = 0.012$, t-test).

Puncture measurements revealed variation in fracture moduli across the surface of individual leaves. The fracture moduli for the mid-vein was significantly greater than the fracture moduli for the lamina, across all leaves tested ($p < 0.001$, t-test, $n = 38$ leaves). The average thickness of the leaf was not correlated with the average fracture modulus ($R^2 = 0.001$) or the bend modulus ($R^2 = 0.033$). The average thickness of the lamina was not correlated with the average lamina fracture modulus ($R^2 = 0.055$). The average thickness of the mid-vein was not correlated with the average mid-vein fracture modulus ($R^2 = 0.017$).

Discussion

Correlating bend and fracture moduli with SLA

There was no relationship between specific leaf area (SLA) and the fracture and bend moduli across all species tested. Only 24% of the variation in fracture modulus and 14% of variation in bend modulus was explained by SLA. Additionally, no relationship was found between fracture and bend moduli and SLA within the functional groups tested, with the exception of the trees. A negative linear relationship was found between bend modulus and SLA for the tree functional group; 99.1% of the variation in bend modulus was explained by SLA. However, subsequent analysis revealed this to be non-significant, likely due to the low sample size. As such, we concluded that there was no relationship between bend or fracture moduli and SLA for the functional groups, in addition to the lack of relationship across all leaves tested.

We expected to see a relationship between SLA and the strength of leaves, as we anticipated that leaves with a higher biomass allocation would have greater biomechanical protection; however, this was not demonstrated in our results. This indicates that biomass allocation is not a driver of durability in alpine and subalpine plants. Therefore, alpine plant durability must be the product of another driving force. Additionally, this study only looked at the leaf component of plants and did not consider the durability of whole life-forms. Entire plant structure can reveal persistence strategies, for instance low-growing forms of cushion plants and feldmark utilise short stature to avoid the impact of high winds.

Other explanations for these results include leaf tissue density and costs of leaf replacement. Méndez-Alonzo *et al.* (2013) found that leaf mechanical properties depend strongly on tissue density. Tissue density was not investigated in this study; whilst SLA measures the ratio of leaf length to dry-mass, it does not measure tissue density. Alternatively, leaf toughness may be more closely related to the net cost of leaf replacement than to biomass allocation of leaves. Westbrook *et al.* (2011) found that slow-growing shade-tolerant juvenile plants had tough leaves which, although a high cost to replace, were negatively correlated with plant mortality rates. In this instance, the cost of replacing leaves was higher than producing tougher leaves due to the light limitations of preferentially growing in shade. Comparatively, the species tested in our study were not light limited; however, the low-nutrient content of alpine and subalpine soils may be an equivalent limitation in producing new leaves.

It is also possible that the small sample size (n = 38) of this study affected our results. Future studies with a greater number of test leaves and species would be beneficial in effectively testing the variation of toughness strategies in alpine and subalpine plants, with greater confidence in the results.

Understanding the biomechanics of plant durability will assist in predicting which species will benefit from changes to the environmental stresses of the alpine zone. Periodic severe stress can reorganise the structure of native Australian alpine vegetation; for instance, snow, drought and insect stress can lead to the damage of tall herbfield and grassland, which may be naturally replaced by broad-leafed herbs (Costin *et al.* 2000). However, due to the introduction of non-native species and the spread of invasive natives, these plant communities may be replaced by invasive species – particularly herbs – and may be unable to recover due to out-competition (Godfree *et al.* 2004). The most successful native and invasive species may be those with durable structures that can cope with harsh climatic fluctuations. This is particularly relevant due to the impact of climate change, which is predicted to enable lower elevation plants to migrate up the elevation gradient into the alpine and subalpine zones, as well as to reduce viable habitat for current alpine species (Pickering *et al.* 2008).

Furthermore, changes to the snow distribution, depth and duration due to climate change have implications for vegetation. A longer growing season is predicted to favour species with larger leaf size in snowpatch communities (Venn *et al.* 2011). Under normal snow conditions, snowpatch species benefit from the snow cover for protection from freezing temperatures and strong wind during the winter; however, change to snow duration exposes vegetation to greater temperature and dynamic force stresses, which may favour species with durable leaf traits.

Bend and fracture moduli

There was large variation in the fracture moduli of the leaves tested (SD = 2235.5 MPa). The average fracture modulus of the mid-vein was greater than that of the lamina. The greater mid-vein strength is indicative of greater investment into vascular tissues than photosynthetic tissues of the lamina. The average strength of the lamina and mid-vein did not correlate with thickness. The strength of the mid-vein, coupled with the lack of correlation with thickness, indicates that mid-vein strength is a product of its constituent materials.

A study into Australian woody evergreen species found that the cuticle was a dense, strong, stiff material, which contributed to lamina strength and rigidity (Onoda *et al.* 2012). Additionally, the authors theorised that the high input cost of a thick cuticle would be paid off by increasing leaf lifespan through withstanding external mechanical stresses. Alpine and subalpine leaves would benefit from protection against mechanical stress, as this would protect the leaves from the extreme climate and insect herbivory. The lack of flexibility found in our study may be indicative of strong cuticles. Alternatively, the lack of bend flexibility in our samples might be due to methodological errors caused by the size of the stand on which the samples were bent; leaves under 5 cm in length could not be properly affixed to the stand and therefore could not be correctly tested. In fact, 40% of the leaves tested were under 5 cm in length. Whilst this proved problematic for testing bend modulus, it also reveals that a large portion of alpine and subalpine leaves are small. Having a small surface area reduces water loss via evapotranspiration, reduces heat loss via transformation of heat into the air and reduces the chance of the leaf freezing (Givnish 1979; McDonald *et al*. 2003). Small leaf size is therefore likely to be an advantageous trait of alpine and subalpine leaves, due to temperature and water stresses. Investigation into leaf size and whole plant longevity and success would be an interesting area of future research in the Australian alpine zone.

The lack of correlation between mid-vein thickness and mechanical resilience suggests that a different structural attribute is responsible for durability in the leaves tested. Perhaps composition toughening plays a role in durability of alpine leaves. The primary source of toughening in plants is derived from cellulose microfibrils in either a hemicellulose or lignin matrix (Lucas *et al.* 2000). The cellulose content in alpine leaves could be tested and compared with leaves from other ecosystems. Toughness is a major defence against herbivory, although puncture resistance traits in leaves are only protective against chewing, not sucking, insects (Peeters *et al.* 2007). Additionally, silica acts as a deterrent to herbivory and reduces digestibility (Massey *et al.* 2007). The combination of cellulose-based structural

toughening and the presence of silica may contribute to alpine and subalpine plant toughness. Indeed, silica is readily available in the Ordovician parent bedrock of the Australian Alps, and this underlying material contributes heavily to the soil characteristics of the Snowy Mountains (Kirkpatrick *et al.* 2014). It may eventuate that compositional materials within alpine leaves are important for durability.

Functional groups

Of the leaves tested, nearly two-thirds were herbaceous, nearly one-third were shrubs, and the remaining 7% were from the tree *Eucalyptus pauciflora*. There was no difference in fracture modulus and SLA between functional groups but the functional groups did differ in their bend modulus. A common fracture modulus and SLA among varying functional groups from the same environment type may indicate a widespread alpine and subalpine strategy of leaf puncture toughness and biomass. SLA tends to be low in resource poor areas, which is indeed the case for the alpine and subalpine zone, and may therefore represent a homogeneous limitation for all the species tested. Differences in bend modulus across functional groups could be a product of the degree of dynamic force those species are typically subject to; due to their height, trees could face greater wind force than herbs and shrubs, which are typically low-growing in clusters and often protected by snow for a large portion of the year.

As mentioned previously, sample size posed a significant limitation in this study. Whilst the small number of tree species was unavoidable, as there is only one species present at the study site, the small number of samples within that functional group ($n = 3$) limited the power of our analyses. Furthermore, we attempted to look at broad-scale alpine and subalpine trends in leaf biomechanics with this limited sample size. A future study may benefit from narrowing the focus to a single species or functional group. For example, investigating the toughness of *Eucalyptus pauciflora* leaves across their maturity may reveal life-history strategies of leaf protection in this ubiquitous subalpine species.

Different plant communities from different climates have different structural properties (Onoda *et al.* 2012); they vary in terms of their structural and morphological properties that best enable them to thrive in their environment. Alpine environments have a large range of thermal climates and water availability. This mosaic leads to different strategies in the varying alpine and subalpine vegetation communities. Further research into alpine vegetation could take into account the variation of climate in microclimate zones of the alpine mosaic; for instance, the role of shrubs in protecting, and hence facilitating, less stress-tolerant species (Ballantyne and Pickering 2015). More broadly, it would be instructive to compare alpine and subalpine vegetation with other plant communities. Balsamo *et al.* (2006) showed that drought tolerance correlated with leaf tensile properties in *Eragrostis*. Likewise, it might be the case that alpine and arid plants, among other water-limited environments, share leaf tensile properties. Alternatively, plant toughness could be compared according to the herbivory pressure experienced by that vegetation community; for instance, temperate regions experience more mammal herbivory, whereas alpine herbivores are predominantly insects.

Conclusions

Plants with a low specific leaf area (SLA) tend to be long-lived, and as such have strategies for protecting valuable high resource-input tissues. We theorised that alpine and subalpine plants would protect leaves with high fracture and bend moduli traits. Leaf replacement is costly for energy and resources, therefore mechanical resilience can be an alternative strategy in environments with low nutrient availability. We found no correlation between fracture and bend moduli and SLA of the leaves tested. Mid-vein strength and material toughness was a consistent trait of the species tested, indicating a strategy for durable circulatory tissues. Leaf traits reveal plant responses to environmental conditions, with flow on effects for plant–animal interactions, due to the resulting tissue-toughness and nutrient availability effects on herbivory.

Acknowledgements

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