Trait variation and potential climate sensitivity of endemic alpine plants in Aotearoa

by

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Abstract

For alpine plants to persist under climate change they must be able to adapt to new temperature and water regimes in their current ranges or migrate upslope to track temperatures within their tolerances. In addition, climate change is predicted to move across landscapes at rates that exceed those of dispersal in many specialists and endemic alpine species[5]. These multiple and interactive drivers of environmental change make it difficult to predict future species ranges from environmental data alone. Plant trait plasticity may provide a mechanism for species to persist in the face of rapid environmental change.

In this study, I aimed to quantify variation in phenotypic plasticity in four endemic alpine species at local and bioregional scales, in order to make predictions about their potential to respond to climate change. Eight trait variables associated with plant life history strategies and resource acquisition were measured, and permutation tests used to detect significant variation in trait values with elevation. Plasticity was seen in all species, indicating all may have some 'bandwidth' to able to adapt with rapidly changing mountain environments. I then discussed the resulting trait–environment relationships and what they may mean in regard species persistence under global climate change.

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Introduction

Globally, mountains make up ~24% of global continental land surface and provide essential ecosystem services, such as fresh water and flood regulation, to lower altitude regions [1–3]. These benefits serve more than just the communities in mountain foothills – more than half the fresh water in the world originates in mountain chains [1]. Under anthropogenic climate change, mountains and their associated ecosystems have been warming more quickly than lowland regions, and their complex topography combined with altitude is predicted to result in more pronounced impacts than other biomes [4,5]. Due to past geographic events, mountains are also home to plant communities with high degrees of local and regional endemism [2–4,6].

In mountains, the alpine zone is the region above the treeline and below the permanent snowline. The plant communities that typically inhabit temperate alpine zones – such as those in Aotearoa – are characterised by low statured plants which have evolved to tolerate the harsh environmental conditions [7–10]. Plants, on the whole, are unable to move to escape unfavourable environmental conditions in the short term, and will only survive if they evolve physiological and morphological traits to cope with their environment which enable their survival and reproduction. Any plant that exists today is a product of millennia of evolution in the climatic and environmental contexts experienced by its lineage, and plants that occupy environments considered harsh by humans have responded with a high degree of specialisation [11–14]. The effects of climate change on alpine zones threaten to drastically change the environmental conditions that these species have adapted to.

Climate change induced warming of alpine zones is accompanied by upslope range expansion of woody plant species, increases in nitrogen deposition, changes in snowfall and snowmelt timing, soil microbial changes, and altered precipitation regimes [4,15–17]. To compound potential effects on alpine life, these factors can also act synergistically, and alterations in abiotic conditions are predicted to shrink alpine ecosystems by ~63% globally by 2100 at intermediate warming projections of ~3.3°C [5,13,18,19]. The steep topography of the islands makes the situation particularly acute in Aotearoa, which faces losses of ~80% of its alpine zones [4]. Alpine regions face acute pressures from the combined effects of climate

change, yet the responses of plants inhabiting these environments remains poorly resolved [4,11,12].

For alpine plants to persist under climate change they must be able to adapt to new temperature and water regimes in their current ranges or migrate upslope to track temperatures within their tolerances [20–23]. Both scenarios are complicated by the concurrent range expansion of other species into alpine ecosystems, be they indigenous or exotic [24,25]. In addition, climate change is predicted to move across landscapes at rates that exceed the rates of dispersal of many specialist and endemic alpine species [5]. These multiple and interactive drivers of environmental change make it difficult to predict future species ranges from environmental data alone.

Traits and trait plasticity

For centuries, ecologists have sought to disentangle the adaptations of species to their environment and the resulting variation in individuals, populations, communities and ecosystems; a pursuit that can help to potentially predict plant responses to future climate scenarios across these same scales [14,26–29]. One approach involves measuring plant phenotypic plasticity. A plants phenotype is directly reflected in its functional traits; these being the morphological, chemical, and phenological responses of plants over time to their environment, all of which are measurable [14,26,30]. In other words, quantifying the traits of a species and determining whether those traits are adaptive, reflects the species life history strategy [14,29,31,32].

Phenotypic plasticity of plant traits is a response to environmental cues, but the traits that respond and the degree to which they can change, is governed by plant genetics [33]. Plasticity can be adaptive (promotes individual fitness), neutral (provides neither benefit nor adversely affects individual fitness), or non-adaptive (adversely affects individual fitness), and there is debate in the literature concerning when plasticity either prevents adaptation of genotypes, or provides opportunities for selection to act on [34–37]. In general, if the response of a functional trait to environmental cues results in stable, or increasing, resource acquisition (or other fitness measures), it is considered to be adaptive [34,36]. For example, specific leaf area (SLA; the mass of a leaf by its photosynthetic area) is considered to be

adaptive, as it indicates a coupling of traits to optimise light capture and biomass allocation [38–40]. A growing body of research is finding SLA, among other commonly measured leaf traits, to have largely predictable responses to environmental conditions in the direction of an increase in fitness for individuals [41]. However, the responses of SLA have not been found to be adaptive across all species and environments [40,41], suggesting that further research into species specific trait responses is necessary in order to build an understanding of potential genus and family-wide responses.

Three-quarters of all variation in plant traits can be attributed to a spectrum of plant growth forms and the leaf economics spectrum (LES) [42]. The LES is the trade-off in plants which balances growth potential of the whole plant against the resource cost to produce leaves [42,43]. LES is well established for leaves of plants adapted to full light, and recent research is showing shade adapted species to follow the same pattern [44]. SLA is one of the most important and well-studied leaf functional traits on the LES [42,45–47], and is strongly correlated with leaf longevity, stress tolerance, and relative growth rate [48,49]. Plants with higher SLA values have more leaf surface area per unit of biomass invested in leaf organs [50]. It can be a highly variable trait within a species and within lifespan adaptability to maximise photosynthetic capabilities confers considerable fitness advantages. Higher general SLA values and responsiveness to environmental variables has been found to be a common feature in invasive plants [51,52], and SLA has consistently been shown to decrease with elevational and temperature gradients [39,49]. Specific root length (SRL; the mass of a root by its absorptive length) is commonly considered to be the belowground equivalent to SLA but the drivers of morphological responses in plant roots are still being resolved [53,54], though Ostonen et al (2007) did find that SRL in woody species responded negatively to elevated CO_2 and temperature [55].

Plant growth form refers to the size, volume (biomass), and habit (how a plant grows) of a species. Plant growth form is one of the key features that is selected upon by the alpine environment, so although there is a wide variety of growth forms globally, in constrained environments such as alpine zones there are only a limited number of functional (trait-based) survival strategies. Only a few leaf shapes are suited to alpine conditions, and the number of plant growth forms present in all alpine zones globally is 10 [8,11,56]. For vascular plants, these forms include low stature or prostrate woody shrubs, tussock forming grasses

and sedges (graminoids), rosette forming herbaceous perennials (forbs), and cushion plants [11]. The short stature and dense growth habits of woody shrubs and cushion plants create microhabitats which experience spring temperatures similar to lowland communities [8,10,57], which then facilitates the germination of seeds blown into the dense foliage [8,11,58]. Some species present in high elevation sites may only be there because of their arrival in one of these microhabitats, and are otherwise outside their environmental optima without them.

Measurable functional traits that are associated with plant growth form include biomass, plant height and lateral spread. Plant biomass is a direct result of photosynthetic and resource gathering activity. Where a plant allocates biomass to, and in what proportions, reflects the growth strategies of the species to aid survival, and both above and belowground biomass have been shown to be affected by environmental conditions [59,60]. Consequently, most LES traits closely correlate with biomass distribution, total biomass, and plant size [42].

Traits associated with the concepts of growth form and LES are often correlated with one another, *i.e.* one trait will change in response to the environmentally induced changes in another. For example, SLA and root mass fraction (RMF; a measure of plant aboveground versus belowground biomass distributions) change in tandem in order to balance resource demands, *i.e.* higher SLA equals higher photosynthetic rates which results in higher water and mineral nutrient demands from belowground organs in order to create more biomass with the products of photosynthesis [61]. Some of these products can be passed to symbionts in the soil, but a more efficient response is to manage biomass and resource acquisition to minimise waste. Some traits will also change as a by-product of adaptive plasticity in other traits, but are not adaptive themselves. Where this cannot be tested for genetically, trait values with a large variance most often reflect non-adaptive responses, and are likely a product of ties to other adaptive traits [62]. However, ongoing research is finding that LES traits aren't constrained by plant growth form, making predictions of LES responses based on plant growth form unviable [63,64].

Some traits are more plastic than others, and whether a particular trait evolves to respond to environmental cues plastically depends on the degree of environmental variation experienced by that trait [65]. In alpine landscapes, environmental conditions are highly variable at small scales, so ecological theory suggests there are advantages to expressing

more variable phenotypes [66,67]. This is reflected in the theory that plant species tolerate a range of different local environments by expressing trait values that are optimal for each location (*i.e.* a trait–environment relationship). For instance, many tree species that occupy montane environments are typically tall trees at lower elevations (within their environmental optima) but become shorter and wider in stature and form smaller leaves when they occupy higher altitudes [10]. Phenotypic plasticity in height and leaf area therefore enables these tree species to extend the range of environments in which they can live, allowing survival outside the species' environmental optima. In some cases, the tree may not be able to collect the resources needed to reproduce, however it does demonstrate that the capacity for plastic responses can confer high environmental tolerances. In alpine landscapes, variation in slope, aspect, and rock cover over a few metres can influence landscape scale patterns of temperature, wind exposure, radiation load, snow distribution and water run-off, requiring plants as individuals and communities to be adaptable within a lifecycle as well as across generations [2,7,68]. A seed may find itself in a very different environment to the parent plant even if it is only a few meters from it, and physical disturbances may result in adult plants being exposed to new conditions after several years of a particular microhabitat. This ability to alter certain characteristics within a life-time to adapt to otherwise suboptimal conditions, or maximise optimal ones, can confer a considerable fitness advantage to both individuals and species [69].

Traditionally, functional ecology (the study of traits contributing to the fitness of organisms) has focused on community measures of trait variation in an effort to understand bioclimatic drivers of plant community assemblies and to build vegetation-environment models [70]. These models aim to predict community and ecosystem changes in response to climate change effects on abiotic factors. Comparatively recently, the variation in functional traits within a species (intraspecific variation) has become a steadily increasing area of research to complement, inform, and expand modelling. By studying the changes in traits, and scaling of correlated traits, within individual species and linking those to environmental changes, researchers can look for patterns in variation that may be applied to families, ecosystems, or growth forms. Finer scale, species level data adds more certainty to modelling, and can inform conservation decisions concerning rare plants or ecosystems.

Local vs. bioregional trait variation

While intraspecific variation of traits along local bioclimatic gradients has been an increasingly common subject of research, broader scale biogeographical studies to complement them have been less common. This has left a gap in attempts to understand the drivers of phenotypic plasticity across scales, as intraspecific traits are consistently shown to respond independently of community level traits in response to environmental gradients [71,72]. This suggests that phenotypic plasticity at local scales may obscure abiotic linkages to traits, and that variation in LES traits cannot always be reliably interpreted as an adaptive response in order to increase resource capture [71]. To disentangle the true extent of adaptive variation within species, studies quantifying changes in functional traits at broader scales are necessary [73,74].

Bioregions are biogeographically defined areas with distinct climates, precipitation regimes, and ecological assemblages. Functional biogeography is the study of the geographic (or bioregional) distribution of trait variation and diversity within and between species [74,75]. This approach is essential in order to synthesise plant trait–environment relationship patterns across scales and provide accurate data to produce generalisations applicable to community level responses to changing environments [74,76,77]. By associating trait variation with environmental and community factors bioregionally, researchers can link the current distributions of species to predicted abiotic changes in the environments they are found in to model future distributions [74,77]. Trait-based bioregional studies can also help model species physiological tolerances more simply [74].

When adaptive, phenotypic plasticity directly contributes to the ability of a species to persist and reproduce when faced with novel environments. As climate change progresses through the 21st century, all species will eventually be subject to changes in the environments they evolved in [34]. By utilising functional ecology to assess the trait–environment relationships within species across spatial scales, we can produce more reliable predictions at each level. At a species level, it becomes more possible to predict the ability of taxa to respond to changes in the environments which they currently inhabit, and the extent of change they can persist under. At community and ecosystem levels, trait-based approaches can aid in

producing models to map predicted changes in species and community distributions in response to climate change, and at biogeographic and global scales, adding species specific data can greatly improve predictive vegetation–climate modelling.

Study aims

Trait functional ecology is increasingly being used to determine how a species, genus, or family of plants may (or may not) be able to adapt to climate change as it progresses. These studies are important, as phenotypic plasticity may be a vital component of the ability of species to endure rapidly changing climates, and species scale data feeds into community and ecosystem level predictions [78]. This is a rapidly progressing field, but there is still a bias in alpine specific studies towards Northern Hemisphere and tropical mountain ranges, and studies examining intraspecific trait variation across biogeographic ranges are still few [74,79]. In addition, studies on trait variation in the indigenous plants of Aotearoa are currently scarce.

For this study, I sought to understand local and regional trait variation in four alpine plant species endemic to Aotearoa. I characterised the trait–environment relationship for nine traits and compared the variation in trait values expressed locally to that expressed over bioclimatic zones (bioregions) nationally. I explore the environmental variables potentially driving patterns of variation in plant traits in the bioregional data. Finally, I consider what the observed trait–environment relationships might infer about species tolerances to future climate warming.

By analysing trait data among bioregions, I will be able to determine if there are broad responses of plant traits either within bioregions or across Aotearoa generally. This data, contrasted with localised data, will help to quantify the link between the environment and traits within these species which is important to understanding the consequences of climate change on species and communities. For example, should a species show trait plasticity in specific traits across bioregions, it may correlate with the ability to phenotypically adapt to temperature shifts and possibly biotic invasion, depending on the traits that respond flexibly. Trends in trait variation across broad scales also strengthen assumptions of adaptive phenotypic plasticity in a species.

Herbaria remain an under-utilised tool for understanding species phenotypic plasticity and variable responses to climate and environment [80,81]. As sampling each species across

Aotearoa was not practicable, herbarium specimens were used to collect bioregional trait data in a more timely manner.

Study questions

Using this approach, I aim to answer the following questions:

1. *How do the traits of each species differ along a single elevation gradient?*
2. *How do these traits differ across bioregions of Aotearoa, and do they differ from the changes seen in the localised study?*

As this study is focussed on intraspecific trait variation, I developed hypotheses for each species for question one, which will be addressed in the proceeding section.

However, functional biogeography is a comparatively new field [74] so relatively little research is available to inform specific hypotheses for question two. In light of this, I propose two hypotheses based on the potential habitat generalism or specialism of each plant.

Given that local populations will be genetically related and theoretically more similar in the extent of variation their genotypes can express, I hypothesise that generalist plants will exhibit greater trait variability at bioregional scales than local, but that the traits that vary will be the same. Generalist plants in this study are *Celmisia gracilenta* and *Rytidosperma setifolium*.

As introduced earlier, research has found that specialist alpine plants express high local variability due to their evolutionary history in landscapes which are climatically distinct but highly heterogeneous at small scales. For specialist species, I hypothesise that plants will exhibit higher local variability comparative to bioregional variation. Specialist plants in this study are *Gentianella bellidifolia* and *Luzula colensoi*.

Study species

All species chosen for this study are endemic to Aotearoa, present across both Te-Ika-a-Māui and Te Waipounamu (South Island), and consistently present above 1,200m elevation across their geographic ranges. Additionally, all are long-lived and wintergreen; meaning plants do not drop their leaves and set new leaf buds before the growth season is over. This is ideal for leaf measurements, as traits will be less affected by seasonal differences as leaves do not need to unfurl and expand over the growing season [48].

Celmisia gracilenta

Figure 1. Celmisia gracilenta growing at 1560m elevation on Tukino slope

Known in Te Reo Māori as pekapeka, *Celmisia gracilenta* is a small composite forb in the Asteraceae family [82]. The species is recorded as being highly variable, with large ranges in reported physical sizes. Preferred habitats are recorded as lowland to low alpine, and the species is present from sea level up to 1700m [83].

The highest recorded presence for *C. gracilenta* at Tukino was 1595m; the lowest was in the Rangipo desert at 1085m. Species abundance appeared to be influenced by microtopography and the presence of cushion forming plants.

Although *C. gracilenta* has a high variability in morphological traits reported, these annotations do not often include details of observed changes in relation to habitat. This tendency, combined with its presence at a broad elevational range across Aotearoa suggests the species is an alpine generalist, and may possess a reasonable degree of trait plasticity to be able to inhabit a broad selection of cold habitats.

Local variation hypothesis: I hypothesise that *C. gracilenta* will express local trait variation in LES traits but with larger variance, suggesting a lower likelihood of genetically controlled adaptive response to specific environments.

Gentianella bellidifolia

Figure 2. Gentianella bellidifolia growing at 1700m elevation on Tukino slope

Gentianella bellidifolia is a small, rosette forming non-composite forb in the family Gentianaceae [84]. It is also reported as highly morphologically variable, with several forms previously considered subspecies recently synonymised under *G. bellidifolia* [85].

Preferred habitats are recorded as sub- to high alpine, with the species present from 600 to 1700m [83]. The highest recorded presence at Tukino was 1817m, while the lowest was 1085m. The species appeared to be most abundant from ~1300 to ~1700m.

In contrast to *C. gracilenta*, observations on physical variability also include remarks on variability occurring in conjunction with habitat, which is suggests potential adaptive phenotypic plasticity. Though *G. bellidifolia* is common throughout alpine regions in Aotearoa, it has a more constrained elevational presence and locally restricted populations occur in some areas [83].

Local variation hypothesis: I hypothesise that *G. bellidifolia* is an alpine specialist that will express local phenotypic plasticity in both growth form and LES traits across a range of traits with low variance, as it is likely to have evolved within complex heterogeneous alpine environments and be more tightly adapted to them than a generalist species. Traits are also more likely to increase along the elevation than decrease as a result of corresponding decreases in temperature.

Luzula colensoi

Figure 3. Luzula colensoi growing at 1820m elevation on Tukino slope

Luzula colensoi is an alpine rush in the family Juncaceae, which forms dense cushions of rosettes up to 2.5cm tall [86]. Habitat preferences are described as low to high alpine, present at 1200 to 2000m [83].

The highest recorded presence at Tukino for *L. colensoi* was 1995m, while the lowest was 1285m. It is the only species which appeared to not be present at the lowest point of Tukino slope in the Rangipo desert (1085m).

Given its comparatively narrow range and high elevation presence at Tukino and geographically, it is likely that *L. colensoi* is also an alpine specialist. The species also possesses traits suggesting evolutionary adaptation to cold environments with high winds, such as a dense cushion habit comprised of small leaved rosettes, and flowering stems which remain sunk in the centres of the leave rosettes, which do not extend past leaves even when the fruit matures. Reported variation in physical trait sizes is small.

Local variation hypothesis: I hypothesise that *L. colensoi* will express local phenotypic plasticity across growth form traits accompanied by low variance, with trait values more likely to increase with elevation in response to lower temperatures.

Rytidosperma setifolium

Figure 4. Rytidosperma setifolium growing at 1890m elevation on Tukino slope. Anisotome aromatica present in the foreground

Also known as bristle tussock, *Rytidosperma setifolium* is a short, yellow-green to bright green grass in the family Poaceae [87]. It forms dense clonal tussocks which are ubiquitous at all elevations at Tukino, where its lowest elevation was in the Rangipo desert at 1085m and its highest was 1995m. It has the widest apparent elevational tolerance of all species sampled, and was present in high abundances at all sample sites.

Preferred habitat is described as lowland to high alpine, and the species is present from sea level up to 1700m [83].

Rytidosperma setifolium is described as widespread but most common in nutritionally depleted habitats such as rockfields and fellfields. This tendency indicates that soil factors will have the largest influence on LES traits, while its broad elevational range distribution both at Tukino and throughout Aotearoa suggests temperature will limit traits related to growth form in the opposite direction to more cold adapted species.

Local variation hypothesis: I hypothesise that *R. setifolium* will show a decrease in growth form traits in response to elevation, and an increase in traits related to the LES. I also expect traits to have a larger variance, due to its broad thermal tolerances and habitat generalism.

Figure 5. Rangipo Desert looking towards Maunga Ruapehu

Methods

Whole plant sampling for this research took place at five sites along Tukino slope on Maunga Ruapehu – an active volcano in Tongariro National Park, on the central volcanic plateau of Te-Ika-a-Māui (North Island). The park is famous for its high winds, with the name Tongariro meaning "taken by the cold south wind". Mean annual temperature across the park as a whole is 1.5°C warmer and mean annual fall is 5mm less than past years [88,89].

Tukino slope is located along the leeward eastern face of Ruapehu, meeting the Rangipo desert at the base of the mountain (600-1000m elevation). The upper reaches of the slope have a median annual air temperature of 6° C, and are characterised by large open swathes of volcanic gravelfield supporting very low growing plant communities in sheltered or stable sites [90,91]. The lower slopes transition to finer, sandier soils with no gravel top layer, which are strongly shaped by fluvial and aeolian processes [91]. The median annual air temperature here is 8° C, and plants form larger, more dense communities where taller species can persist. Summer temperatures can reach 25° C. Rangipo desert experiences around 270 ground frosts per year. These can occur at any time of the year and this freeze-thaw cycle is a greater danger to plant tissues than an insulating snow pack over winter. Soils across the whole face are porous and very well-draining, so while annual rainfall is not low (1,200–2,500 mm per year), water does not remain in the soil for long, and this drying effect is exacerbated by the prevalent north-westerly winds [91].

Sites

Samples were collected over two seasons in January and December of 2020. The highest, lowest and median elevations where each species was present along Tukino slope were identified, and five sites chosen along the slope to capture all species distribution ranges efficiently while minimising sampling locations (Figure 6, Table 1).

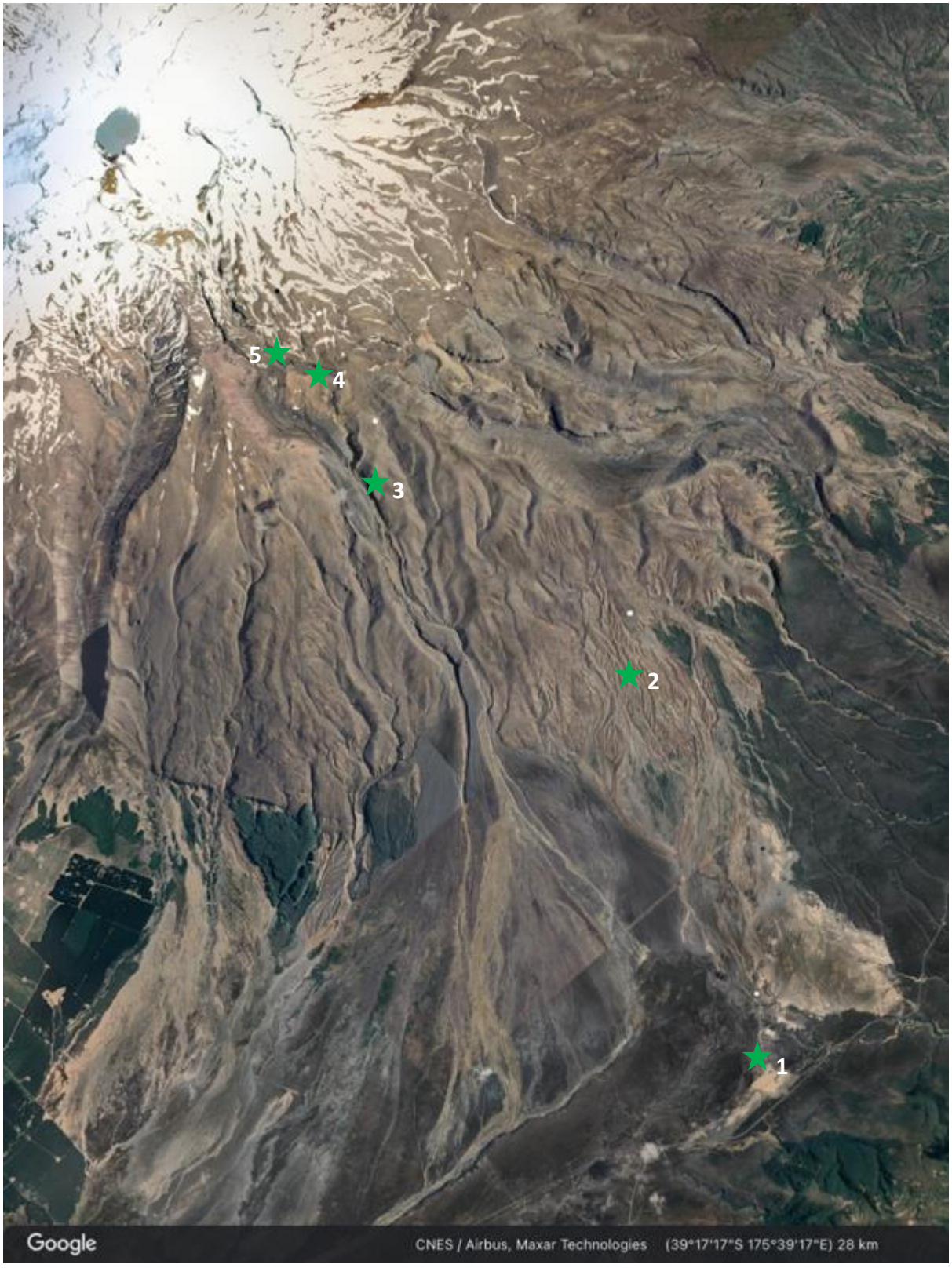


Figure 6. Map of sampling sites along Tukino slope

Table 1. Site elevations and corresponding elevation range points for all species, followed by distance and elevation gain for each species range

Site #	Elevation	*Celmisia gracilenta*	*Gentianella bellidifolia*	*Luzula colensoi*	*Rytidosperma setifolium*
1	1070m	low	low		low
2	1285m	mid		low	
3	1598m	high	mid	mid	mid
4	1806m		high		
5	1930m			high	high
Distance	~11.19km	~9.12km	~10.74km	~7.16km	~11.19m
Elevation gain	860m	528m	736m	645m	860m

Field sampling

Each site was found by hiking to each elevation along the slope and identifying an area within a 10m elevational band that contained all required species for collection at that elevation at reasonable abundances.

The plants sampled for this study were representative of the average population size for the site. Chosen plants were measured for the highest vegetative point above the soil, and then harvested by digging directly downwards at an approximately 10cm radius around the plant until the majority of root biomass ceased at ~20cm [92,93]. Roots extending beyond this radius were eased out as far as practicable. Samples were then refrigerated at ~4°C in sealed Ziploc bags between damp tissues within four hours.

Field sample traits

From the whole plant samples collected from Tukino, nine traits from each species were measured; seven aboveground, and two belowground traits (Table 2).

Aboveground traits chosen were; biomass, height, leaf area, leaf width, leaf length, leaf thickness and specific leaf area (SLA). Belowground traits chosen were biomass and specific

root length (SRL). By measuring multiple correlated traits, I will potentially be able to see if some trait values are driving others, *e.g.* changes in aboveground biomass may increase concurrently with SLA or leaf thickness, suggesting that the gain in biomass is being driven by thicker leaves upslope.

Table 2. Traits, abbreviations and units of measurement for whole plant samples from Tukino (local variation)

Trait	Abbreviation	Unit	*Celmisia gracilenta*	*Gentianella bellidifolia*	*Luzula colensoi*	*Rytidosperma setifolium*
Aboveground biomass	**ABV**	g	✿	✿	✿	✿
Leaf area	**AREA**	mm^2	✿	✿	✿	✿
Belowground biomass	**BLW**	g	✿	✿	✿	✿
Vegetative height	**HGT**	cm	✿	✿	✿	✿
Leaf length	**LEN**	mm	✿	✿	✿	✿
Specific leaf area	**SLA**	mm^2-mg	✿	✿	✿	✿
Specific root length	**SRL**	cm-mg	✿	✿	✿	✿
Leaf thickness	**THK**	mm	✿	✿	✿	
Tillers	**TILL**	count				✿
Leaf width	**WDT**	mm	✿	✿	✿	

Lab methods

Once in the lab, samples were gently cleaned under tap water, separated into above- and belowground portions, and stored in separate Ziploc bags with damp tissues to maintain turgor. Plants kept this way have been shown to last up to 3 weeks [85].

Three to six fully expanded leaves were removed from each sample for leaf trait measurements [92]. Each set of leaves were then measured for thickness using a IP65 Mitutoyo External Micrometre before scanning on an Epson V370 flatbed scanner at 1200 dpi [92]. For *G. bellidifolia*, thickness was measured between the leaf margin and the midrib at the widest point of the leaf, while measurements from both *C. gracilenta* and *L. colensoi* were taken from the centre of their length and width. Leaf thickness was not measured in *R. setifolium*. Whole tiller sets were counted for *R. setifolium* samples before leaves were removed for scanning.

Roots were sieved under tap water until clean of soil, then 1–2 full length segments were selected for scanning. For the forb species (*C. gracilenta* and *G. bellidifolia*), preference was for sections with the most fine-root mass present. *Luzula colensoi* and *R. setifolium* roots are mostly uniform in size and appearance, and neither species possess differing belowground structures for extra nutrient storage. Root sections for SRL were laid flat in a wide, shallow petri dish (15cm diameter) half-filled with distilled water and scanned at 1200dpi [92]. Remaining belowground biomass and root sections were stored in separate paper bags and dried in a ConTherm at 72C for 2 days. All dried samples were then weighed on an ABT 220-4M Kern precision balance.

Remaining trait measurements were carried out on the scanned images using ImageJ software [94] Prior to processing, leaves of *C. gracilenta* were outlined in white using Adobe Photoshop [95], as glare from the scanner lights made it difficult for ImageJ to accurately locate the edges of the leaves. Leaf width (mm) was measured at the widest point of the leaf for all species except *R. setifolium*. Leaf length (mm) excluded petioles for all species except *G. bellidifolia* [85].

Leaf area was recorded in millimetres squared. Specific leaf area is the ratio of leaf area to dry leaf mass and is calculated as:

SLA = total leaf area (mm2) / total leaf weight (mg)

One dimensional root length was measured in millimetres. Specific root length is the belowground equivalent ratio to SLA and is calculated as:

SRL = total root length (cm) / total BLW mass (mg)

Herbaria methods

Herbaria samples were sourced from collections at Te Papa Tongarewa, Manaaki Whenua (Landcare Research), and Auckland Museum. Herbarium specimens were removed from the sample set if they had insufficient information on the location of sampling to derive the elevation of the sample. This excluded all samples collected prior to 1980 as well as many

samples collected post-1980. All specimens which met the location criteria and were in good condition were measured. A full set of trait data could be collected for of *C. gracilenta* and *G. bellidifolia.* A partial set of measurements for *Rytidosperma setifolium* were possible. *Luzula colensoi* was poorly represented in herbarium samples and it's cushion growth habit led to poor preservation of the traits of interest, consequently it was excluded.

Eight traits were measured for herbarium specimens, but for the purpose of this study, three traits were used that overlapped with traits measured for the local variation dataset. Doing this allows for direct comparisons in variation between the same traits and simplifies variables involved. Traits are; leaf length, leaf width, vegetative height (Table 3). Only *C. gracilenta* and *G. bellidifolia* were measured.

Table 3. Traits, abbreviations and units of measurement for herbaria samples (bioregional variation).

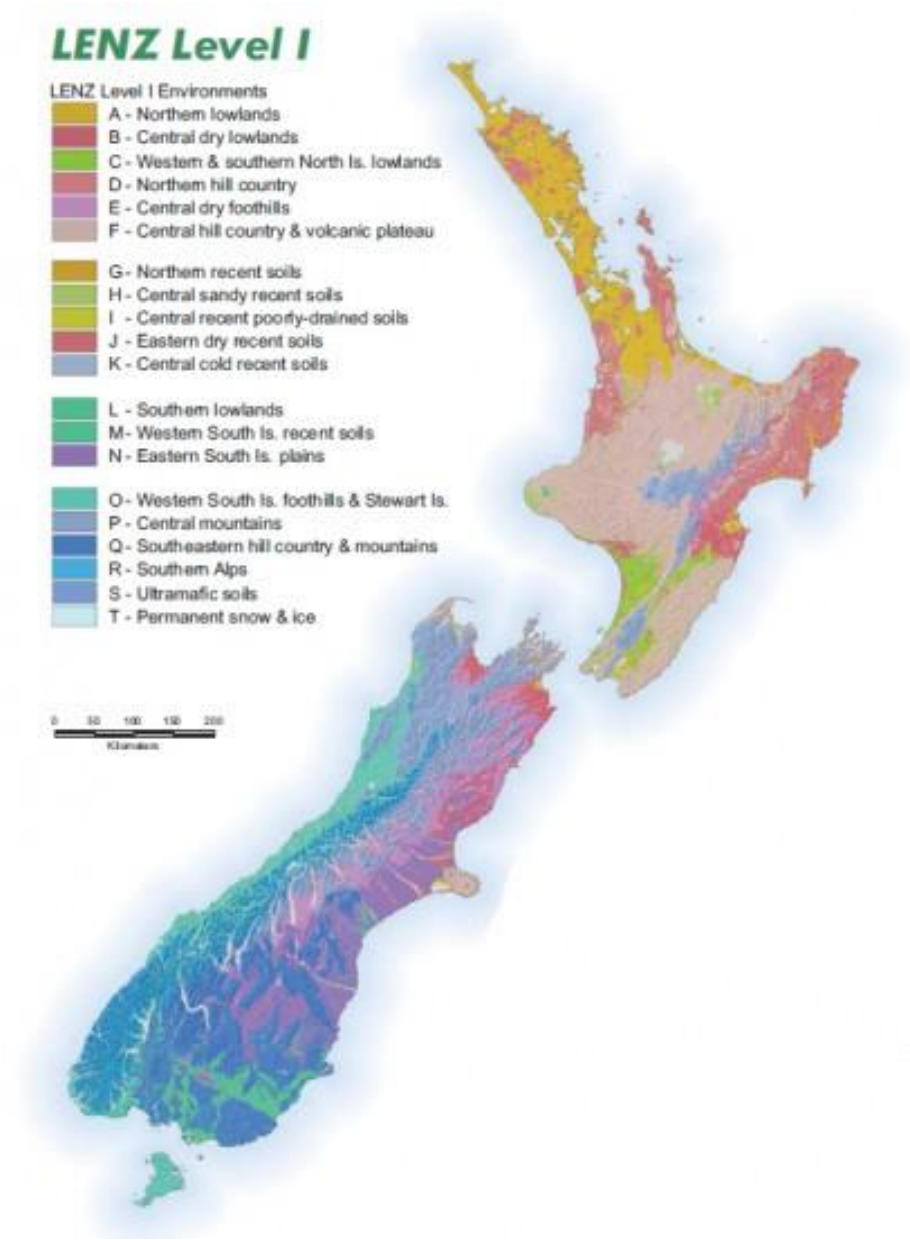
Trait	Abbreviation	Unit	*Celmisia gracilenta*	*Gentianella bellidifolia*
Vegetative height	**VEG**	mm	✿	✿
Leaves	**LEA**	count	✿	✿
Leaf length	**HLEN**	mm	✿	✿
Leaf width	**HWDT**	mm	✿	✿
Culm length	**CUL**	mm	✿	✿
Flowers	**FLW**	count	✿	✿
Stem width	**STW**	mm	✿	
Rosette width	**RSW**	mm		✿

Icons denote which traits were measured for each species.

All specimens were photographed on sheets at each herbarium using in-house imaging equipment, and the images were used to measure traits. As with Tukino samples, images were processed in ImageJ, and all measurements were recorded in millimetres.

Environmental data and bioregions

Land Environments of New Zealand (LENZ) Level II environments were used for bioregion differentiation of herbaria samples. Using numerical data layers from multiple datasets to classify fifteen aspects of climate, landform and soils, LENZ is a map of the key environmental types found across Aotearoa. It was created with the intent of identifying abiotic factors likely to influence the distribution of species [96,97].



LENZ data is available at 4 classification scales; Level I (Figure 7) contains 20 environment groups which consequent levels are nested within at increasing scales of detail. Level II contains 100 environment groups. Herbaria specimens were mapped against LENZ Level II using recorded latitude/longitude in Google Earth Pro [98] (Figure 8).

Figure 7. Map of environments described in Level I (20 environment groups). Image credit: Manaaki Whenua, 2021.

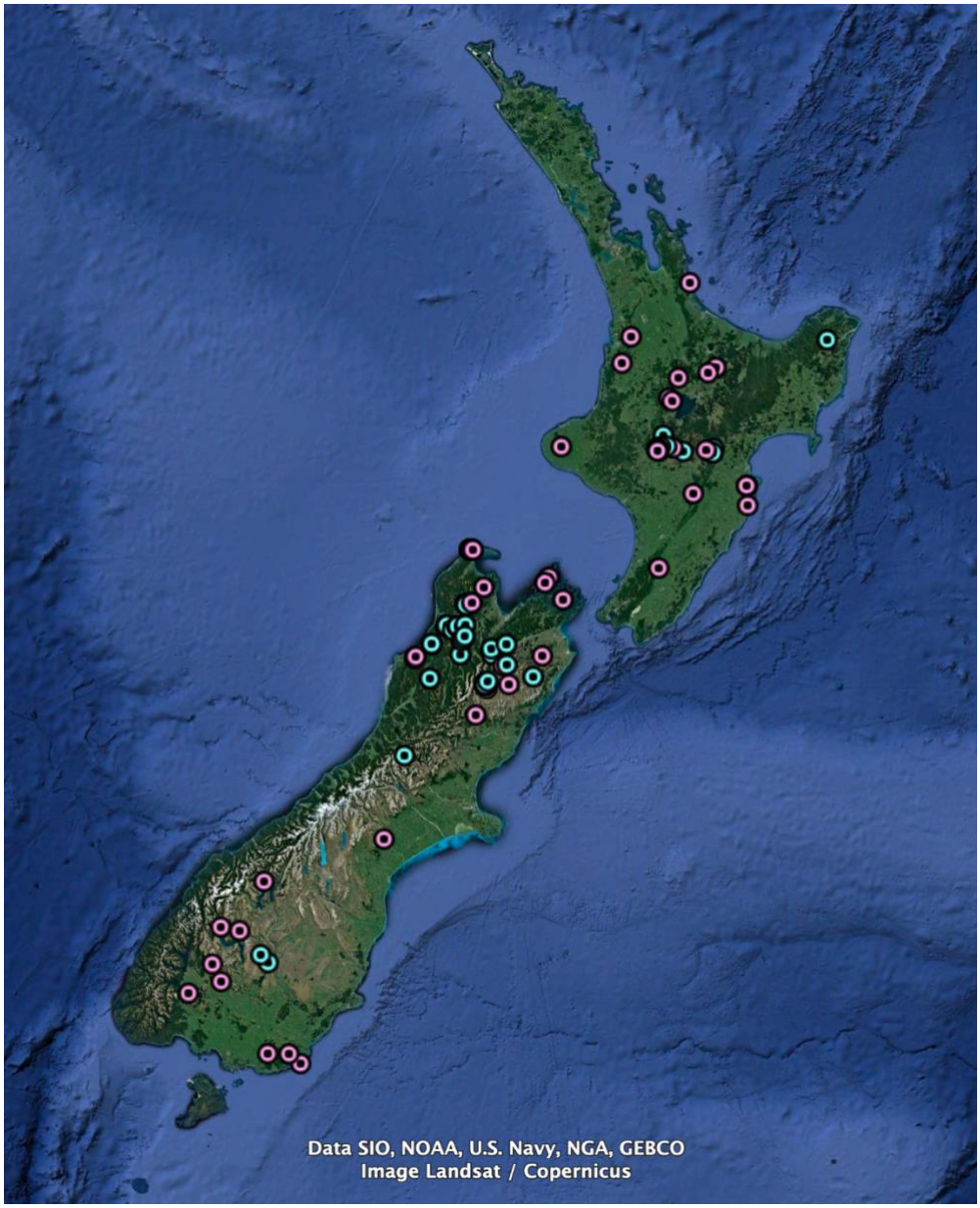


Figure 8. Map of herbarium specimen locations across Aotearoa.
Pink icons denote Celmisia gracilenta, blue icons denote Gentianella bellidifolia.

Figures 9 to 12 show the distributions across Aotearoa of all imaged herbaria specimens. This includes specimens where a precise collection location was unknown, so the location was entered as the centre of the mountain range given by the specimen collector.

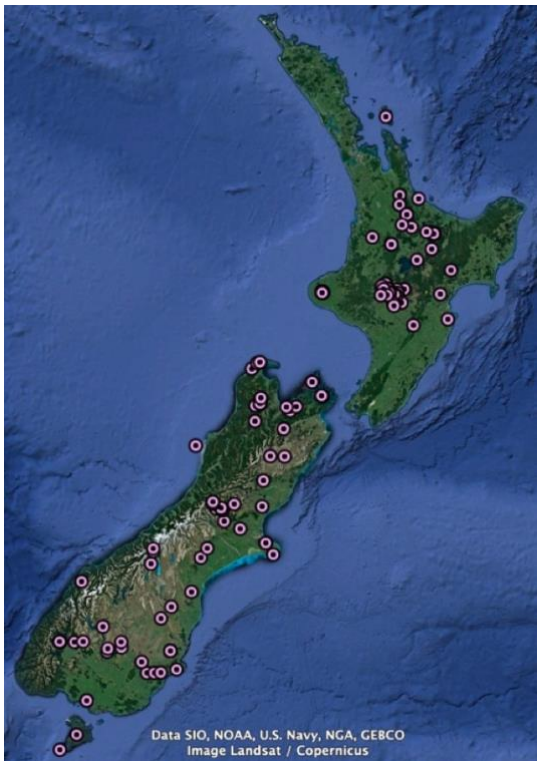


Figure 10. Distributions of all imaged herbaria specimens of Celmisia gracilenta

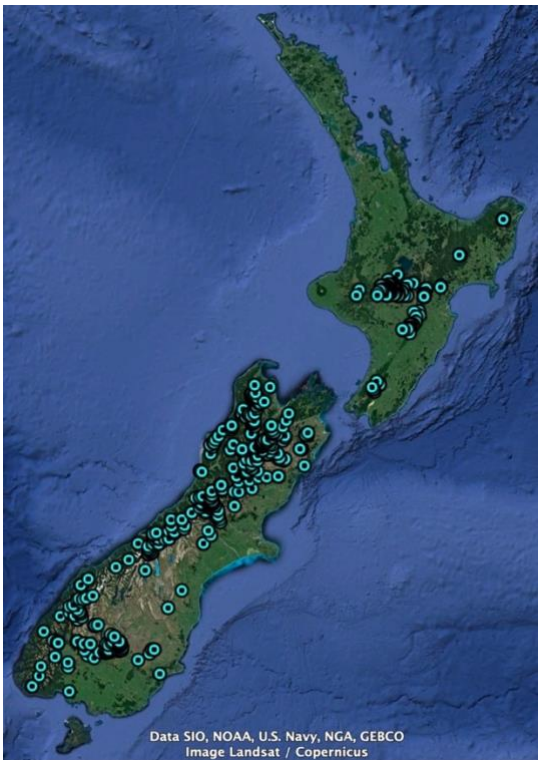


Figure 9. Distributions of all imaged herbaria specimens of Gentianella bellidifolia

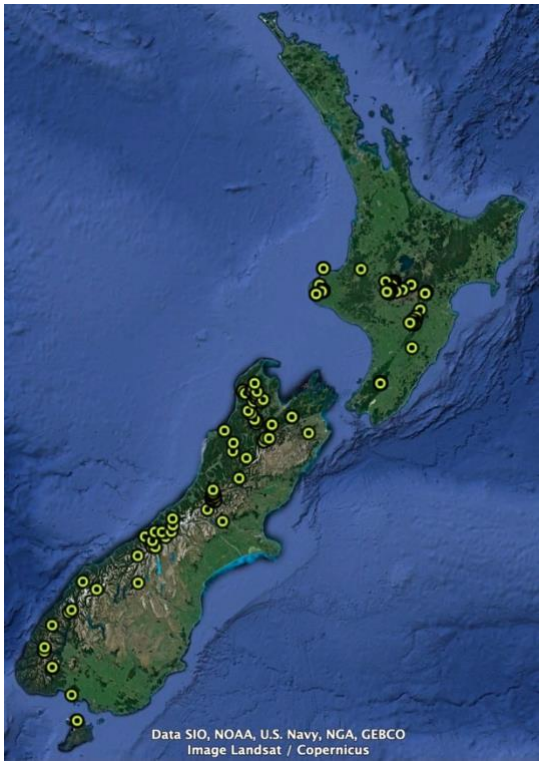


Figure 11. Distributions of all imaged herbaria specimens of Luzula colensoi

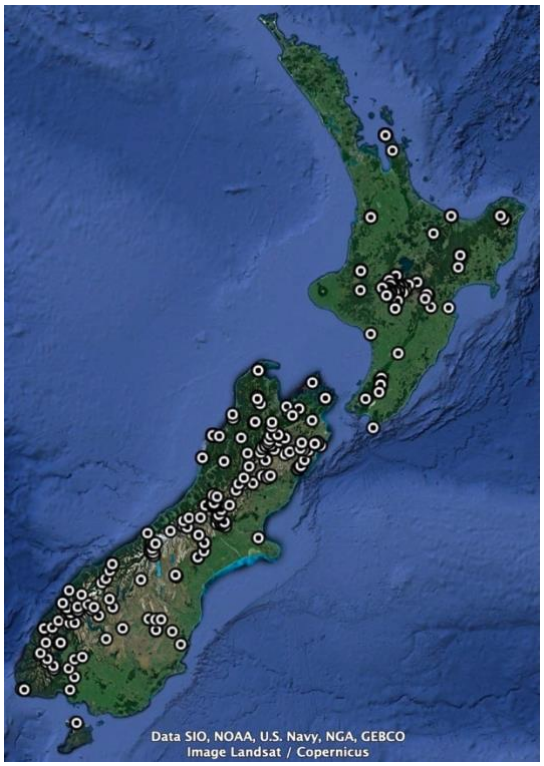


Figure 12. Distributions of all imaged herbaria specimens of Rytidosperma setifolium

The climate variables encompassed in the LENZ methodology are: mean annual temperature, mean minimum temperature of the coldest month, mean annual solar radiation, mean winter solar radiation, October vapour pressure deficit, mean annual water deficit, and monthly water balance ratio. Temperature and solar radiation levels influence plant potential productivity within a growth season. Vapour pressure deficit refers to air "dryness" and has a linear relationship with plant evapotranspiration rates. Mean annual water deficit is the sum of any deficits between rainfall and potential evaporation and signifies potential drought limitations to plants (*i.e.,* a larger deficit equals a higher likelihood of plants enduring drought conditions in a year). Monthly water balance ratio is the average of the monthly ratios of rainfall vs. potential evaporation, and is a measure of the relative "wetness" of an environment.

The LENZ landform variable is slope, while the soil variables are: drainage, acid soluble phosphorus, exchangeable calcium, soil particle size, induration, soil age, and chemical limitations to plant growth. Slope is a large factor in soil drainage and microclimate, while drainage itself influences oxygen availability in the upper soil layers. Acid soluble phosphorus and exchangeable calcium are key mineral nutrients required by plants, and soil particle size indicates rates of nutrient release from chemical weathering. Induration is a measure of soil hardness and how resistance soils are to weathering. Soil age is a categorical variable with two classes; one for older, less fertile soil matrices, and one for younger soils. Chemical limitations to plant growth ranks the presence of saline or ultramafic substrates within environments.

Level II environments containing less than six specimens of a species were removed. The remaining specimens were found in Level I environment groups B, E, F, P, and Q. Level II environments are nested within Level I and are denoted by a number after the letter (Table 4). All variable values for each environment are listed in Table 5.

Table 4. Herbarium specimen counts by LENZ Level II environments.

LENZ Level II	Environment Type	*Celmisia gracilenta*	*Gentianella bellidifolia*
B1	Central Dry Lowlands	6	
E4	Central Dry Foothills	8	
F1	Central Hill Country & Volcanic Plateau	12	
F5	Central Hill Country & Volcanic Plateau	13	
P1	Central Mountains	6	29
P2	Central Mountains		13
P3	Central Mountains	9	27
P4	Central Mountains	10	12
P6	Central Mountains	8	
P7	Central Mountains		6
Q1	Southeastern Hill Country & Mountains	18	
Q3	Southeastern Hill Country & Mountains		7
Q4	Southeastern Hill Country & Mountains	10	
	TOTAL	**100**	**94**

Environment B is found mostly at low elevations in both Te Ika-a-Māui and Te Waipounamu, chiefly in the east. **B1** is the most widespread Level II environment within B, and is characterised as "warm, with high solar radiation, moderate vapour pressure deficits and low annual water deficits". Soils are poorly drained and low fertility [99].

Environment E is also found primarily in the east of both islands, though it is most common throughout Waitaha (Canterbury) and Tauihu (Marlborough) at mid-elevations (~700–800m). **E4** is the most common Level II sub-type, and only occurs in inland valleys throughout Waitaha. General temperatures are cooler and solar radiation is high, with moderate vapour pressure deficits and low annual water deficits. Soils have high natural fertility and are well-drained [99].

Environment F occurs across low to mid-elevations in both islands, largely in the foothills of ranges such as the Raukumara in Tairawhiti (Gisborne). **F4** is the most common Level II sub-type and is most common in Te Ika-a-Māui. The climate is described as "mild with high solar radiation and slight annual water deficits", with well-drained but low fertility soils [99]. **F5** is found at lower elevations in Taranaki, Mohua (Golden Bay), and the inner Marlborough Sounds. The climate is warm, with high solar radiation, and only a slight water deficit. Soils are well-drained with high fertility [99].

Table 5. Variable values of LENZ Level II environments in which herbarium specimens occur

LENZ Level II Environment	Variable														
	Mean annual temperature (°C)	Mean minimum temperature of coldest month (°C)	Mean annual solar radiation (MJ/m²/day)	Mean winter solar radiation (MJ/m²/day)	October vapour pressure deficit (kPa)	Monthly water balance ratio	Mean annual water deficit (mm)	Slope (°)	Drainage (1 = very poor to 5 = good)	Acid soluble phosphorus (1 = poor to 5 = good)	Exchangable calcium (1 = very low to 5 = very high)	Particle size (1 = clay/silt to 5 = boulders)	Induration (1 = non-indurated to 5 = very indurated)	Soil age (1 = recent/raw, 2 = older)	Chemical limitations (1 = low, 2 = saline, 3 = ultramafic)
B1	12.3	2.5	14.7	5.0	0.4	2.4	90.6	6.9	3.8	1.7	1.8	4.4	3.6	2.0	1.0
E4	8.4	-2.2	14.1	4.6	0.4	2.7	64.2	6.5	5.0	3.9	1.1	3.0	2.1	2.0	1.0
F1	11.5	2.7	14.2	4.8	0.3	3.6	20.6	13.6	4.6	1.5	1.3	4.7	3.8	2.0	1.0
F5	12.5	4.4	14.7	5.0	0.3	4.0	16.3	2.2	4.9	4.6	1.9	1.3	2.2	3.0	1.0
P1	5.8	-3.2	14.1	4.7	0.3	5.3	1.4	27.1	5.0	2.9	1.0	4.0	4.0	2.0	1.0
P2	4.7	-4.2	13.9	4.6	0.2	7.8	0.0	28.2	3.0	3.0	1.0	4.0	4.0	2.0	1.0
P3	7.8	-1.6	13.5	4.2	0.1	9.9	0.0	24.9	5.0	1.2	1.0	4.9	4.8	2.0	1.0
P4	5.7	-1.4	14.1	5.0	0.1	7.8	0.0	14.3	4.9	3.5	1.5	3.0	3.4	2.0	1.0
P6	9.8	0.1	15.0	4.7	0.3	4.9	2.1	23.6	4.9	2.5	1.4	4.2	4.0	2.0	1.0
P7	8.5	0.3	14.5	5.2	0.3	4.3	0.5	18.1	5.0	1.7	1.5	1.9	2.6	2.0	1.0
Q1	5.6	-3.3	13.3	3.7	0.3	4.4	2.0	22.1	4.8	3.0	1.4	4.0	4.0	1.0	1.0
Q3	6.5	-3.1	13.0	3.6	0.3	3.2	22.5	9.5	3.5	3.0	1.8	4.0	4.0	1.0	1.0
Q4	9.1	0.4	12.4	3.3	0.3	3.2	20.6	7.0	4.4	3.1	1.8	3.2	3.2	1.0	1.0

Environment P is the most common environment group in Aotearoa, as reflected by the bulk of herbarium specimens falling into this group. It occurs consistently at high elevations and covers most mountain chains. **P1** is extensive throughout both islands at mid to high elevations (1060–1325m). The climate is cold, with high solar radiation, moderate vapour pressure deficits, intermediate monthly water balance ratios, and very low annual water deficits. Soils are low fertility and well-drained.

P2 occurs at high elevations (~1465m) in mountain valleys. Temperatures are very cold, with moderate solar radiation and low vapour pressure deficits. Soils are not always present due to the elevation and landforms it contains, but they are poorly drained and low in natural fertility where they are present [99]. **P3** is found at mid elevations (740–875m) in Te Waipounamu. The climate is characterised by cool temperatures, moderate solar radiation, very low vapour pressure deficits and high monthly water balance ratios. Soils are well drained but experience leaching, leading to low fertility. **P4** encompasses the volcanic cones of Maunga Taranaki, Maunga Ruapehu and Maunga Tongariro. Winter temperatures are warmer than other P environment sub-types, with high solar radiation, low vapour pressure deficits and high monthly water balance ratios. **P6** is found at mid elevations (525–660m) in Te Tai-o-Aorere (Tasman) and Tauihu. It has very high solar radiation, low vapour pressure deficits and moderate monthly water balance ratios, with cool temperatures. Soils are moderately fertile and well-drained. **P7** occurs chiefly through central Te Ika-a-Māui. It has cool temperatures, with high solar radiation, low vapour pressure deficits and moderate monthly water balance ratios. Soils are moderately fertile and well-drained [99]. The eastern face of Maunga Ruapehu that holds Tukino slope is a mosaic of P4 and P7 environments.

Environment Q is the main environment of south-eastern Te Waipounamu, from south Waitaha to the coast in the south and the edge of Fiordland in the west. **Q1** occurs at high elevations (1095–1305m), with cold temperatures, moderate solar radiation, moderate vapour pressure deficits, low monthly water balance ratios and slight annual water deficits. As with environment P2, soils are not always present due to slope and landform but are of low to moderate fertility and well drained when present. **Q2** occurs at mid elevations (640–730m) close to Q1 environments. Climatically, the environment is cool with moderate solar radiation, moderate vapour pressure deficits, very low monthly water balance ratios and low annual water deficits. Soils are well-drained and moderately fertile. **Q4** occurs at low elevations (215–330m) in rolling foothills. The climate is cool, with low solar radiation, moderate vapour pressure deficits and low annual water deficits. Soils are moderately to well-drained and moderately fertile [99].

Tukino sampling sites were mapped against LENZ Level IV for finer resolution of their environments (Table 6). For some variables, for example mean annual temperature, this approach did not result in a linear shift from warmer to colder along the slope due to environments being averages and applied to locations via modelling. To address this, temperature data from a WaRM (Warming and Removals in Mountains) project site at Tukino slope was used (Deslippe, J., *unpublished data*). Data loggers from ambient control treatments in the WaRM project collected temperature at 90-minute intervals from 5cm below and 5cm above the soil over the 2019-2020 summer (December – February). Averages for both day and night temperatures were calculated, and a linear curve applied to estimate temperatures for each site (Table 6). This data allows a much more accurate assessment of the temperatures that plants experience both above and belowground during a growth season, which is often vastly different to air temperature means [57]

Table 6. Variable values of LENZ Level IV environments in which sampling sites at Tukino slope occur

Variable	Site #				
	1	2	3	4	5
Day time air temperature *	19.85	18.84	17.36	16.38	15.79
Night time air temperature *	8.31	8.07	7.71	7.47	7.33
Day time soil temperature *	15.96	15.30	14.35	13.72	13.34
Night time soil temperature *	15.81	14.37	12.27	10.88	10.04
LENZ Level 4 Environment	P7.1a	P4.1a	P7.1c	P4.1b	P4.1b
Mean annual temperature (^{o}C)	8.1	6.1	6.8	3.6	3.6
Mean minimum temperature of coldest month (^{o}C)	0.1	-1.4	-0.9	-2.5	-2.5
Mean winter temperature (^{o}C)	-7	-13	-19	-33	-38
Mean annual solar radiation (MJ/m^{2}/day)	14.5	14.1	14.2	14	14
Mean winter solar radiation (MJ/m^{2}/day)	5.2	5	5.1	5	5
October vapour pressure deficit (kPa)	0.32	0.18	0.22	0.07	0.07
Monthly water balance ratio	4.1	6.8	5.4	9.3	9.3
Mean annual water deficit (mm)	0.56	0	0.01	0	0
Slope (^{o})	24.4	9.5	18.4	21.4	21.4
Drainage (1 = very poor to 5 = good)	5	5	5	4.7	4.7
Acid soluble phosphorus (1 = poor to 5 = good)	1	3.2	2	3.8	3.8
Exchangable calcium (1 = very low to 5 = very high)	1	1.3	1.2	1.6	1.6
Particle size (1 = clay/silt to 5 = boulders)	4	2.4	1	4.2	4.2
Induration (1 = non-indurated to 5 = very indurated)	4	3.4	2	3.8	3.8
Soil age (1 = recent/raw, 2 = older)	2	2	2	2	2
Chemical limitations (1 = low, 2 = saline, 3 = ultramafic)	1	1	1	1	1

Variables marked with * are derived from WaRM data (Deslippe, J., *unpublished data*)

Analyses

Analysis was conducted using R statistical software [100], and figures were produced using the ggplot2 package [101]. For any trait with multiple observations per individual (*e.g*. leaf length), medians were used to reduce observations to within-plant values as medians are more robust than the mean to the centre of the data. Due to small sample sizes, trait values were not normally distributed and non-parametric permutation testing was therefore used instead of logarithmic transformation, as permutation tests allow for a rigorous analysis of variance without the strict assumptions of standard linear models [102]. Permutation tests were run at 5,000 resamples using code written by David Howell [103].

Each trait was analysed separately per species. Three permutation tests were run on each to determine if there was a probability that;

1. elevation affected variation in trait values
2. collection date affected variation in trait values
3. the effect of collection date and elevation interacted to influence plant trait values

Bonferroni-adjusted pairwise permutation t-tests determined that slight differences in ranges between collection dates were common, so all trait data by collection was adjusted by subtracting the mean for the corresponding collection day to remove the variability explained by differences in the collection date – this increases the ability of the t-tests to determine significant differences between elevations, and removes the interaction effect in permutation tests. Permutation t-tests were run using the MKinfer package in R [104]. Root to shoot ratios were calculated and run though the same process. Variance from the mean was calculated for each trait, as high variance around the mean can be an indicator of non-adaptive response of traits to an environment [62].

Scatter plots with linear smoothing were used to plot pairs of traits (within a species) that showed a significant effect of elevation in permutation tests. Where these suggested a relationship, Spearman's partial correlation coefficient was computed using permutation methodology to assess the strength and direction of the relationship after controlling for elevation [105]. Correlation tests were carried out using the rcompanion package [105].

Results

Local variation: *How do traits differ along a single elevation gradient?*

Analyses of all above and below ground traits showed statistically significant differences within all species for several traits (Table 7). Overall, more significant changes in leaf traits and aboveground plant traits were detected than for belowground traits. For example, all species showed significant differences in trait values for at least three aboveground traits whereas only *C. gracilenta* exhibited a significant response to elevation for SRL. Forbs were larger, achieving greater aboveground biomass at the highest elevation site and tended to produce thicker leaves with lower SLA at height. For *C. gracilenta* this pattern extended belowground with a tendency to produce thicker roots with lower specific root length at altitude.

Spearman's tests indicated moderate positive relationship in *L. colensoi* between leaf area and width (rho = 0.648, 95% CI), and G. bellidifolia between plant height and leaf thickness (rho = 0.617, 95% CI). A moderate negative relationship between aboveground biomass and SRL was detected in *C. gracilenta* (rho = -0.552, 95% CI). Weak positive relationships were found in *G. bellidifolia* between leaf length and SLA (rho = 0.456, 95% CI), leaf length and leaf thickness (rho = 0.480, 95% CI), and between plant height and leaf length (rho = 0.406, 95% CI). *Rytidosperma setifolium* also showed weak positive correlations between aboveground biomass and leaf length (rho = 0.415, 95% CI), aboveground biomass and plant height (rho = 0.468, 95% CI), plant height and leaf length (rho = 0.472, 95% CI). No other trait pairings showed any significant relationships.

Root to shoot ratios for all species showed no significant effect of elevation on values, but Spearman's tests between above and belowground biomass showed a strong positive relationship in *L. colensoi* (rho = 0.751, 95% CI) and a moderate positive relationship in *G. bellidifolia* (rho = 0.656, 95% CI). *Celmisia gracilenta* and *R. setifolium* both indicated weak positive relationships (rho = 0.422 and 0.468, respectively. 95% CI).

Variance in aboveground biomass values for *C. gracilenta* and *G. bellidifolia* were seen to increase with elevation, whereas the variance for *L. colensoi* was small for low and median elevations but much higher for the highest elevation (Table 9). *Rytidosperma setifolium*

showed the opposite response to the forbs for aboveground biomass, with lower variance at higher elevation. Leaf length for *R. setifolium* exhibited extremely high variance across all elevations.

Table 7. Traits which showed a significant effect of elevation on values, including results for effect of collection date and interaction of elevation and collection date.

Species	Trait	Elevation	Collection	Interaction	Direction
Celmisia gracilenta	**ABV**	**	ns	ns	↟
	HGT	*	ns	ns	u-shaped
	SRL	*	**	ns	↡
	THK	**	***	ns	↟
Gentianella bellidifolia	**ABV**	*	ns	ns	↟
	HGT	***	ns	***	↟
	LEN	**	***	ns	u-shaped
	SLA	*	ns	ns	u-shaped
	THK	**	**	ns	↟
Luzula colensoi	**ABV**	*	ns	ns	↟
	AREA	**	**	ns	↟
	WDT	***	ns	ns	↟
Rytidosperma setifolium	**ABV**	**	ns	ns	↡
	HGT	**	ns	ns	↡
	LEN	*	**	ns	↡
	SLA	*	***	**	↟

"Direction" refers to the direction of the trait change with elevation *i.e.,* aboveground biomass for *Celmisia gracilenta* increased (↟) with the increase in elevation.

ABV = aboveground biomass; AREA = leaf area; HGT = plant height; LEN = leaf length; SLA = specific leaf area; SRL = specific root length; THK = leaf thickness; WDT = leaf width.

Significance stars: ns = not significant; * = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001

Table 8. Pairwise test results between elevation pairs for traits which showed a significant effect of elevation on values.

Species	Trait	low-med	low-high	med-high
Celmisia gracilenta	ABV	ns	***	*
	HGT	*	ns	**
	SRL	ns	**	**
	THK	ns	**	*
Gentianella bellidifolia	ABV	**	*	ns
	HGT	ns	**	***
	LEN	ns	ns	***
	SLA	**	ns	ns
	THK	ns	**	***
Luzula colensoi	ABV	ns	ns	ns
	AREA	ns	***	**
	WDT	ns	***	***
Rytidosperma setifolium	ABV	ns	***	**
	HGT	***	*	ns
	LEN	*	*	ns
	SLA	*	ns	ns

Significance stars: ns = not significant; * = P ≤ 0.05; ** = P ≤ 0.01; *** = P ≤ 0.001

Table 9. Mean values and variance for all traits in which a significant effect of elevation was found by species, per elevational range point

		Mean			Variance		
Species	Trait	low	median	high	low	median	high
Celmisia gracilenta	ABV	0.3922	0.6927	1.3190	0.03	0.34	0.78
	HGT	4.96	3.89	5.29	3.79	0.73	3.71
	SRL	1.88	1.48	0.81	1.59	0.63	0.20
	THK	0.65	0.66	0.73	0.06	0.05	0.06
Gentianella bellidifolia	ABV	0.4607	0.9353	1.0444	0.17	0.17	0.46
	HGT	3.18	2.91	5.71	3.34	0.99	6.35
	LEN	24.96	20.55	28.22	78.25	25.06	63.90
	SLA	20.55	15.21	17.15	34.48	10.15	23.83
	THK	0.30	0.31	0.39	0.01	0.46	0.002
Luzula colensoi	ABV	1.0858	1.0915	2.3808	0.45	0.69	4.87
	AREA	10.55	12.00	15.97	10.52	18.10	16.27
	WDT	1.17	1.20	1.59	0.02	0.03	0.07
Rytidosperma setifolium	ABV	8.5106	5.9550	2.8698	33.75	8.91	1.04
	HGT	15.38	10.42	11.65	25.34	3.68	28.51
	LEN	95.73	78.19	77.44	500.80	349.21	266.02
	SLA	8.51	9.88	9.58	0.88	4.91	6.75

ABV = aboveground biomass; AREA = leaf area; HGT = plant height; LEN = leaf length; SLA = specific leaf area; SRL = specific root length; THK = leaf thickness; WDT = leaf width

Celmisia gracilenta

Above and belowground traits responded idiosyncratically to elevation in *C. gracilenta*, with aboveground biomass, plant height and leaf thickness increasing with elevation, specific root length declining with elevation, and non-significant changes in other traits (Table 7, Figure 13). Though SLA, leaf width and leaf thickness did not show significant results, SLA saw a strong downwards trend with elevation, while leaf width and thickness increased with elevation. Pairwise comparisons identified a difference between low and high sites for leaf width, but permutation tests revealed no significant effect of elevation on this difference.

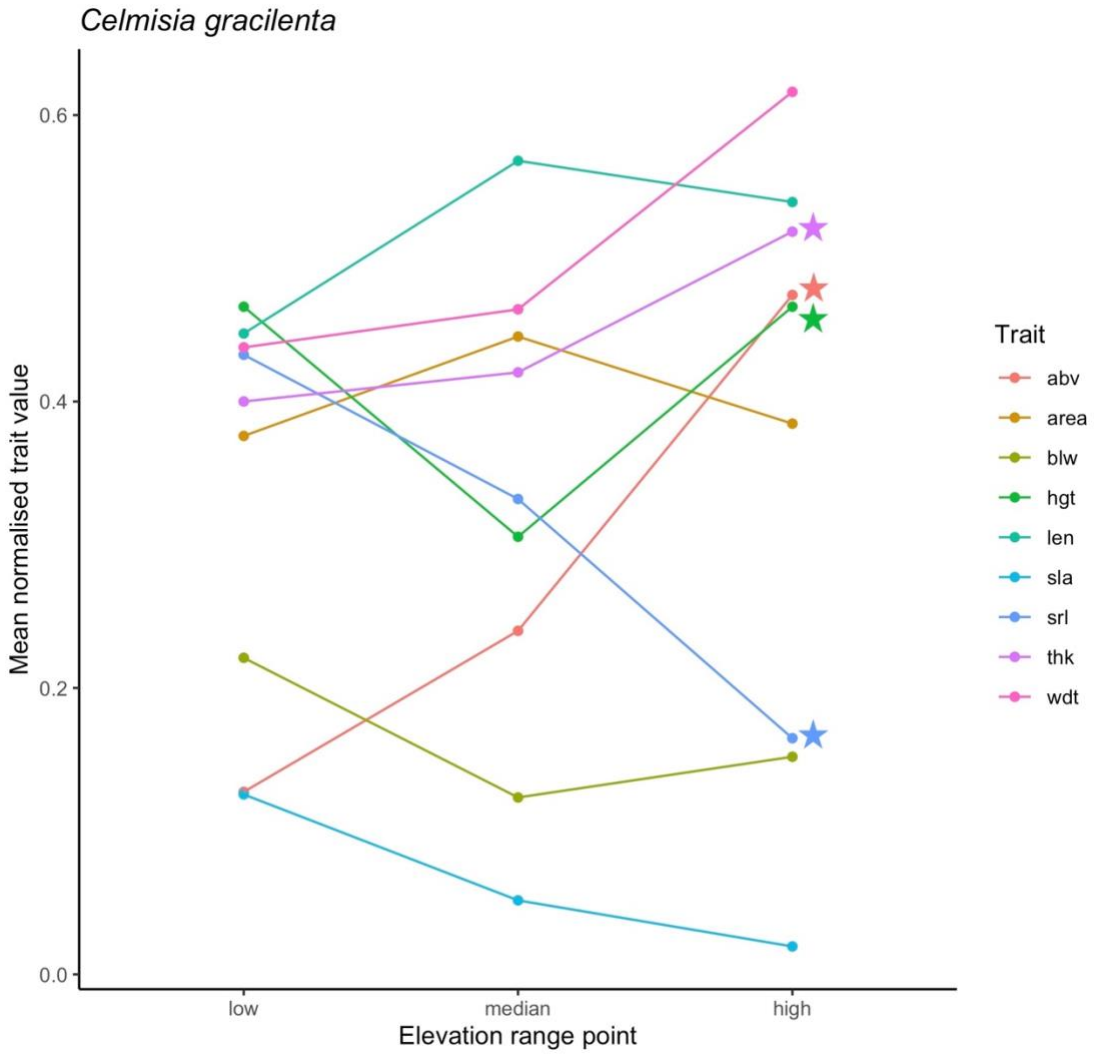


Figure 13. Normalised mean trait values for Celmisia gracilenta in response to elevation

ABV = aboveground biomass; AREA = leaf area; BLW = belowground biomass; HGT = plant height; LEN = leaf length; SLA = specific leaf area; SRL = specific root length; THK = leaf thickness; WDT = leaf width

Stars denote traits which showed an effect of elevation on values in permutation tests

Aboveground Biomass

Permutation tests indicated a significant difference in plant aboveground biomass between sites which was affected by the increase in elevation (p = 0.003; Table 7, Figure 14). Pairwise comparisons indicated that plants had significantly more aboveground biomass at the high elevation compared to both the median (p = 0.05) and low elevation sites (p = <0.001; Table 8). Mean aboveground biomass at the low elevation site was 0.3922g, whereas plants had a mean weight of 1.3190g at the highest site – a difference of 0.9268g (Table 9). The pattern of variation in aboveground biomass was also strikingly different among elevations, with the dispersion around the mean much greater at the high elevation site than at the low elevation site (Table 9).

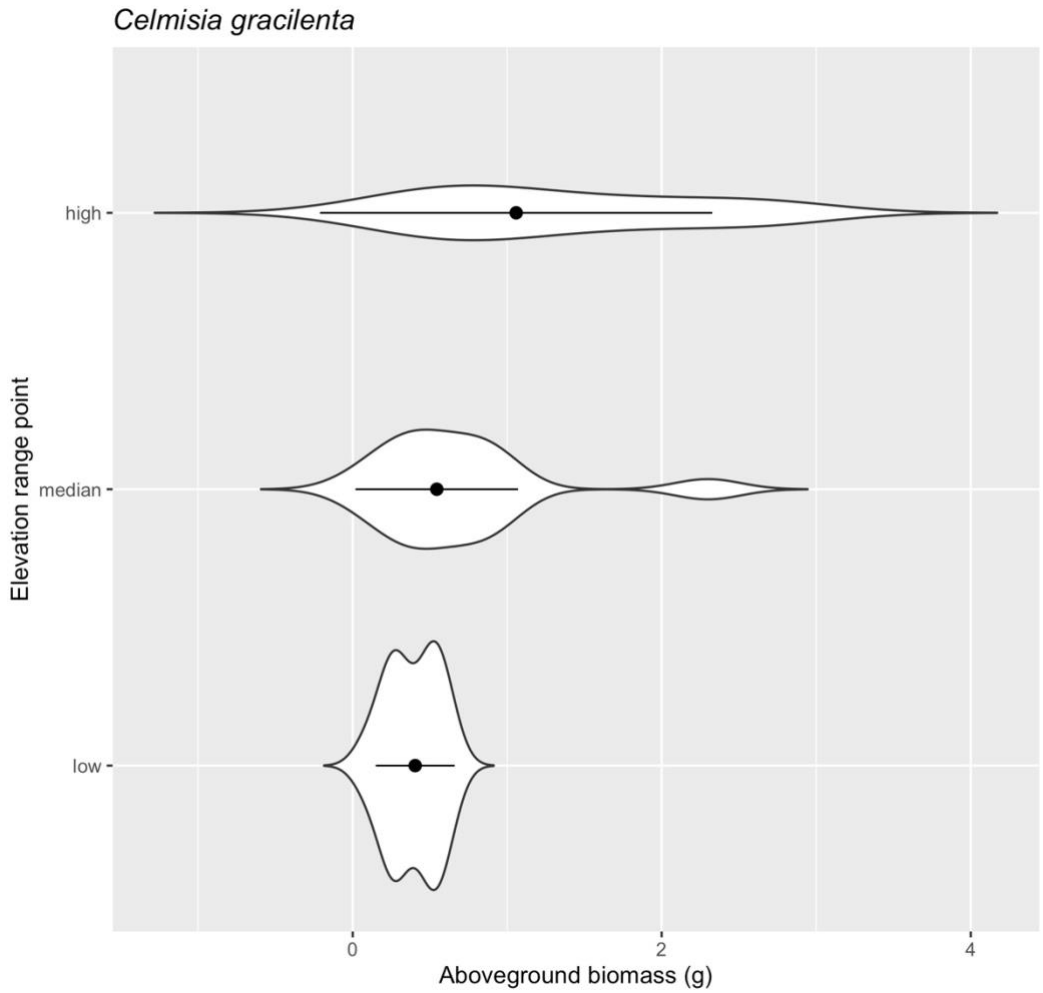


Figure 14. Violin plot illustrating the pattern of variation in aboveground biomass over elevation in Celmisia gracilenta

Plant Height

Permutation tests indicated a significant effect of elevation on plant heights (p = 0.04; Table 7, Figure 15). Pairwise comparisons revealed a u-shaped response to elevation, showing plants to be much shorter at median elevation than at lower (p = 0.05; Table 8) or higher sites (p = 0.01). Mean plant height at the median site was 3.89cm compared to 3.18cm at the lowest point and 5.71cm at the highest. Variance was also much lower at the median site than for either high or low elevations (Table 9).

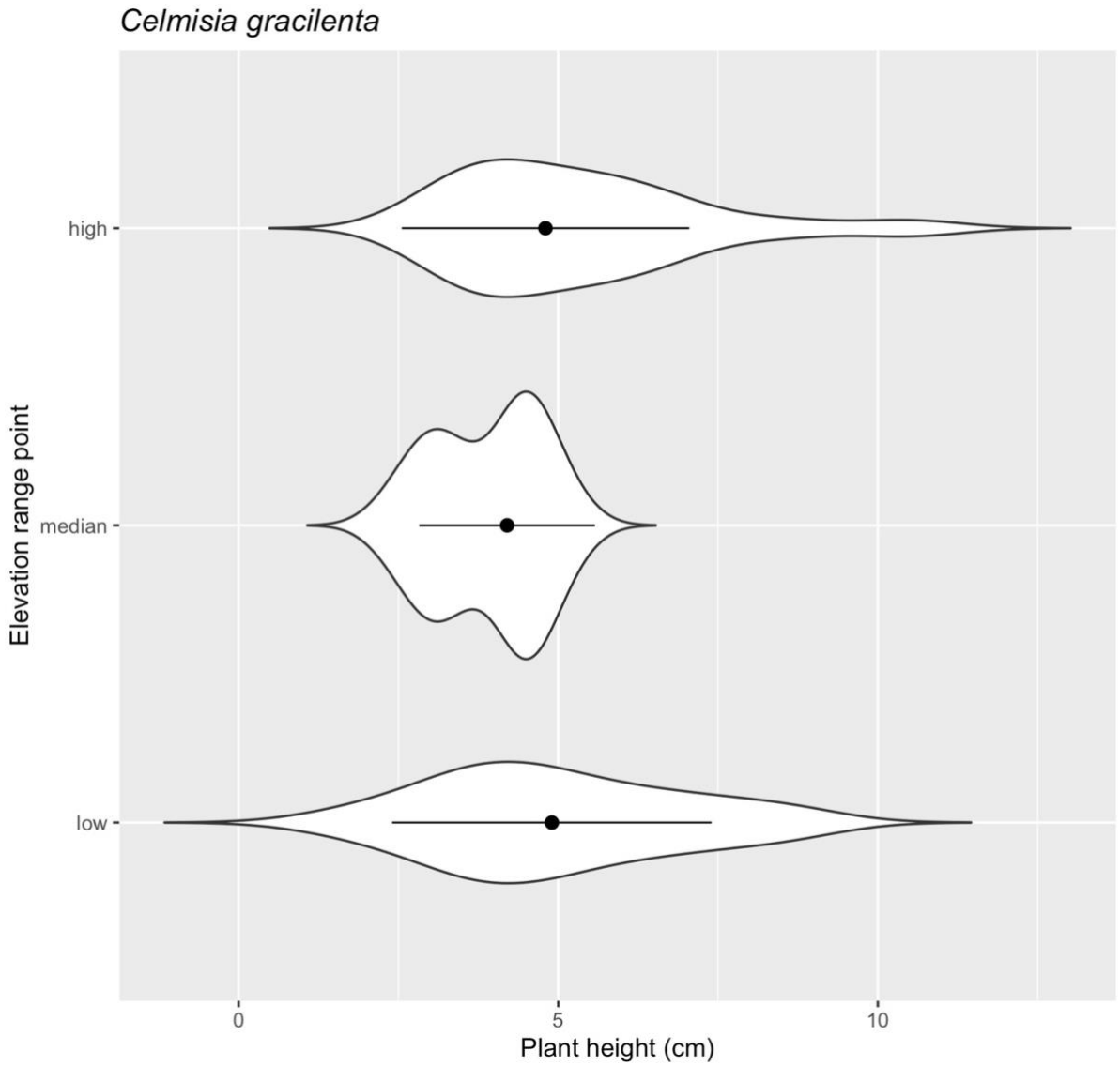


Figure 15. Violin plot illustrating the pattern of variation in plant height over elevation in Celmisia gracilenta

Specific Root Length

Permutation tests reported a significant effect of elevation on the difference in SRL values between sites (p = 0.02; Table 7, Figure 16). Pairwise comparisons showed SRL to be significantly lower at high elevation compared both the median and the lowest (both p = 0.01; Table 8), indicating that roots were shorter per unit of biomass at the highest range point (*i.e.,* roots were shorter and thicker at higher elevations). In contrast to aboveground biomass, variance was greater at the low elevation site compared to both middle and high sites (Table 9).

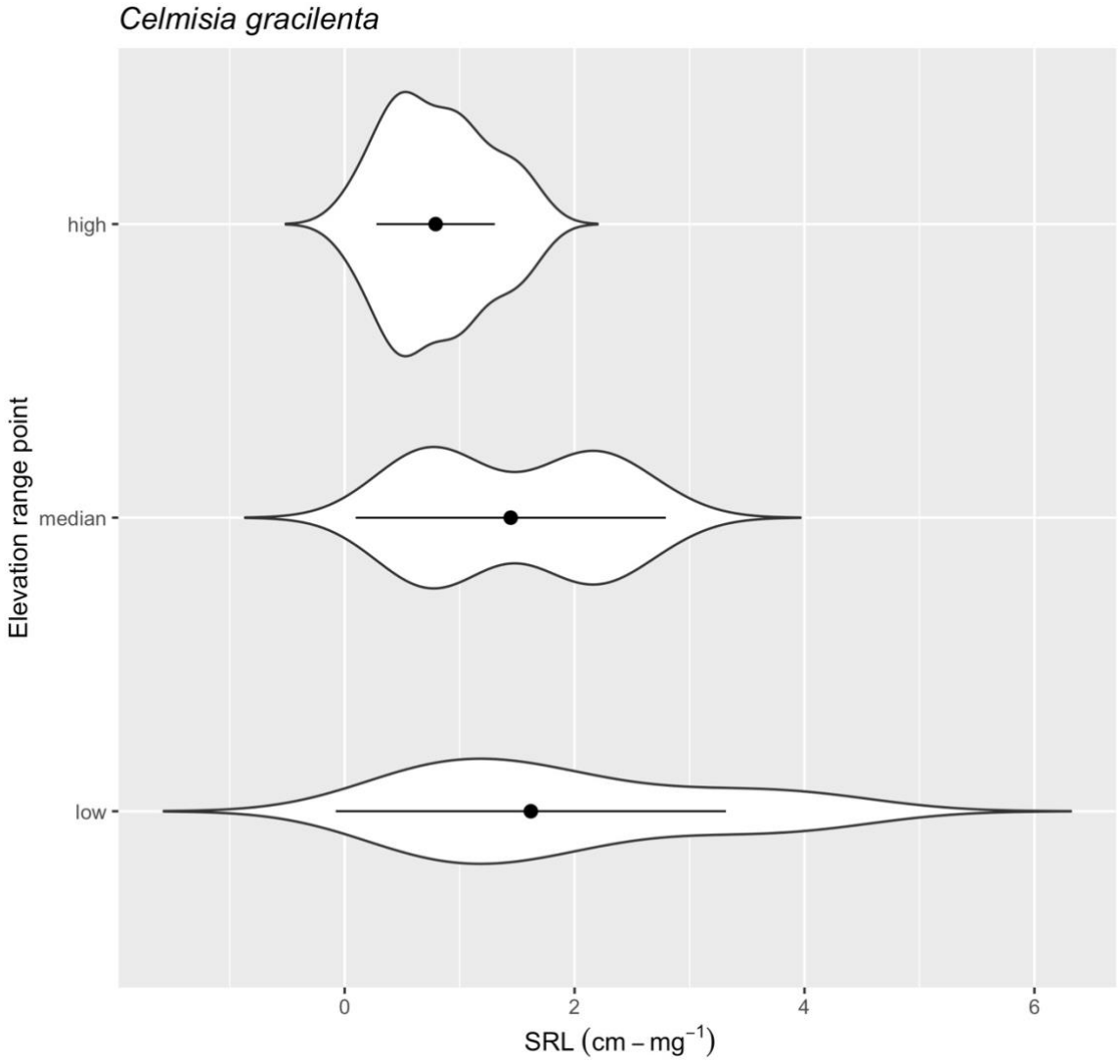


Figure 16. Violin plot illustrating the pattern of variation in specific root length (SRL) over elevation in Celmisia gracilenta

Leaf Thickness

Permutation tests reported a significant effect of elevation on the difference in leaf thickness between sites (p = 0.01; Table 7, Figure 17). Pairwise comparisons revealed leaves to be significantly thicker at the highest elevation compared both median and low elevations (low, p = 0.003; median, p = 0.02; Table 8). Variance differences among elevations were minor (Table 9).

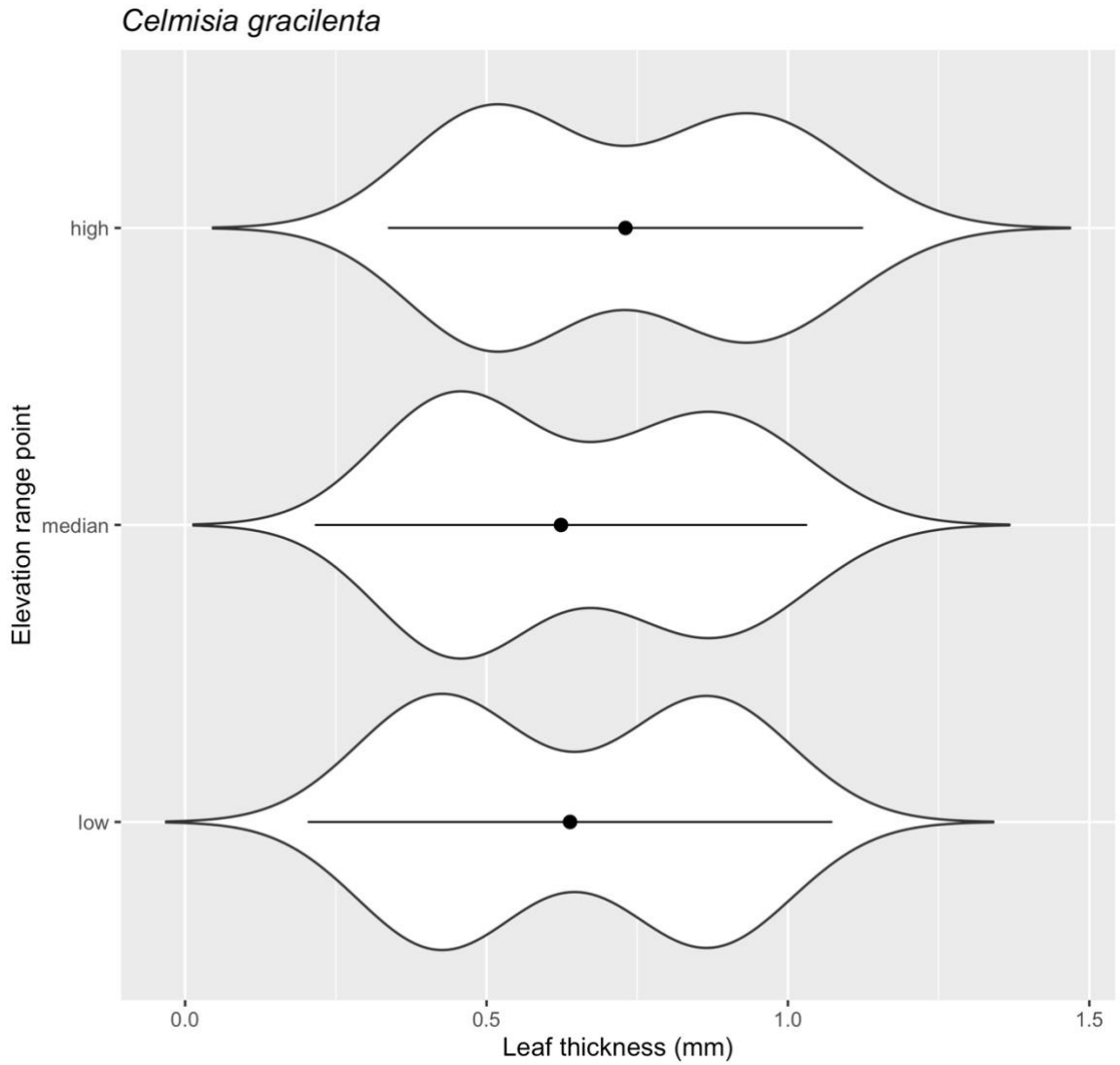


Figure 17. Violin plot illustrating the pattern of variation in leaf thickness over elevation in Celmisia gracilenta

Gentianella bellidifolia

In contrast to *C. gracilenta*, many traits of *G. bellidifolia* reached minimum or maximum values at the median elevation, suggesting an overall u-shaped response to the environment (Figure 18). Significant increases in plant traits among elevations were identified for aboveground biomass, plant height, and leaf thickness, while u-shaped responses were revealed for leaf length, and SLA (Table 7). Leaves were longer and thicker at the highest elevation (length; p = 0.01, thickness; p = 0.005) resulting in lower SLA at the highest elevation site (p = 0.03). Pairwise comparisons identified a difference between median and high sites for leaf area, but permutation tests revealed no significant effect of elevation on this difference.

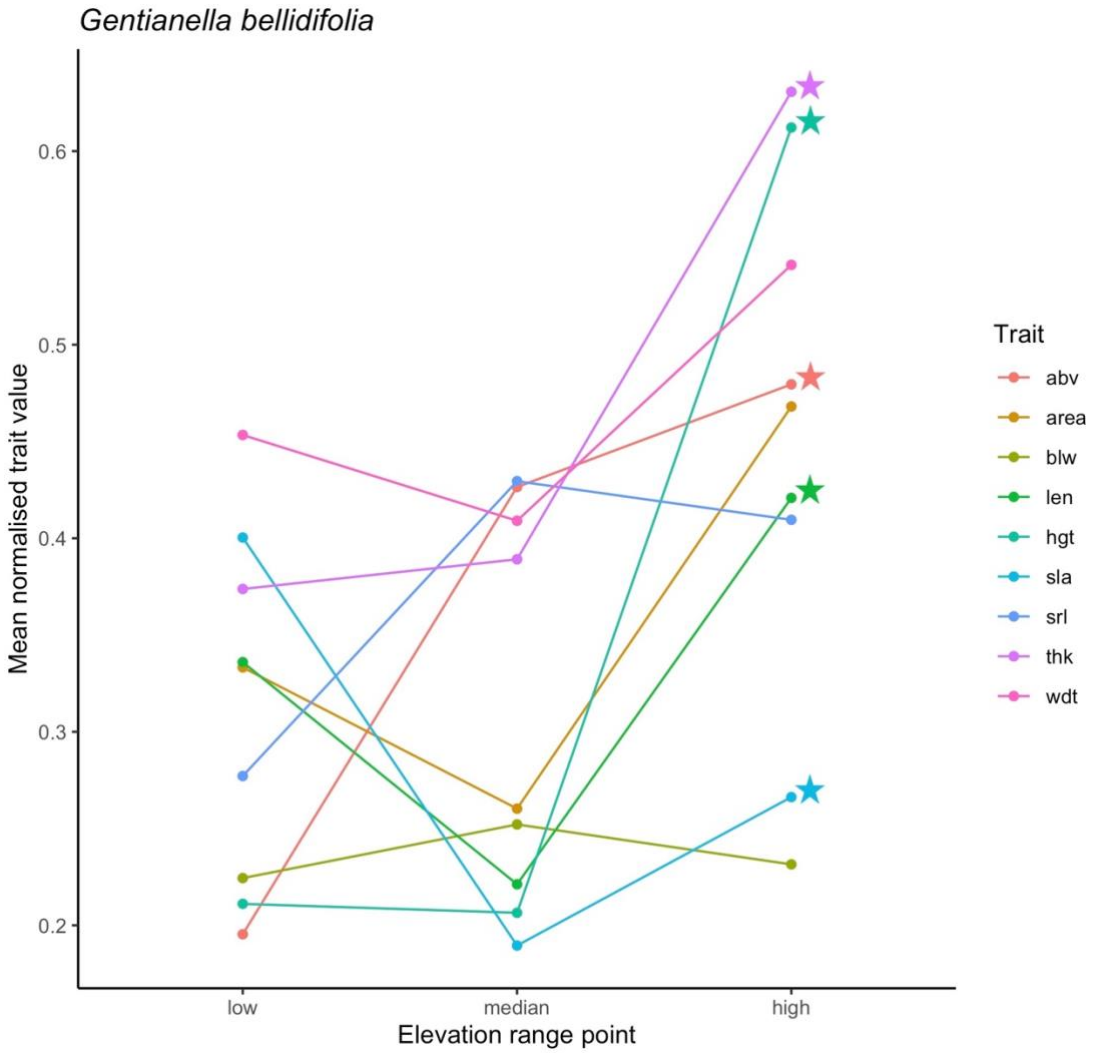


Figure 18. Normalised mean trait values for Gentianella bellidifolia in response to elevation

ABV = aboveground biomass; AREA = leaf area; BLW = belowground biomass; HGT = plant height; LEN = leaf length; SLA = specific leaf area; SRL = specific root length; THK = leaf thickness; WDT = leaf width

Stars denote traits which showed an effect of elevation on values in permutation tests

Aboveground Biomass

Permutation tests reported a significant effect of elevation on the differences in aboveground biomass between elevations (p = 0.02; Table 7, Figure 19). As seen with *C. gracilenta*, pairwise comparisons revealed aboveground biomass to be significantly smaller at the lowest elevation compared to the highest (p = 0.005; Table 8) and median (p = 0.03). Mean aboveground biomass for plants at the highest elevation was 1.0444g, in contrast with 0.4607g at the lowest site – a 0.5837g difference, the bulk of which occurs between the median and high sites. Variance was only slightly higher for the high site compared to low and median sites, which reported similar variance (Table 9).

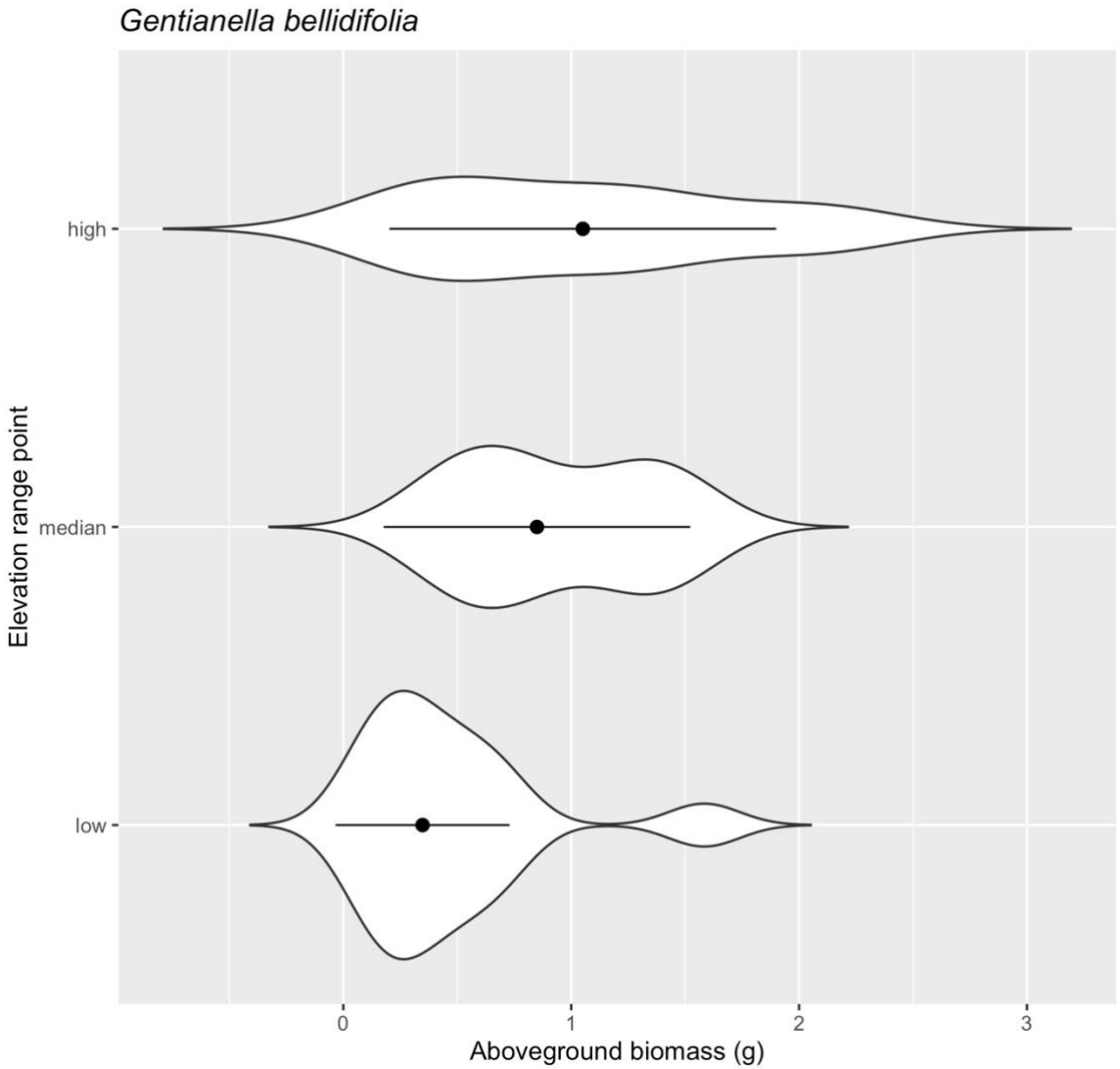


Figure 19. Violin plot illustrating the pattern of variation in aboveground biomass over elevation in Gentianella bellidifolia

Plant Height

Permutation tests revealed a significant effect of elevation on plant height (p = <0.001; Table 7, Figure 20). Though the trait–environment relationship was very slightly u-shaped, plants were found to be significantly taller at the highest elevation compared to both low (p = 0.003; Table 8) and median sites (p = <0.001), with a 2.80cm height difference between the median and high elevation sites (Table 9). Variance was much higher at the high site (Table 9).

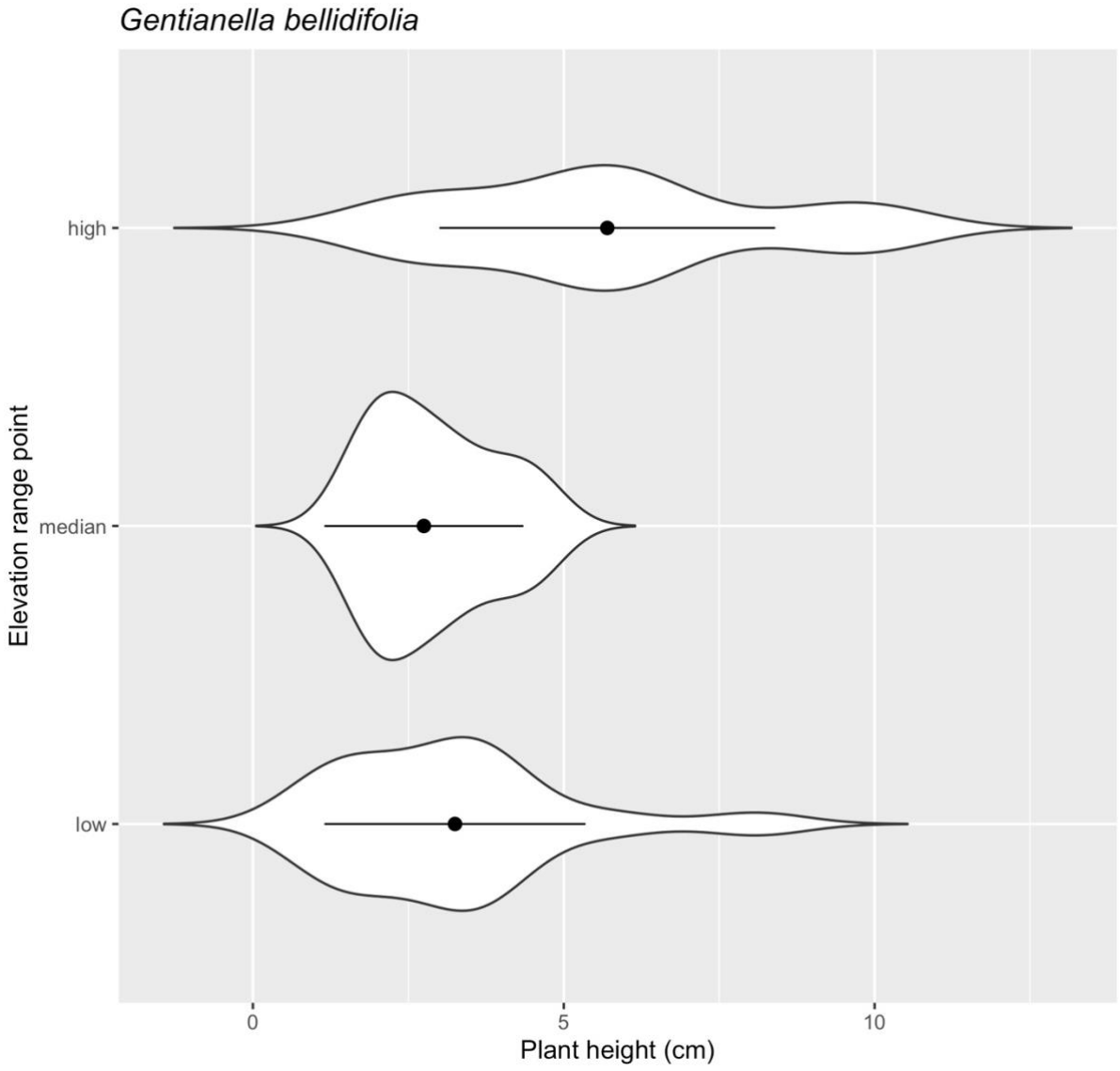


Figure 20. Violin plot illustrating the pattern of variation in plant height over elevation in Gentianella bellidifolia

Leaf Length

Permutation tests reported a significant difference in leaf lengths between sites driven by elevation (p = 0.01; Table 7, Figure 21). Pairwise comparisons revealed a u-shaped relationship, with shorter leaves at the median elevation compared to the high site (p = <0.001; Table 8). Though mean leaf length for plants at the median site was shorter than the low site, pairwise comparisons showed no significant difference between the two. Variance at the median site was low compared to both high and low elevations, but all reported values were high (Table 9).

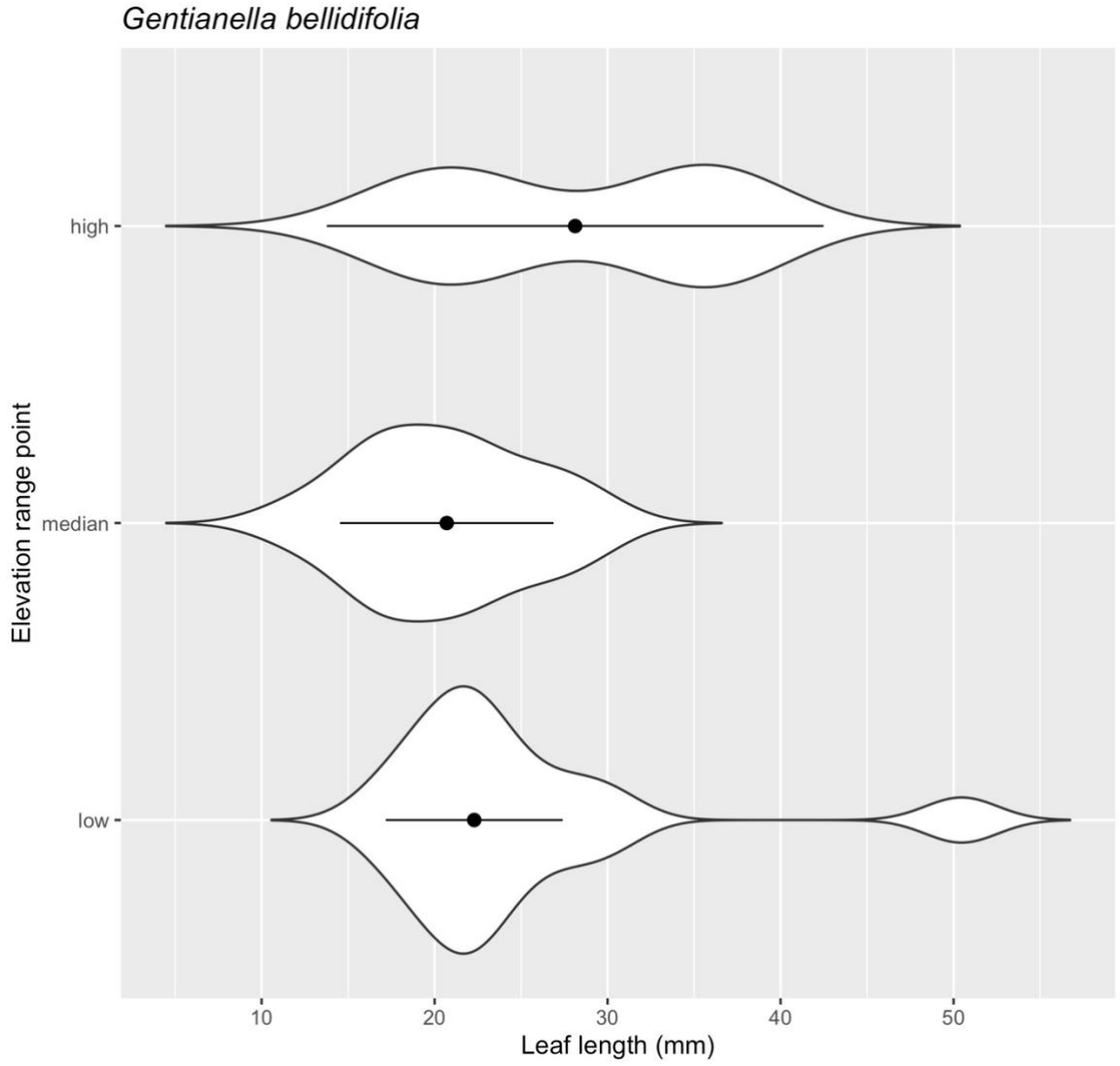


Figure 21. Violin plot illustrating the pattern of variation in leaf length over elevation in Gentianella bellidifolia

Specific Leaf Area

Permutation tests reported a significant effect of elevation on differences in SLA values between sites (p = 0.03; Table 7, Figure 22). Pairwise comparisons revealed significantly lower SLA values at low elevation compared to the median site (p = 0.02; Table 8), but showed no other differences among pairs. Variance was lower for the median elevation than both high and low (Table 9).

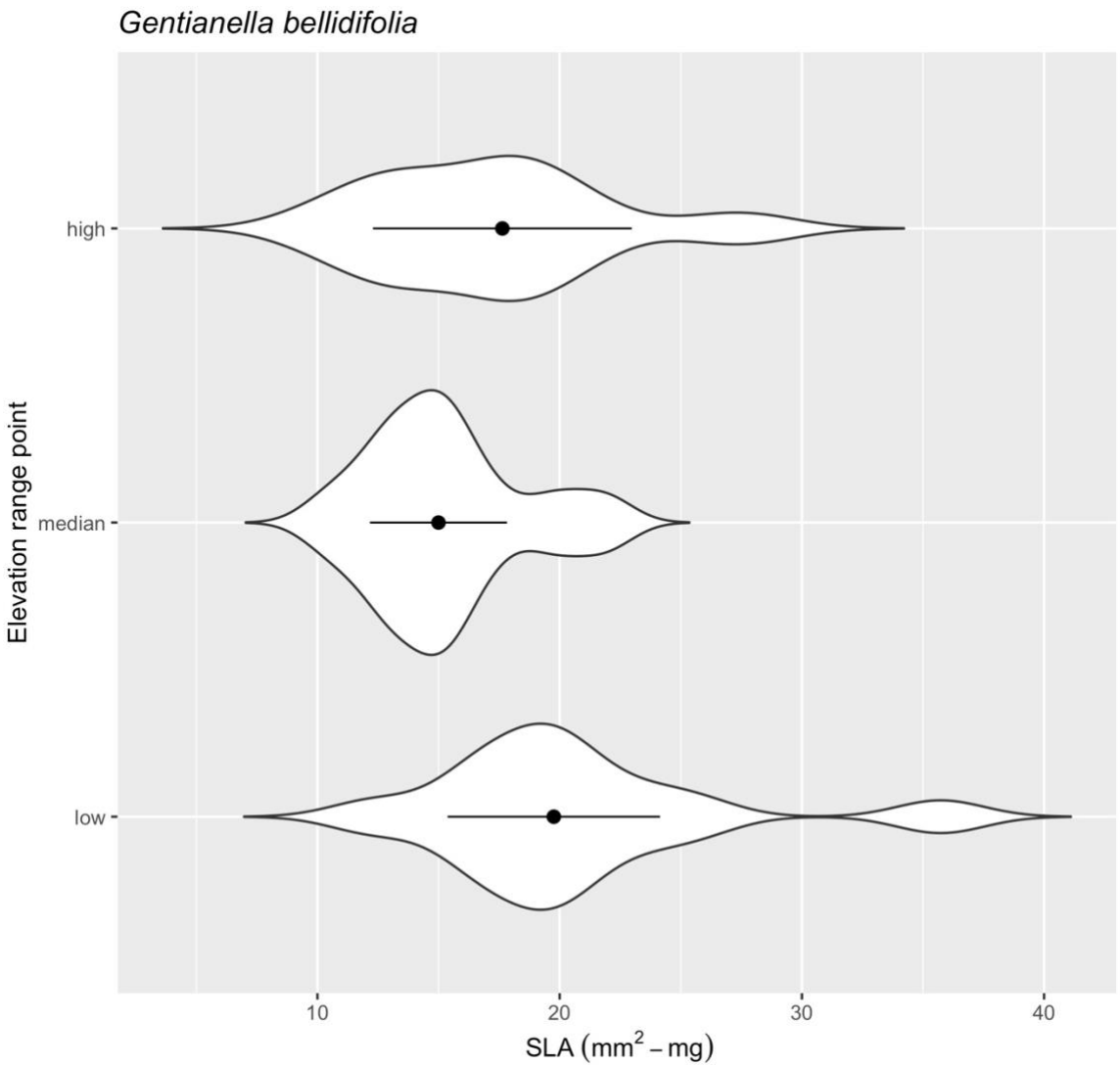


Figure 22. Violin plot illustrating the pattern of variation in specific leaf area (SLA) over elevation in Gentianella bellidifolia

Leaf Thickness

Permutation tests reported a significant effect of elevation on leaf thickness (p = 0.005; Table 7, Figure 23). Pairwise comparisons revealed leaves to be thicker at the high elevation site compared to both the median (p = 0.001; Table 8) and low sites (p = 0.002). Mean leaf thickness was 0.39mm at the highest elevation, compared to 0.33mm at the lowest (a 0.06mm difference). In contrast to leaf length and SLA within *G. bellidifolia*, dispersion around the mean was smaller for low and high sites, but larger for the median (Table 9).

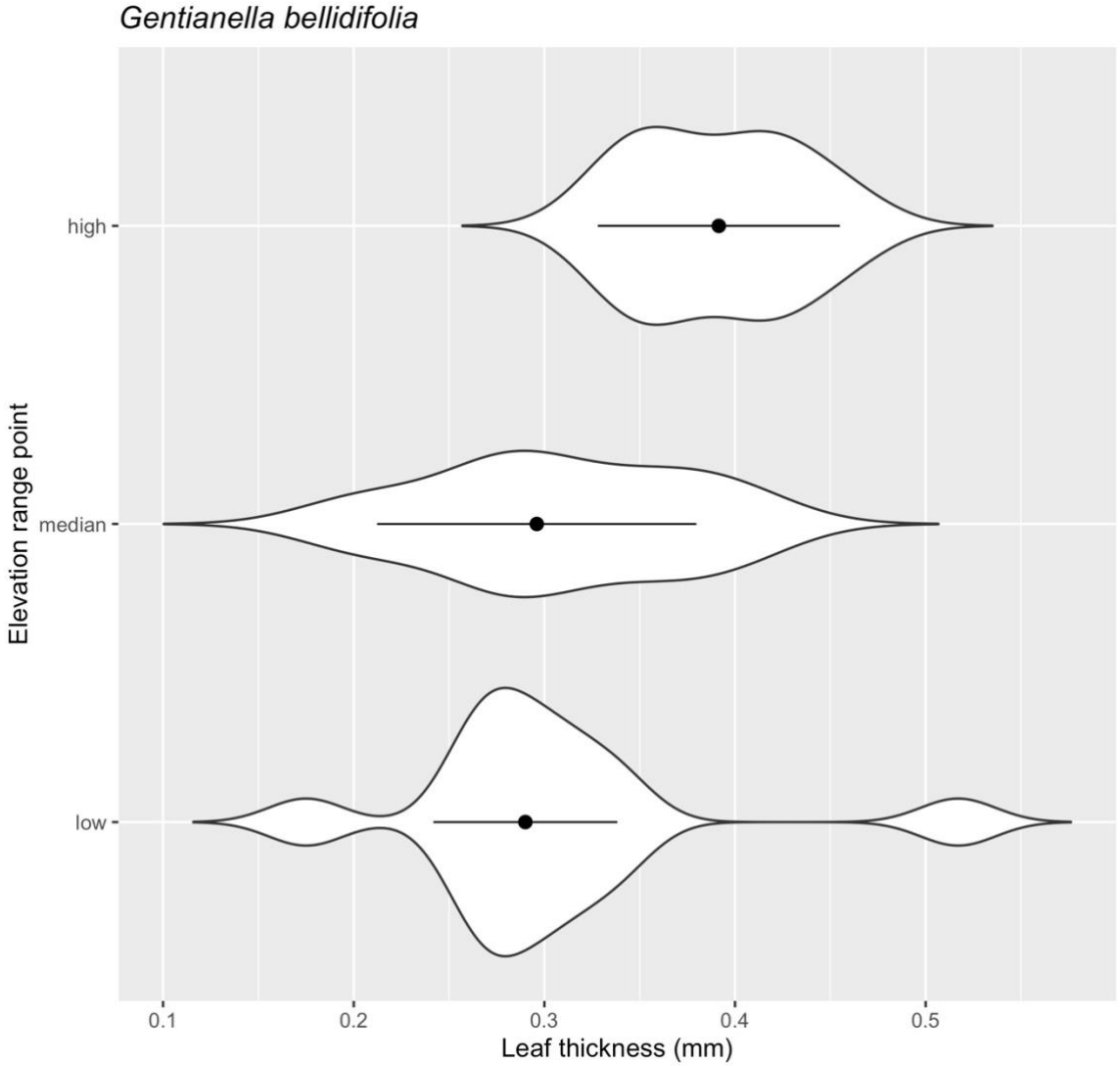


Figure 23. Violin plot illustrating the pattern of variation in leaf thickness over elevation in Gentianella bellidifolia

Luzula colensoi

Overall, *L. colensoi* exhibited mostly linear responses to elevation, with high elevation values much greater than those at low and median sites. Significant differences between elevations were identified for aboveground biomass, leaf area and leaf width, however pairwise comparisons among elevations showed no significant differences comparisons for aboveground biomass (Table 8). Leaf area and width increased with elevation (leaf area, p = 0.002; leaf width, p = <0.001; Table 7, Figure 24). Pairwise comparisons identified a difference between low and high sites for leaf length, but permutation tests revealed no significant effect of elevation on this difference.

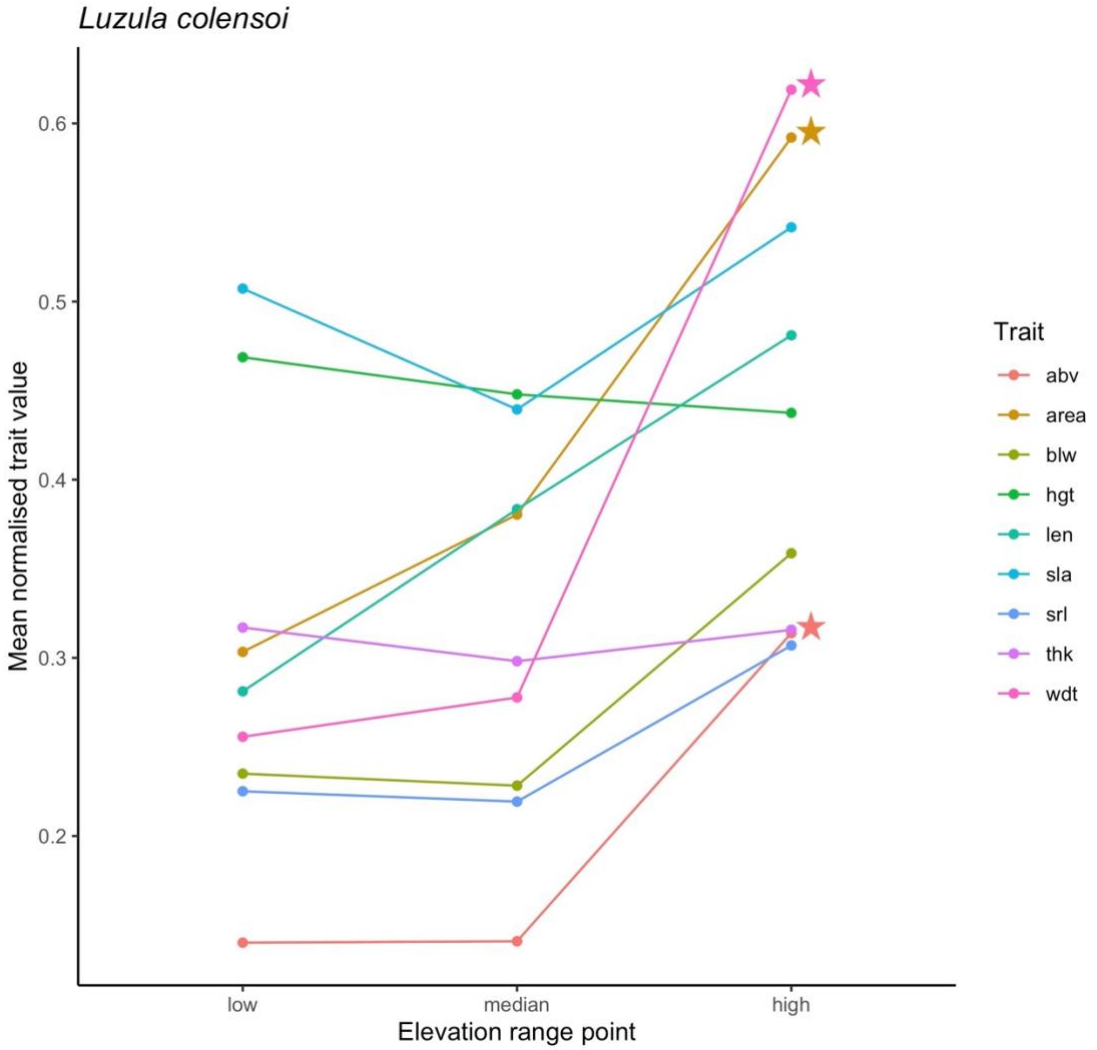


Figure 24. Normalised mean trait values for Luzula colensoi in response to elevation

ABV = aboveground biomass; AREA = leaf area; BLW = belowground biomass; HGT = plant height; LEN = leaf length; SLA = specific leaf area; SRL = specific root length; THK = leaf thickness; WDT = leaf width

Stars denote traits which showed an effect of elevation on values in permutation tests

Leaf Area

Permutation tests reported a significant probability of an effect of elevation on leaf area (p = 0.002; Table 7, Figure 25). Pairwise comparisons showed leaves to have significantly greater surface area at the highest elevation compared to both low (p = <0.001; Table 8), and median sites (p = 0.01). Mean leaf area of plants at the low site was 10.55mm^2 compared to 15.97mm^2 at the high site. Variance around the mean was moderate for all three sites, and is the highest variance of the traits showing significant changes in *L. colensoi*. (Table 9).

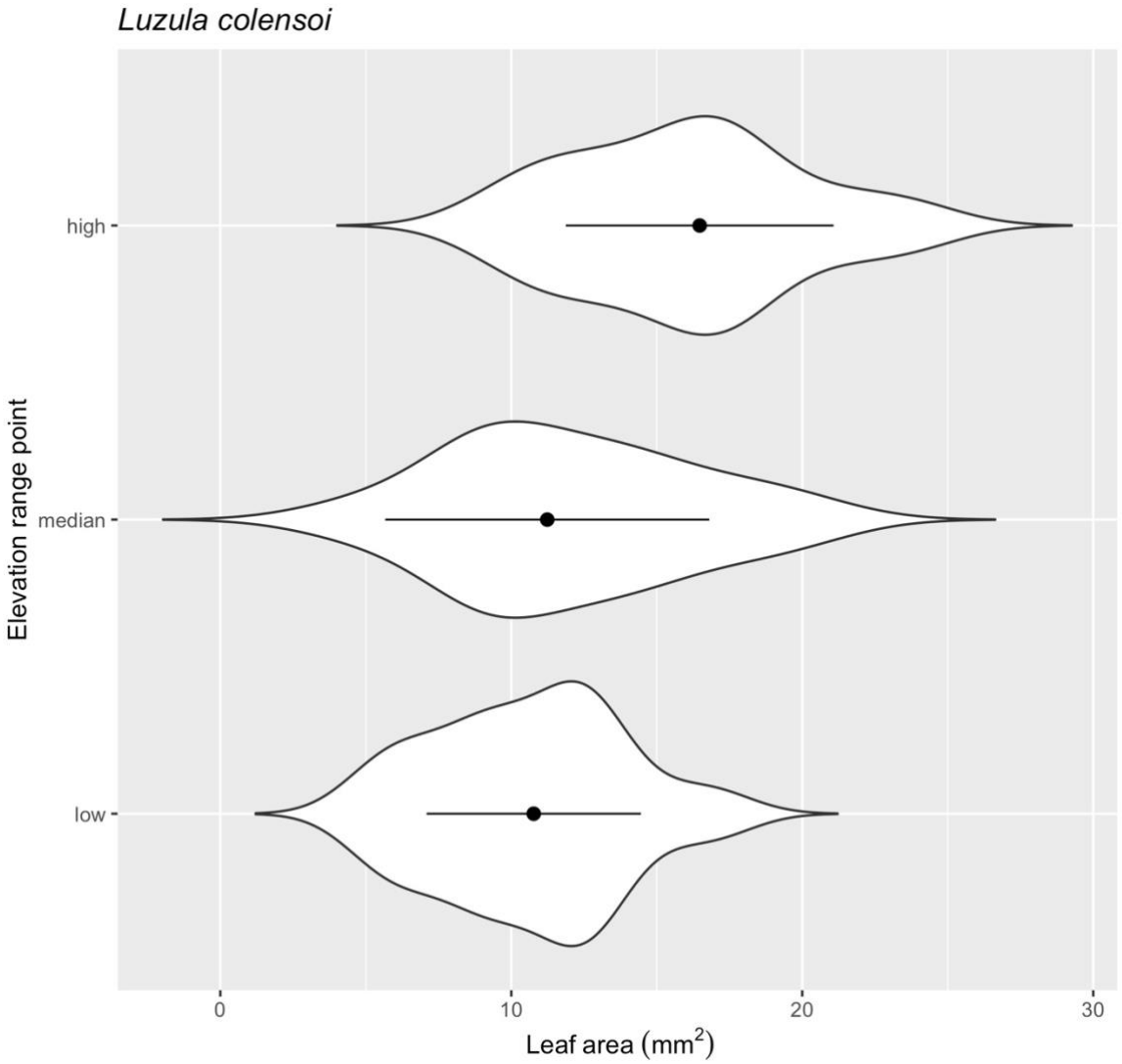


Figure 25. Violin plot illustrating the pattern of variation in leaf area over elevation in Luzula colensoi

Leaf Width

Permutation tests reported a significant difference in leaf width between sites driven by elevation (p = <0.001; Table 7, Figure 26). Leaves were considerably wider at higher elevations compared to the lowest (p = <0.001; Table 8) or median site (p = <0.001). Mean width was 1.59mm at the high site versus 1.17mm at the lowest (a 0.42mm difference). Dispersion from the mean was small across all three elevations (Table 9).

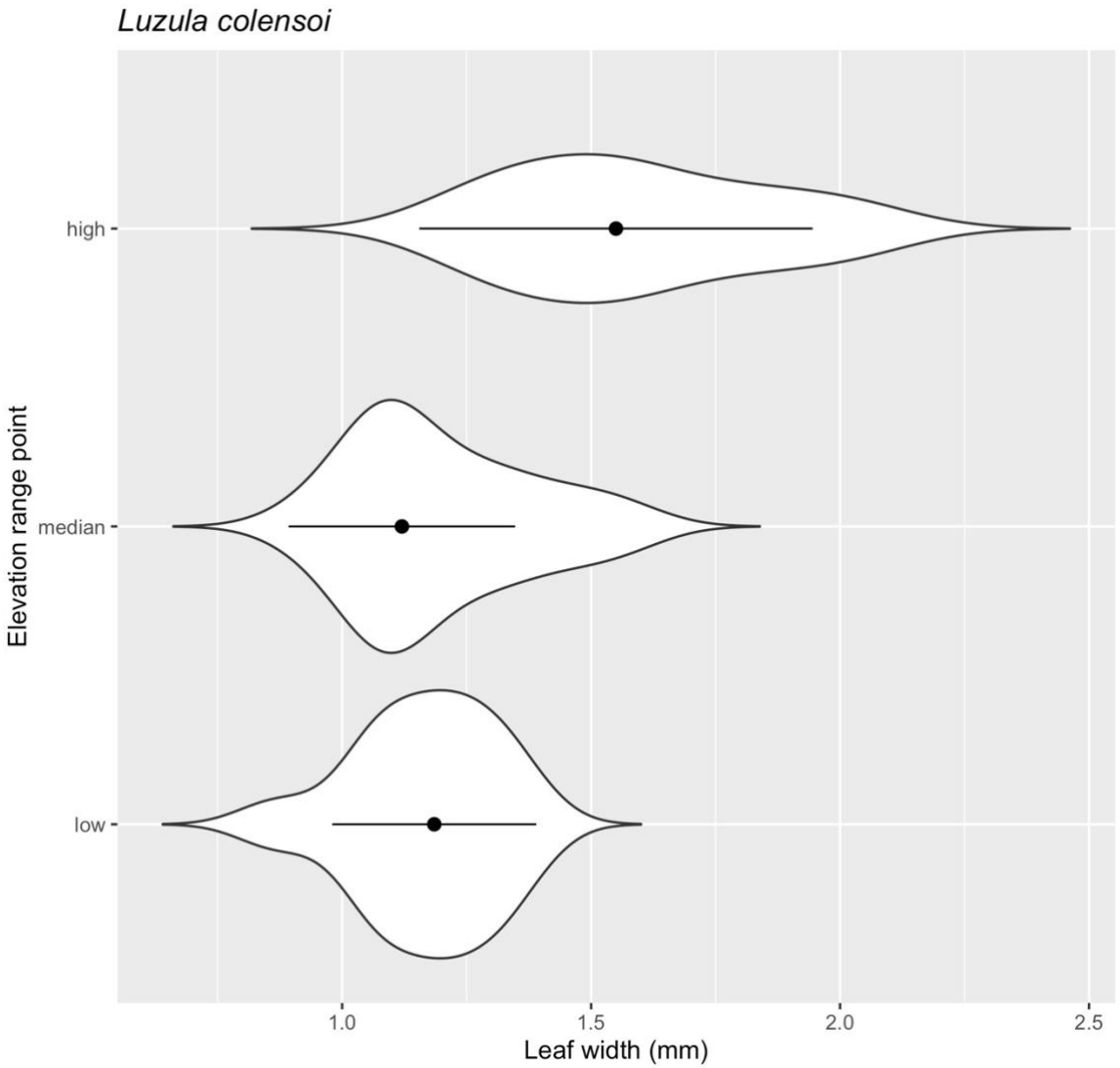


Figure 26. Violin plot illustrating the pattern of variation in leaf width over elevation in Luzula colensoi

Rytidosperma setifolium

In contrast to the other three species, trait values of *R. setifolium* tended to decline with elevation except for SRL and SLA, which exhibited slight increases, and a u-shaped response from belowground biomass (Figure 27). Declines in values were significant for aboveground biomass, plant height, leaf length, and SLA (Table 7), showing that both individual leaves and plant biomass overall declined with elevation. Pairwise comparisons revealed no significant differences between elevations for SLA despite the significant permutation test result.

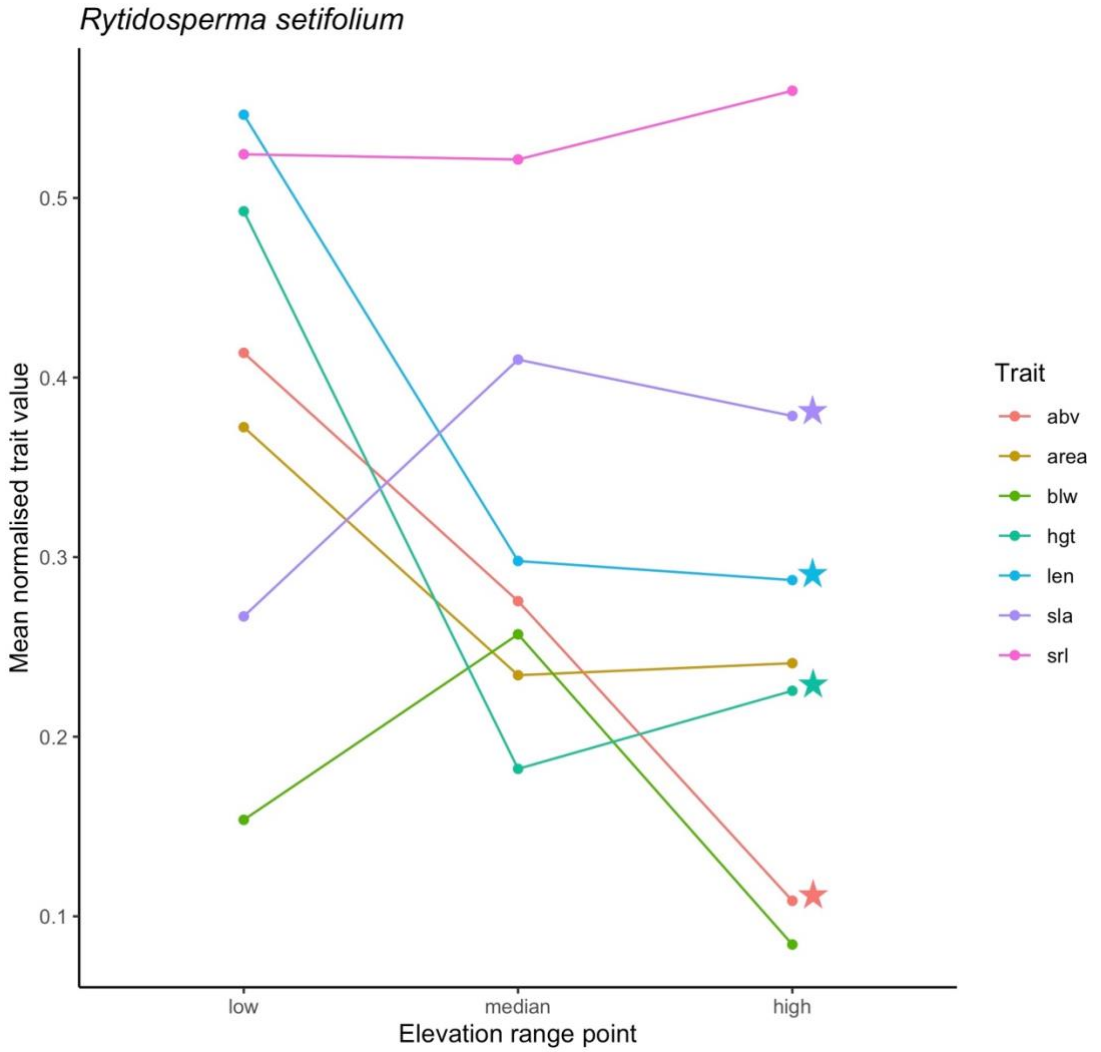


Figure 27. Normalised mean trait values for Rytidosperma setifolium in response to elevation

ABV = aboveground biomass; AREA = leaf area; BLW = belowground biomass; HGT = plant height; LEN = leaf length; SLA = specific leaf area; SRL = specific root length; THK = leaf thickness; WDT = leaf width

Stars denote traits which showed an effect of elevation on values in permutation tests

Aboveground Biomass

Permutation tests reported a significant difference in aboveground biomass between sites driven by elevation (p = 0.01; Table 7, Figure 28). As opposed to *C. gracilenta* and *G. bellidifolia*, aboveground biomass linearly decreased. Plants at higher elevations were significantly smaller compared to plants at the median (p = 0.002; Table 8) and lower sites (p = 0.001). Mean biomass at the high site was 2.8698g, as opposed to a mean aboveground biomass of 8.5106g at the lowest site, where dispersion around the mean was also much greater (Table 9).

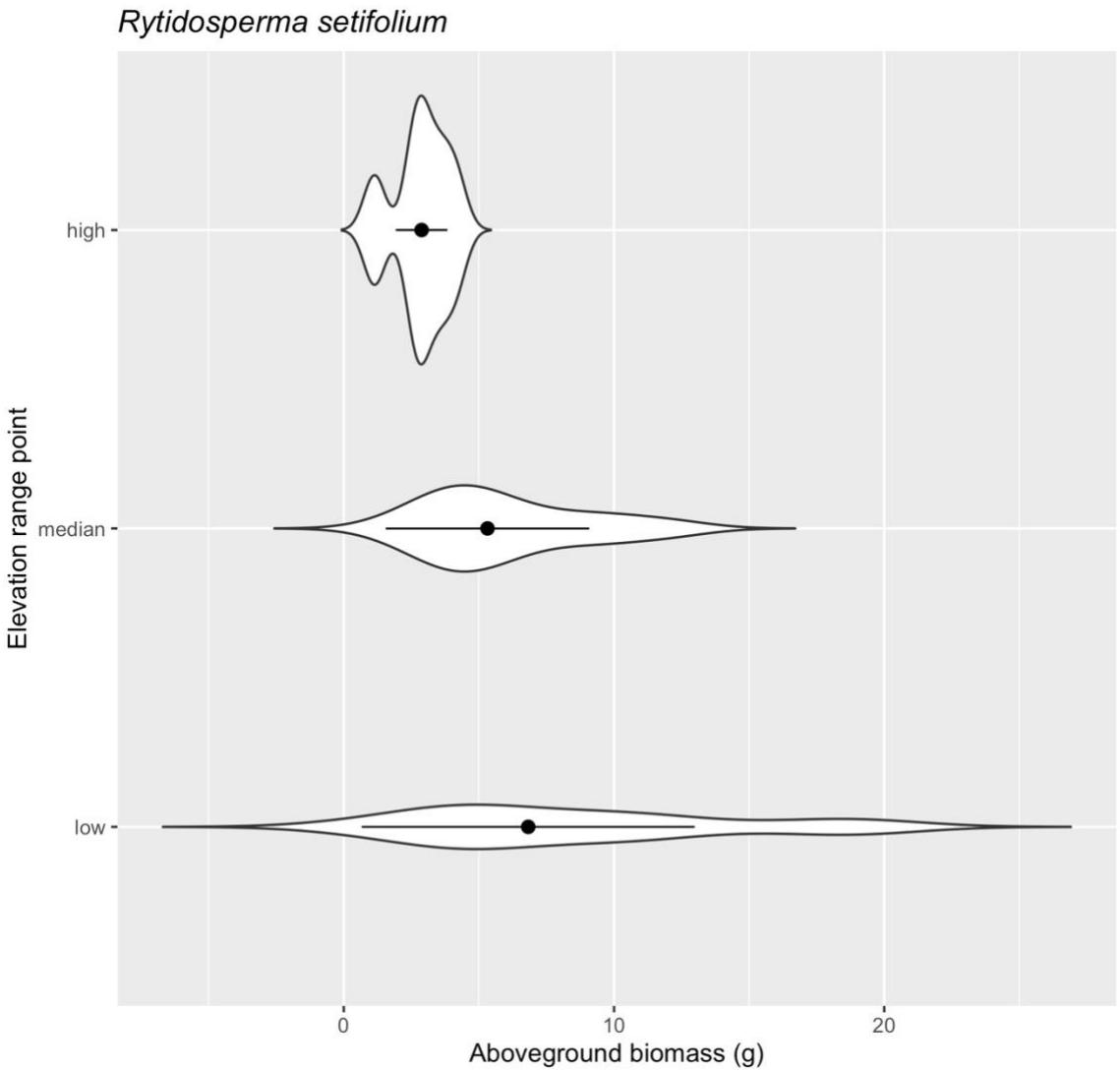


Figure 28. Violin plot illustrating the pattern of variation in aboveground biomass over elevation in Rytidosperma setifolium

Plant Height

Permutation tests revealed a significant effect of elevation on plant height (p = 0.01; Table 7, Figure 29). Visual inspection indicated a u-shaped response to elevation, however pairwise comparisons revealed plants to be significantly taller at the lowest elevation compared to both the median (p = <0.001; Table 8) and high sites (p = 0.04), with plants being 4.96cm shorter at the highest elevation than those at the lowest (Table 9). Variance was moderate at low and high elevations, but low for the median site (Table 9).

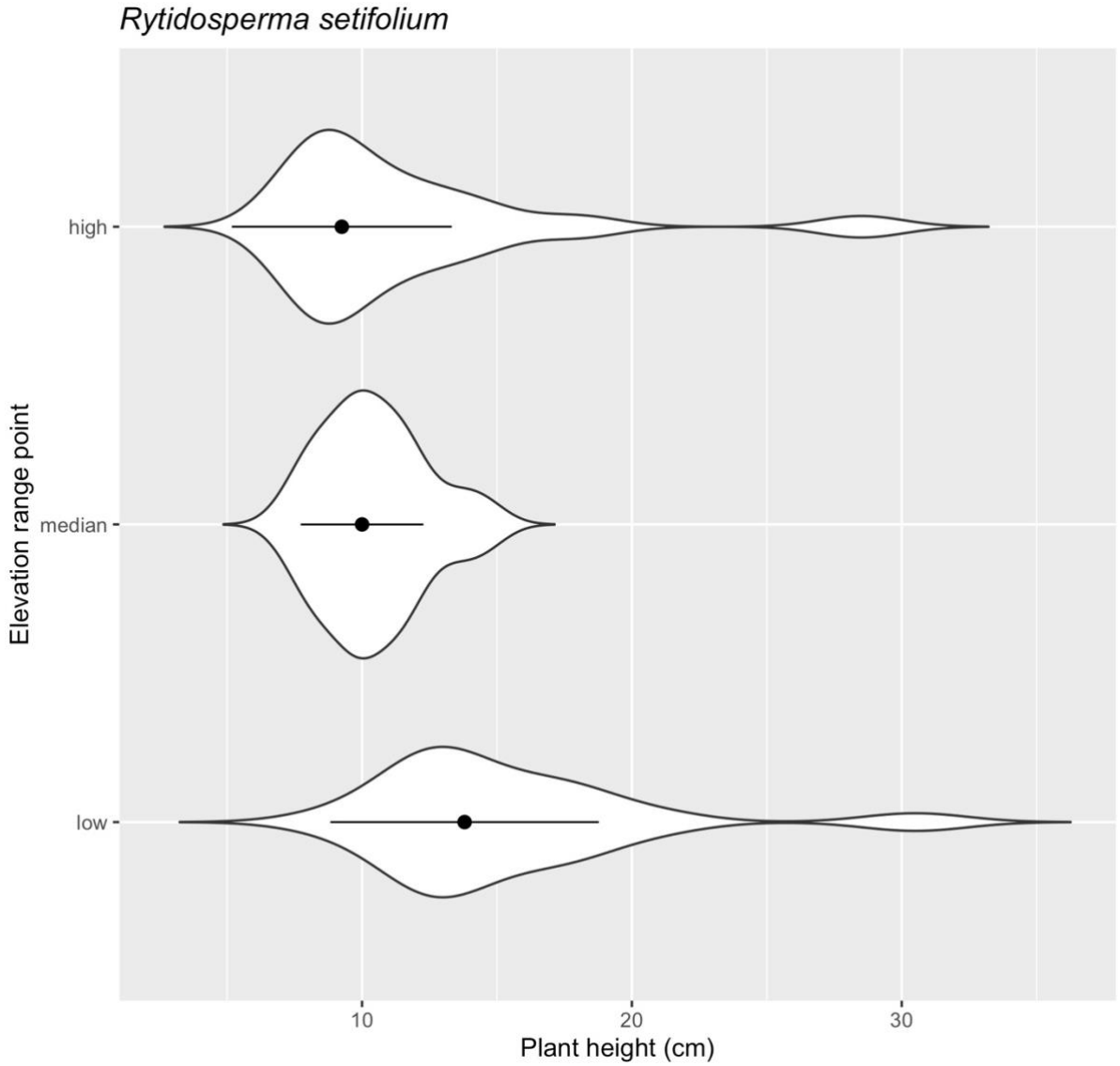


Figure 29. Violin plot illustrating the pattern of variation in plant height over elevation in Rytidosperma setifolium

Leaf Length

Permutation tests reported a significant effect of elevation on differences in leaf lengths between sites (p = 0.02; Table 7, Figure 30). In conjunction with the decline in aboveground biomass, leaves were significantly shorter at high elevation compared to the low (p = 0.02; Table 8) and median sites (p = 0.03). On average, leaves were 18.29mm shorter at the high site than the low, with the bulk of the difference occurring between low and median elevations (17.54mm difference, Table 8). Variance for all three elevations was huge (Table 9).

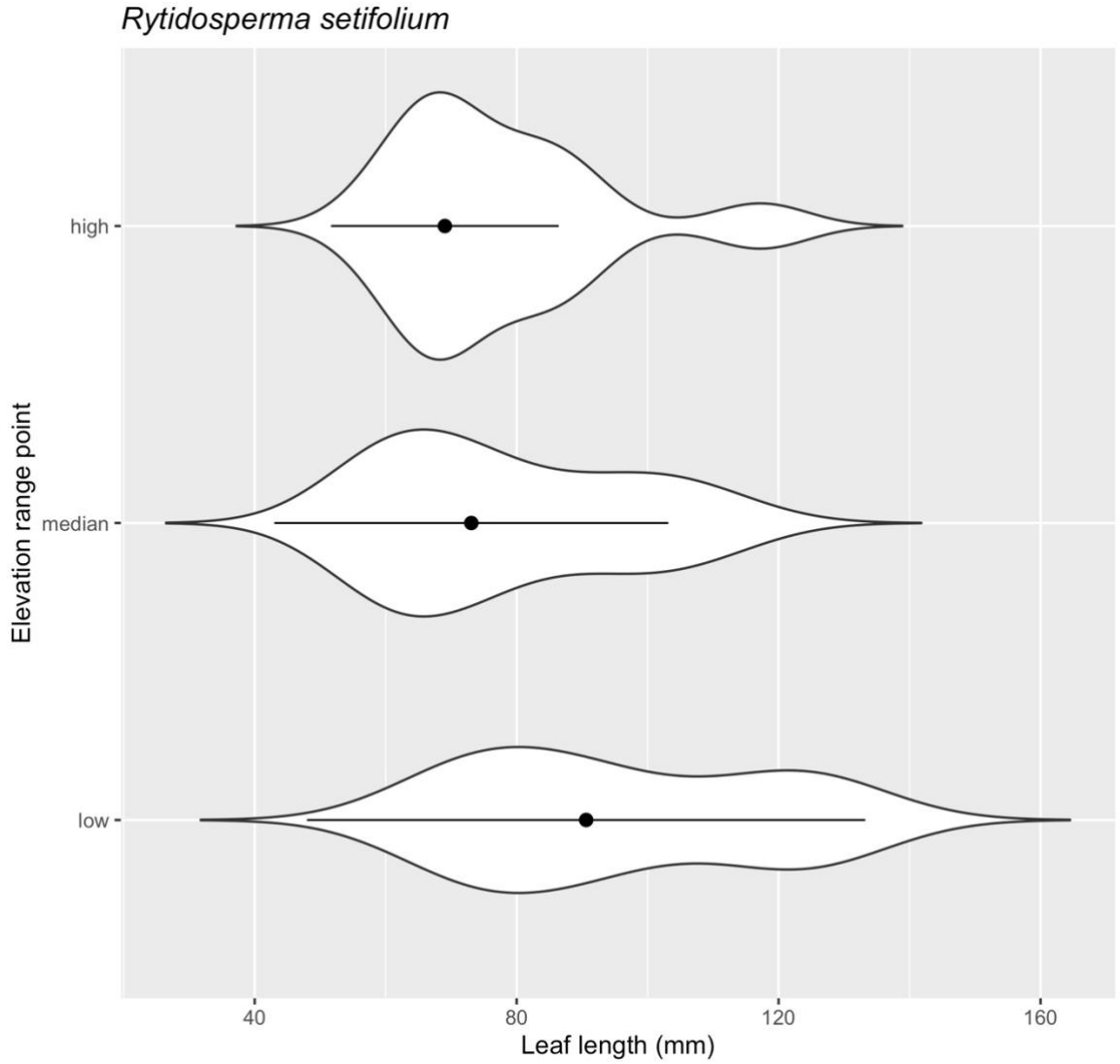


Figure 30. Violin plot illustrating the pattern of variation in leaf length over elevation in Rytidosperma setifolium

Bioregional variation: *How do these traits differ across bioregions of Aotearoa, and do they differ from the changes seen in the localised study?*

Summary statistics of traits within bioregions were generated to be used descriptively to identify any potential differences between regions (Table 10 and Table 11). These were produced due to the limited number of specimens with reliable location or collection information, resulting in insufficient numbers to perform any meaningful analyses of variation in traits between environments.

Table 10. Descriptive statistics for Celmisia gracilenta in LENZ Level II environments

Species	LENZ Environment	Minimum elevation (m)	Maximum elevation (m)	Mean elevation (m)	Statistic	LEN.H	VEG	WDT.H
Celmisia gracilenta	B1	370	1400	1038	Mean	117.25	119.98	1.97
					Variance	5299.13	5353.25	0.75
					Minimum	47.05	45.57	0.97
					Maximum	205.91	192.02	2.87
	E4	91	100	n/a	Mean	74.87	97.88	1.38
					Variance	487.83	931.20	0.03
					Minimum	46.49	64.76	1.08
					Maximum	113.00	143.15	1.59
	F1	91	940	434	Mean	121.02	130.31	2.24
					Variance	5659.84	5118.57	0.89
					Minimum	43.01	46.88	1.19
					Maximum	268.36	272.20	4.28
	F5	2	120	90	Mean	76.76	104.95	2.56
					Variance	1379.58	2897.43	1.00
					Minimum	34.25	48.06	1.17
					Maximum	138.28	196.87	4.41
	P1	1000	1700	1298	Mean	144.71	176.44	2.85
					Variance	2057.74	2431.88	0.37
					Minimum	87.70	118.39	1.89
					Maximum	220.72	241.50	3.50
	P3	29	650	236	Mean	82.33	97.89	2.90
					Variance	899.98	923.94	0.42
					Minimum	47.58	54.30	1.67
					Maximum	130.27	154.03	3.84
	P4	548	1000	874	Mean	65.58	85.44	1.75
					Variance	1166.45	1720.74	2.81
					Minimum	27.59	43.62	0.74
					Maximum	141.03	155.01	5.31
	P6	556	556	n/a	Mean	61.86	79.88	0.90
					Variance	95.51	88.39	0.01
					Minimum	48.25	67.17	0.77
					Maximum	79.96	92.50	1.11
	Q1	10	1400	1009	Mean	74.33	95.15	1.72
					Variance	444.18	806.72	1.25
					Minimum	41.71	48.57	0.71
					Maximum	129.42	178.30	6.00
	Q4	0	710	350	Mean	93.83	96.35	1.56
					Variance	1657.86	813.78	0.17
					Minimum	45.25	55.81	0.80
					Maximum	172.27	157.18	2.33

LEN.H = leaf length; VEG = vegetative height; WDT.H = leaf width

Table 11. Descriptive statistics for Gentianella bellidifolia in LENZ Level II environments

Species	LENZ Environment	Minimum elevation (m)	Maximum elevation (m)	Mean elevation (m)	Statistic	LEN.H	VEG	WDT.H
Gentianella bellidifolia	P1	1219	1889	1604	Mean	28.82	41.90	6.20
					Variance	174.96	429.19	5.96
					Minimum	13.90	22.54	2.57
					Maximum	64.82	121.52	14.35
	P2	1480	1767	1614	Mean	51.72	76.09	9.73
					Variance	1179.79	2617.91	38.87
					Minimum	21.62	33.05	5.13
					Maximum	116.22	171.73	24.65
	P3	1230	1432	1329	Mean	26.46	39.54	5.66
					Variance	116.09	239.66	5.20
					Minimum	7.97	14.78	2.65
					Maximum	50.42	77.88	11.45
	P4	1290	1700	1426	Mean	36.33	50.89	6.22
					Variance	1103.89	1660.96	17.06
					Minimum	19.52	25.48	3.12
					Maximum	140.31	174.30	18.58
	P7	1360	1650	1408	Mean	37.95	49.46	8.42
					Variance	391.67	439.82	4.17
					Minimum	22.20	30.57	5.33
					Maximum	77.26	87.34	11.18
	Q3	1523	1665	1604	Mean	44.85	58.56	12.05
					Variance	150.78	378.11	16.29
					Minimum	28.50	41.07	7.90
					Maximum	62.10	88.89	20.09

LEN.H = leaf length; VEG = vegetative height; WDT.H = leaf width

Table 12. Mean, minimum and maximum elevations recorded for each species across all herbarium collections

Species	Minimum elevation (m)	Maximum elevation (m)	Mean elevation (m)	Most commonly reported elevation (m)
Celmisia gracilenta	0	2539	715	~800
Gentianella bellidifolia	63	3020	1329	~1889
Luzula colensoi	163	2539	1330	~1547
Rytidosperma setifolium	0	3027	964	~1273

Not all variables derived from LENZ Level IV environment data had any reasonable difference between sites (annual solar radiation, winter solar radiation, drainage, soil age, chemical limitations, exchangeable calcium). Though sites 3 and 4 were found in the same Level IV environment, no single species was collected from both those sites.

Mean minimum temperature of the coldest month and mean winter temperature were not used due to these being confounded by snow pack at higher elevations and freeze-thaw

cycles at the lower sites. In discussing local trait changes at Tukino, temperature data from the WaRM experiments are used in place of mean annual temperature from the LENZ environments.

Environments in which *C. gracilenta* is present tend to have slightly warmer annual mean temperatures (an average of 8.9^{o} C) accompanied by very well drained and highly indurated soils with large particle sizes. Water balance ratios and annual water deficits vary widely, though vapour pressure deficits are generally low. Variance was high across all traits and environments, though values were generally smaller in P environments. Traits from plants in environment P6 had the smallest variance and range in sizes from 8 specimens. Plants in environments B1, F1 and P1 were considerably taller on average. B1 and F1 are similar environments which are warmer and with higher water limitations than P1, which is generally cooler and wetter.

Gentianella bellidifolia collections reflected a stronger set of environmental preferences, as almost all measurable specimens were collected from P environments. However, distributions of total available herbarium samples show that *G. bellidifolia* is widespread throughout Aotearoa (Figure 10). Mean annual temperatures were generally cooler (an average of 6.5^{o} C) and water limitations low, with low vapour pressure deficits, annual water deficits and water balance ratios. Soils are consistently well drained with large particle sizes, but varying rates of induration. Variance for all traits were high across all environments, with large ranges in both leaf length and plant height. Plants in environment P2 were the tallest and had the longest leaves. P2 has the lowest annual temperature of the environments *G. bellidifolia* was present in, but all other variables were consistent with overall trends.

Discussion

Overall, results indicate that phenotypic plasticity is occurring in response to elevation in all species, and this variation was particularly notable in plant aboveground traits associated with resource acquisition. Almost all traits significantly affected by elevation increased in value with the elevation, barring SRL in *C. gracilenta*, SLA in *G. bellidifolia*, and aboveground biomass, plant height, and leaf length in *R. setifolium*, all of which decreased in values with elevation gain. Additionally, plant height in *C. gracilenta*, and leaf length and SLA in *G. bellidifolia* demonstrated u-shaped trait–environment responses.

At Tukino slope, higher elevations represent a colder, wetter climate than lower elevations. Mean annual air temperature is ~3.6^{o} C and higher water balance ratios are combined with a lower vapour pressure deficit (VPD), meaning plants at higher elevations experience less water limitations than downslope. Lower elevations represent a warmer, drier climate with mean annual air temperatures of ~8.1^{o}. Though the area experiences high annual rainfall, the high winds and porous soils mean monthly water balance ratios and annual water deficits are lower. These factors combined with higher VPD suggest plants are under higher water stress in the Rangipo desert compared to higher elevations.

Overnight soil temperatures collected from WaRM data showed that soil at lower elevations retained most of the heat accumulated over the day (0.15^{o} loss overnight at site 1, 0.93^{o} loss at site 2); whereas the middle elevations lost more than 2^{o} overnight, and the highest site lost 3.3^{o}. Daily air and soil temperatures both decline at a steady rate upslope, with a 4.09^{o} drop in air temperature between sites 1 (1070m) and 5 (1930m), and a 2.62^{o} drop in soil temperature. Surprisingly, site 4 (1806m) had the least difference between day air and soil temperatures at 2.66^{o} difference, as opposed to ~3^{o} at sites 1 through 3 (1598m) and 5.5^{o} at the highest site. Night air temperatures were an average of ~9.86^{o} lower than during the day across all five sites, with the difference between the two deceasing with elevation.

Trait changes

All species showed significant increases in biomass with elevation gain. Though pairwise comparisons found no significant differences between elevations for *L. colensoi*, this is likely

due to small sample sizes impeding the ability of the t-tests to determine differences despite the permutation approach. However, the overall trend was an increase in aboveground weights with elevation, though low and median mean values were similar and presented as a plateau before rising sharply between the median and highest sites. This pattern was mirrored closely by its belowground biomass and SRL values, though neither were shown to be affected by elevation in permutation tests.

There was only one positive correlation between aboveground biomass and leaf traits (in *R. setifolium*), indicating that no one leaf trait is driving changes in plant sizes for any species. It is possible that the observed biomass gain is influenced by plant age in the higher elevations at Tukino, as life cycles and relative growth rates are slowed by temperature limitations [11]. Variance for aboveground biomass was largest at the highest range point in those species which increased in size with elevation while the low and median sites remained comparatively low, suggesting a broader range of sizes for sampling at these sites increasing within site variability. *Rytidosperma setifolium* exhibited the opposite by having a much higher variance at its lowest elevation than the other two sites.

For *C. gracilenta*, the increase in aboveground biomass was the most distinct change in all traits reported for the species. The difference in means was numerically largest between the median and high sites, and the largest corresponding difference in temperature variables between sites 2 and 3 was in overnight soil temperature (2.1^{o} difference). Though correlation testing found no interaction, the increase in above ground weight in *C. gracilenta* is matched by increases in leaf thickness.

Both leaf length and aboveground biomass decreased with elevation in *R. setifolium*, and the two traits were weakly correlated in Spearman's tests. This suggests that changes in leaf length may be driving the accrual of aboveground biomass at lower elevations. Though it is possible that wind shear may interact with temperature to drive this difference, the combination of positive correlations between aboveground biomass, plant height, and leaf length suggest that variability is more likely described by a phenotypic response. This finding is also supported by other intraspecific trait research on alpine Poaceae which found significant decreases in leaf length [106]. The same study saw an increase in lateral spread correlated with plant height. I did not measure lateral spread of *R. setifolium* but the positive correlations among aboveground traits suggest that plants are generally smaller at

higher elevations (*i.e.,* lateral spread is not occurring) and environmental factors such as temperature and acid soluble phosphorus may be constraining growth. Temperature decreases and acid soluble phosphorus availability increases considerably with elevation and *R. setifolium* has been described as preferring depleted habitats within environments.

Luzula colensoi was the only species in which a positive effect of elevation on leaf area was reported. On average, leaves had 5.42mm^2 more surface area at its highest elevation (site 5) compared to its lowest (site 2). A positive correlation with leaf width indicates the change in surface area is caused by wider leaves at higher elevation. According to the LES, larger surface area of leaves without corresponding changes in SLA or thickness suggests an increase in photosynthetic potential while keeping construction costs of leaves low, which is correlated with leaf longevity. This indicates that *L. colensoi* replaces more leaves over a lifetime than the other study species at higher elevations. However, a linear increase with elevation was seen in leaf length and SLA maintained moderately high values at all sites. As seen in other studies, intraspecific variation in LES traits does not always follow the established responses seen at larger scales.

Rytidosperma setifolium was significantly shorter at higher elevations, which was reflected in correlated reductions in leaf length and aboveground biomass. Differences in height means resulted in a mild u-shaped response which was likely driven by the small sample size as the difference in means between median (site 3) and high (site 5) elevations was very small (a 1.23cm difference between sites 3 and 5 versus a 5.64cm difference between site 1 and 3).

Both forb species saw significant differences in height with elevation. Mean plant height and within site variance for *C. gracilenta* was similar between its low and high range points (sites 1 and 3), but significantly lower at its mid elevation (site 2). VPD was lower and monthly water balance ratio higher at site 2 compared to site 1 and 3, but all other environmental variables track linearly between those sites and this combination suggests that plants at site 2 have slightly higher water retention than surrounding sites. Though not statistically correlated, shorter plant sizes at mid-elevation are matched by peaks in mean leaf length and mean belowground biomass, suggesting wider rosettes and increased belowground carbon allocation. Aboveground biomass does not match these changes, and maintains similar weights the lower site.

In contrast to the idiosyncratic response of *C. gracilenta*, *G. bellidifolia* plants were much taller at high elevations while low and mid-elevation plants were similar heights. This increase in stature was moderately correlated with leaf thickness and weakly with leaf length, indicating that taller plants also have thicker, longer leaves. Leaf thickness is significantly correlated with aridity and water availability [107] , and the increase in values in *G. bellidifolia* corresponds with increases in water availability and evapotranspiration rates (defined by VPD values) upslope. The positive correlation of leaf thickness with elevation and small within site variance in *C. gracilenta* and *G. bellidifolia* indicates the a sensitivity to water availability. Herbarium specimens of both species exhibit high variation of leaf morphologies, many of which are thinner and wider than those found at Tukino. This is symptomatic of adaptive plasticity and suggests that these forbs may have a large band of variation available to cope with precipitation and water changes in water availability. For *G. bellidifolia*, this is reflected bioregionally in an overall preference for cooler, wetter environments with low water loss rates but good drainage, whereas *C. gracilenta* environments showed no bias for particular water conditions, though drainage is always high.

Gentianella bellidifolia was the only species which showed identifiable variation in SLA correlated with elevation, expressing lower SLA at higher elevations. In alpine research, SLA has been shown to decrease in response to temperature, soil pH, and precipitation [49] suggesting that the reduction in SLA seen here is driven by temperature in *G. bellidifolia*.

Celmisia gracilenta showed a mild u-shaped response to the environment in SRL values, with the overall trend being a decrease in values with the increase in elevation. These results are likely to have been affected by the sampling methodology in combination with the species rooting strategy – *C. gracilenta* has thick, fleshy roots which travel comparatively long distances from the rosette before fine root mass appears, resulting in lower amounts of fine root mass collected with samples. The higher belowground biomass at the lowest elevation site suggests roots may not have to spread as far in order to collect water or nutrients, therefore more was available within the 20cm sampling radius. Decreased SRL values were also moderately correlated with increased aboveground biomass.

Predictions for climate sensitivity

Gentianella bellidifolia and *L. colensoi* inhabit more restricted elevational bands generally than either *C. gracilenta* or *R. setifolium*, and the consistent increase of trait values with elevation combined with the known habitat preferences and elevational presences of both species indicate that they are alpine specialists. I hypothesised that both specialist species would express trait changes with low variation locally but large variation bioregionally. Few traits were correlated with an increase in elevation locally in *L. colensoi*, but aboveground biomass and leaf width showed very low variance among sites, while leaf area variance was moderate. Bioregional variation could not be shown in *L. colensoi* but the lower number of total available herbarium specimens and the distribution of collections does reinforce the suggestion that it exclusively prefers high alpine habitats. These areas are often difficult to reach and are not as often visited by collectors for herbaria. While bioregional variation could not be explicitly tested in *G. bellidifolia*, variance among bioregions was consistently high across all measured traits, in contrast with the low to very low variance expressed locally at Tukino in all traits. These findings are consistent with the hypothesis that specialist species possess tighter physiological coupling with their environment, which may limit their ability to morphologically or physiologically adapt to warming temperatures within their habitats.

I hypothesised that *R. setifolium* and *C. gracilenta* were generalist species. *Rytidosperma setifolium* appears to tolerate lower temperatures but prefer warmer ones as indicated by its overall decrease in trait values upslope, suggesting the species is constrained at colder temperatures. As with *L. colensoi*, bioregional variation could not be shown, but available evidence from its broad geographic and elevational spread suggests it has the capacity to persist under climate change for longer than other species in this study, as is inhabits broad climatic niches. It is possible that the most constraining factor for this species will be mineral nutrients such as acid soluble phosphorus, and given its higher biomass and height at warmer sites, it may expand its range to occupy new environments as they change. *Celmisia gracilenta* appears to be an alpine generalist with a more restricted climatic niche than *R. setifolium*. I hypothesised that *C. gracilenta* would show changes in LES related traits locally, but with high variance. However, my results found that the only economic spectrum related trait that exhibited a correlation with elevation was SRL, which reduced in value upslope

with temperature and increasing water availability. Additionally, all traits showed low variance at all sites, suggesting that C. gracilenta is more tightly adapted to low alpine habitats than hypothesised. Bioregional variation could not be explicitly tested, but the most consistent variable across environments it was found in was high drainage with large soil particle sizes and moderate acid soluble phosphorus, suggesting that water limitations may play a role in influencing trait variation.

As the climate warms, the habitats that *G. bellidifolia* and *L. colensoi* are adapted to will move upslope, and the ranges of specialist species such as these will move to track them. As this can only happen intergenerationally, adult plants will remain at lower elevations until environmental conditions move too far outside their optima. Populations that remain in warming ecotypes will eventually lose the ability to reproduce, as alpine specialist reproduction is tightly linked to the cold environment. For example, timing of flowering is correlated with snowmelt timing and relative daylight hours (which also herald the arrival of pollinators) and seeds often have "chill time hours" that need to be met to be able to germinate. Alpine plants also often have low propagule dispersal rates, and this combined with potential climate change induced reductions in reproduction rates will culminate in an "extinction debt" as adult plants will still persist but the establishment of new generations will not be occurring [108,109].

Future directions

Previous and ongoing research is finding that LES traits measured at intraspecific scales do not always respond in ways expected and established at community or ecosystem scales. This means that known community level responses cannot reliably be applied to species within the same family or growth form to predict responses to changing environments. While my results strongly indicate that variation in key functional traits in response to environmental factors is occurring among all species at a local scale, analyses of broadscale variation between and among bioregions was not possible. This addition is vital to disentangle true adaptive variation within species and improve predictions of their ability to cope with climate change.

There are two key pieces of the puzzle that were unable to be shown here that will strengthen future studies of alpine plant traits. The first is a broadscale sampling of plants throughout the bioregions of Aotearoa, and the second is to establish strong linkages to the abiotic variables influencing these changes both locally and bioregionally. By carrying out robust, trait based, intraspecific studies, researchers can build on species specific variation patterns seen in northern hemisphere studies and potentially use these to identify the alpine species likely to be most sensitive to climate change. To strengthen findings, a systematic review of all available herbaria samples of select species would track trait variation over time as well as space. This may allow future researchers to add time as a dimension to future phenotypic adaptation to climate change and project how long a species may be able to plastically respond for at current rates of warming.

Climate change is already threatening alpine ecosystems globally [3,4], and all predicted changes of climate variables will interact synergistically with biotic and topographic factors in alpine ecosystems to accelerate rates of change [5,110,111]. Additionally, abundances and distribution of specialist species such as *L. colensoi* and *G. bellidifolia*, will be reduced as alpine zones shrink upwards, restricting genetic recombinants possible during reproduction and potentially causing species to linger only as an extinction debt [108,112].

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Supplementary Materials

S1: Localised variation

S1.1: All summary statistics by species

species	range point	elevation	n	statistic	ABV	AREA	BLW	LEN	SLA	SRL	THK	WDT
Celmisia gracilenta	low	1077	12	mean	0.3922	86.7973	1.0182	54.1473	11.2909	1.8842	0.6454	1.6655
				variance	0.03	774.01	1.71	101.80	365.10	1.59	0.06	0.07
				st.dev	0.16	26.53	1.25	9.62	18.22	1.20	0.23	0.25
				min	0.0945	44.15	0.0525	40.49	4.099	0.574	0.357	1.27
				max	0.6334	136.28	4.4215	72.51	68.817	4.169	0.952	2.22
	median	1283	12	mean	0.6927	95.8883	0.5922	61.0542	6.4243	1.4786	0.6601	1.7058
				variance	0.34	1185.48	0.32	274.75	36.53	0.63	0.05	0.18
				st.dev	0.55	32.97	0.54	15.87	5.79	0.76	0.22	0.41
				min	0.0519	37.57	0.0608	28.52	3.229	0.442	0.392	1
				max	2.2992	157.79	2.149	84.44	25.389	2.657	0.989	2.21
	high	1590	12	mean	1.3190	87.9242	0.7163	59.4050	4.3072	0.8060	0.7309	1.9367
				variance	0.78	936.75	0.25	169.45	0.75	0.20	0.06	0.15
				st.dev	0.84	29.30	0.48	12.46	0.83	0.43	0.23	0.37
				min	0.1670	54.7900	0.1779	41.1600	3.0240	0.1420	0.4360	1.4700
				max	2.7231	168.51	1.7294	85.79	6.415	1.544	1.078	2.52

REMEMBER TO CHANGE

species	range point	elevation	n	statistic	ABV	AREA	BLW	LEN	SLA	SRL	THK	WDT
Gentianella bellidifolia	low	1077	12	mean	0.4607	109.71	0.2742	24.9617	20.5483	0.7251	0.3028	7.2300
				variance	0.17	3610.27	0.10	78.25	34.48	0.43	0.01	4.35
				st.dev	0.39	57.53	0.31	8.47	5.62	0.62	0.08	2.00
				min	0.0593	49.82	0.0094	16.84	12.345	0.091	0.175	4.46
				max	1.5836	234.57	1.1894	50.46	35.729	2.253	0.517	10.57
	median	1590	12	mean	0.9353	96.04	0.3069	20.5542	15.2118	1.0737	0.3081	6.9592
				variance	0.17	1771.36	0.01	25.06	10.15	0.46	0.46	2.25
				st.dev	0.40	40.30	0.11	4.79	3.05	0.65	0.06	1.44
				min	0.3536	47.29	0.1694	12.06	10.51	0.218	0.203	5.07
				max	1.5361	185.43	0.4711	29.03	21.881	2.27	0.404	10.23
	high	1795	10	mean	1.0444	134.95	0.2826	28.2210	17.1538	1.0280	0.3907	7.7670
				variance	0.46	2573.64	0.02	63.90	23.83	0.42	0.00	1.38
				st.dev	0.64	48.13	0.13	7.58	4.63	0.61	0.04	1.12
				min	0.2911	73.04	0.0771	18.1	10.413	0.19	0.331	6.02
				max	2.1135	206.73	0.4534	36.74	27.42	2.379	0.461	9.33

species	range point	elevation	n	statistic	ABV	AREA	BLW	LEN	SLA	SRL	THK	WDT
Luzula colensoi	low	1283	12	mean	1.0858	10.55	0.2213	9.0808	16.0612	30.9608	0.2510	1.1692
				variance	0.45	10.52	0.02	2.94	12.35	249.41	0.01	0.02
				st.dev	0.64	3.11	0.13	1.64	3.36	15.12	0.08	0.13
				min	0.1369	5.73	0.0328	6.22	8.7	11.932	0.134	0.87
				max	2.044	16.70	0.4529	11.62	20.152	61.315	0.36	1.37
	median	1590	12	mean	1.0915	12.00	0.2153	10.1250	15.0770	30.3066	0.2437	1.1950
				variance	0.69	18.10	0.02	7.95	13.88	95.00	0.01	0.03
				st.dev	0.79	4.07	0.13	2.70	3.57	9.33	0.08	0.17
				min	0.0414	4.86	0.0118	6.21	10.177	16.609	0.141	0.94
				max	2.6116	19.80	0.532	16.42	23.06	45.494	0.397	1.56
	high	1958	12	mean	2.3808	15.97	0.3316	11.1217	16.5606	40.1773	0.2505	1.5942
				variance	4.87	16.27	0.07	3.41	25.83	770.47	0.02	0.07
				st.dev	2.10	3.86	0.25	1.77	4.87	26.58	0.12	0.25
				min	0.1042	9.66	0.0611	7.75	9.043	5.615	0.128	1.24
				max	7.4941	23.62	0.9033	14.17	23.211	118.206	0.516	2.04

species	range point	elevation	n	statistic	ABV	AREA	BLW	LEN	SLA	SRL
Rytidosperma setifolium	low	1077	12	mean	8.5106	66.74	2.2423	95.7258	8.5092	3.0737
				variance	33.75	957.24	6.90	500.80	0.88	1.13
				st.dev	5.56	29.62	2.51	21.43	0.90	1.02
				min	0.8614	37.36	0.3394	68.52	7.374	0.747
				max	19.349	129.55	8.3344	127.74	10.396	4.479
	median	1590	12	mean	5.9550	52.92	3.5699	78.1900	9.8835	3.0567
				variance	8.91	401.45	17.52	349.21	4.91	0.88
				st.dev	2.86	19.18	4.01	17.89	2.12	0.90
				min	2.0465	29.55	0.5037	57.17	7.962	1.555
				max	12.0865	91.66	13.117	111.09	15.554	4.552
	high	1958	11	mean	2.8698	53.59	1.3501	77.4382	9.5820	3.2810
				variance	1.04	458.76	1.51	266.02	6.75	3.50
				st.dev	0.97	20.42	1.17	15.55	2.48	1.78
				min	1.1153	29.48	0.2671	58.92	5.943	0
				max	4.2478	104.16	4.6854	117.2	14.328	5.862

S1.2: All permutation test results by species

		PERMUTATION TESTS			PAIRWISE TESTS: ELEVATION			PAIRWISE TESTS: JAN~ELEV			PAIRWISE TESTS: DEC~ELEV		
species	trait	elev.	coll.	interact	low-med	low-high	med-high	low-med	low-high	med-high	low-med	low-high	med-high
Celmisia gracilenta	ABV	**	ns	ns	ns	***	ns	ns	ns	ns	ns	*	ns
	AREA	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	BLW	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
	LEN	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	SLA	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	*	ns
	SRL	*	**	ns	ns	**	*	ns	ns	ns	ns	*	ns
	THK	**	***	ns	ns	**	ns	ns	ns	ns	ns	ns	ns
	WDT	ns	***	ns	ns	*	ns	ns	ns	ns	ns	*	ns

		PERMUTATION TESTS			PAIRWISE TESTS: ELEVATION			PAIRWISE TESTS: JAN~ELEV			PAIRWISE TESTS: DEC~ELEV		
species	trait	elev.	coll.	interact	low-med	low-high	med-high	low-med	low-high	med-high	low-med	low-high	med-high
Gentianella bellidifolia	ABV	*	ns	ns	*	ns	ns	ns	ns	ns	*	ns	ns
	AREA	ns	***	ns	ns	ns	**	ns	ns	**	ns	ns	ns
	BLW	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	LEN	**	***	ns	ns	ns	***	ns	ns	**	ns	ns	ns
	SLA	*	ns	ns	*	ns	ns	ns	ns	ns	*	*	ns
	SRL	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	THK	**	**	ns	ns	**	**	ns	ns	ns	ns	*	ns
	WDT	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

		PERMUTATION TESTS			PAIRWISE TESTS: ELEVATION			PAIRWISE TESTS: JAN~ELEV			PAIRWISE TESTS: DEC~ELEV		
species	trait	elev.	coll.	interact	low-med	low-high	med-high	low-med	low-high	med-high	low-med	low-high	med-high
Luzula colensoi	ABV	*	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
	AREA	**	**	ns	ns	**	*	ns	ns	ns	ns	ns	ns
	BLW	ns	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns
	LEN	ns	ns	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
	SLA	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	SRL	ns	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	ns
	THK	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	WDT	***	ns	ns	ns	***	***	ns	*	ns	ns	**	*

		PERMUTATION TESTS			PAIRWISE TESTS: ELEVATION			PAIRWISE TESTS: JAN~ELEV			PAIRWISE TESTS: DEC~ELEV		
species	trait	elev.	coll.	interact	low-med	low-high	med-high	low-med	low-high	med-high	low-med	low-high	med-high
Rytidosperma setifolium	ABV	**	ns	ns	ns	**	**	ns	ns	ns	ns	**	*
	AREA	ns	***	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	BLW	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
	LEN	*	**	ns	ns	*	ns	ns	ns	ns	ns	ns	ns
	SLA	*	***	**	ns	ns	ns	*	*	ns	ns	ns	ns
	SRL	ns	*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns

significance stars:

ns	not significant
*	P ≤ 0.05
**	P ≤ 0.01
***	P ≤ 0.001

S2: Bioregional variation

S2.2: Total LENZ Level II environment counts

L2 ENV	Enviroment Type	*Celmisia gracilenta*	*Gentianella bellidifolia*
B1	Central Dry Lowlands	6	
D2	Northern Hill Country	2	
D4	Northern Hill Country	1	2
E1	Central Dry Foothills	2	
E4	Central Dry Foothills	8	
F1	Central Hill Country & Volcanic Plateau	12	
F4	Central Hill Country & Volcanic Plateau	1	
F5	Central Hill Country & Volcanic Plateau	13	
F6	Central Hill Country & Volcanic Plateau	3	
F7	Central Hill Country & Volcanic Plateau	5	3
K1	Central Upland Recent Soils	1	
O1	Western South Island Foothills & Rakiura	3	
P1	Central Mountains	6	29
P2	Central Mountains		13
P3	Central Mountains	9	27
P4	Central Mountains	10	12
P6	Central Mountains	8	
P7	Central Mountains	2	6
P8	Central Mountains	1	
Q1	Southeastern Hill Country & Mountains	18	
Q3	Southeastern Hill Country & Mountains	1	7
Q4	Southeastern Hill Country & Mountains	10	
R1	Southern Alps		1
S1	Ultramafic Soils	4	4
S2	Ultramafic Soils	4	4
	TOTAL	**130**	**108**