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Phenotypic plasticity and water availability: responses of alpine herb species along an elevation gradient

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Abstract

Background: Alpine regions are particularly vulnerable to the effects of climate change. The Australian Alps are potentially more so than other mountain regions, as they cover a very small geographic area (<0.05% of mainland Australia), with a low maximum elevation (2228 m). Therefore, response to climate change will be primarily determined by the ability of species to survive in-situ through local adaptation or phenotypic plasticity. Existing climate change models project not only warming but increasingly variable precipitation and snow cover across the Australian Alps. Thus, plasticity in water use traits may become increasingly important for the establishment and persistence of Australian alpine plants. Given that plants from lower elevations inhabit a more heterogeneous environment with more frequent frosts, greater temperature extremes, and higher evapotranspiration, we predict plasticity – and particularly adaptive plasticity – may be more common at low relative to high elevation. To test these predictions we investigated the extent of plasticity and the adaptive value thereof in water use traits in three herbaceous Australian alpine plant species. Seeds were collected from low and high elevation alpine sites and grown at ample and limiting water availability under common-garden conditions. For morphological and physiological traits, we compared both their means and phenotypic plasticity across treatments and elevations.

Results: Responses of morphological and physiological traits to water availability were in accord with many previous studies of water response. Although previous work in the same environment demonstrated greater plasticity in response to temperature for low elevation populations, plasticity in response to water availability in our study showed markedly little variation as a function of elevation. Rather, patterns of plasticity were highly variable among species and among traits within species, with few instances of adaptive plastic responses.

Conclusion: We discuss the difficulties in observing adaptive plasticity and the importance of microhabitat variation in shaping the persistence of these Australian alpine species.

Keywords: Altitude, Australia, Climate change, Intra-specific variation, Microhabitat variability, Plant functional traits

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Background

Global climate change predictions include increasing temperatures, changing precipitation patterns and an increase in the frequency of extreme events [1]. Given the limited spatial and elevation scale of alpine landscapes, they are likely to be vulnerable to climate change [2, 3]. Indeed, particularly rapid rates of environmental change within alpine areas have been observed [4]. The Australian alpine region is likely to be particularly impacted because it has a small geographic area (<0.05% of the Australian continent), a limited elevation gradient of approximately 400 m above tree-line, and low summits (maximum of 2228 m.a.s.l) [5–7].

Alpine plants therefore have limited options for range shift, and in addition, some are characterized by poor dispersal capacity [8, 9]. In conjunction with slow growth rates and often fragmented habitats responses to climate change are therefore expected to be primarily determined by the ability to survive insitu through local adaptation or phenotypic plasticity [8–11]. Phenotypic plasticity is the capacity for a given genotype to express different phenotypes under heterogeneous environments [12, 13]. Plasticity may be an important adaptation to spatial and temporal heterogeneity and may allow species to better cope with climate change [12–14]. The extent to which plasticity is adaptive, whereby a fitness benefit is conferred, is expected to vary among and within species as a function of both environmental conditions and evolutionary history [14, 15]. Whether plasticity can facilitate adaptation under changing conditions, through allowing for short-term adjustments to novel conditions, is still debated [11]. As such, more information is required to understand the capacity for, and variability in, adaptive phenotypic plasticity across species within vulnerable habitats, and how this may impact on future distributions and persistence [10, 16].

Intra-specific variation in traits and tolerances is increasingly seen as a potentially fundamental factor in understanding community responses to climate change [17]. Relative performance along environmental gradients, often using elevation as a proxy, is often used as an indicator of potential responses to climate change. At lower elevations, for instance, organisms are exposed to higher mean temperatures, greater temperature fluctuations, longer growing seasons and increasingly variable water availability [18, 19]. For that reason, lower elevations are often seen as indicative of the changes that will potential occur at higher elevations [20]. Thus, one may ask whether low elevation populations, which are already exposed to high levels of environmental heterogeneity, are more likely to display greater levels of adaptive phenotypic plasticity? The answer to this question may provide an indication of whether such plasticity will be favoured under climate change.

In contrast to temperature, water availability in alpine regions often varies in a mosaic-like pattern, as a function of many factors, including climate and topography, soil composition, snow layer presence and aspect [18, 21]. Water availability has not generally been considered a driving factor in the evolution of alpine plants [18]. However, climate change scenarios predict not only higher mean temperatures and a greater variability in temperatures, but also changing spatial and temporal patterns of precipitation and snowfall, thus the present mosaic-like variation in water availability may further intensify [1, 3]. As such, understanding the underlying morphological and physiological responses of plants to varying water availability, not only on a species by species but also at a within-species level, may help inform predictions on distributions and persistence into the future for this vulnerable plant community [22, 23].

Among Australian alpine herbs, lower elevation populations of *Wahlenbergia ceracea* and *Aciphylla glacialis* have been shown to have a greater plasticity in a range of traits in response to warming temperatures [24] and a greater ability to acclimate to cold temperatures than their higher elevation counterparts [25]. In both cases, greater plasticity resulted in a fitness advantage [24, 25]. However, it is possible the evolution of adaptive responses for one abiotic variable may be maladaptive or neutral for another [26, 27]. Consequently, it is important to assess the responses of alpine plants to variable water availability, in order to see whether they display similar patterns of plasticity in response to water availability along elevation gradients as were seen for temperature. Alternatively, given that water availability may vary more as a mosaic within the alpine landscape than as a continuous gradient, there may be less pressure for the evolution of plasticity in response to water availability along elevation gradients.

Here we examine whether three Australian alpine herb species show within-species variation in plastic response to water availability. For two of these species prior studies have shown such variation in plastic response to temperature [24, 25]. We grew plants under common garden conditions exposed to either well-watered or water-limited conditions and examined plant functional traits that are known to vary as a function of water availability, including morphological and physiological traits [28]. Specifically, we looked to answer the following questions: 1) How are plant traits and fitness influenced by reduced water availability, and how does this vary depending upon source elevation? 2) Is there any evidence that the water responses represent adaptive plasticity? Or 3) are the traits themselves under directional selection?

We predicted that plant biomass, leaf count, leaf size and specific leaf area (SLA) would be lower under

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limited water availability. Also, we expected plants grown under low water availability to have more conservative water use strategies, and thus to exhibit reduced stomatal conductance and consequently reduced photosynthetic rates, and potentially greater instantaneous water use in comparison to plant grown under high water availability [29, 30]. Furthermore, as plants decrease photosynthesis in response to reduced water availability, less discrimination of $^{13}CO_2$ in favor of $^{12}CO_2$ occurs due to the reduced CO_2 concentration within the leaf. This results in a less negative $\delta^{13}C$ (an integrated measure of water use efficiency) compared to a plant with stomata more open [31]. Finally, we expected leaf nitrogen content (N) to decrease under water limitation [32].

With regards to intra-specific variation attributable to elevation, we considered two alternative hypotheses; a) that we would observe similar patterns of plasticity for water availability as previously observed for temperature along the elevation gradient [24, 25]. As such, plants sourced from environmentally more heterogeneous lower elevations are predicted to display greater plasticity and more evidence of adaptive plastic responses. Alternatively, that b) as a result of the inherently more mosaic-like distribution of water availability within the Australian alpine landscape, responses to water availability would be measurable but few elevation based differentiation in traits responses would be observed.

Methods
Seed collection and germination
Seeds from three alpine herbs; *Aciphylla glacialis* (F. Muell.) Benth. (Apiaceae), *Oreomyrrhis eriopoda* (DC.) Hood.f. (Apiaceae), and *Wahlenbergia ceracea* Lothian (Campanulaceae), were collected from Kosciuszko National Park, New South Wales, Australia between December 2009 and April 2010 (Additional file 1: Figure S1). For each species, seeds were collected from 30 individual plants within their natural distribution (ranging from 1600 to 2200 m a.s.l,). Seeds were germinated over winter between May and August 2010 under controlled conditions at the Australian National Botanic Gardens,

Canberra. From these seedlings, replicates of four to six maternal lines from the upper and lower 25% of the elevation distribution (henceforth referred to as high and low elevation) of each species were selected for the experiment (Table 1). Seedlings were grown under common conditions in 50 mm/0.2 l pots containing commercial seed raising mix with micronutrients (Debco Pty Ltd., Victoria, Australia) in the nursery for approximately six months with some variation among species depending upon germination timing.

Experimental design: Glasshouse conditions
In March 2011 (autumn), 370 seedlings were transplanted from the nursery into glasshouse conditions with ambient light conditions and a temperature set to cool alpine conditions of 20/10 °C day/night with a natural light cycle. Seedlings were potted into 175 mm diameter/2.8 l pots containing a mix of 80:10:10; commercial potting mix: field soil: and washed river sand with added slow release osmocote for natives (Debco Pty Ltd., Victoria, Australia). Field soil was used to provide the seedlings with a natural inoculum, and was collected from a site within the Kosciuszko National Park where all three species co-occur. Initial leaf number was counted and seedlings of uniform size were selected from each maternal line to minimize variance within and between lines for each species. Following time to adjust to transplanting, pairs of seedlings were allocated to three blocks in a stratified design (May 2011); one member of each pair was randomly assigned to a well-watered treatment, and the other to a dry treatment. Seedlings were placed on a bench in a randomized design (minimum n = 2 per block), however, due to variation in germination and establishment success the design was not perfectly balanced (Table 1).

High and low water treatments were applied using a Water-Pro vapour pressure deficit (VPD) controlled system (MicroGrow GreenHouse Systems, Temecula, California), with each plant watered individually by a dripper at soil level [33]. Drippers were calibrated to a standard flow rate that was checked at the beginning and end of the experiment. Watering events were

Table 1 Study species and their elevation based sampling distributions within Kosciuszko National Park, NSW, Australia

Species	Source elevation	# of maternal lines	High water individuals	Low water individuals
Aciphylla glacialis	Low (1906–1937m)	4	27 (11/15/22)	28 (11/17/22)
	High (2162–2225m)	5	11 (10/10/10)	10 (8/8/10)
Oreomyrrhis eriopoda	Low (1733–1746m)	6	36 (9/3/17)	39 (9/28/18)
	High (1974–2225m)	6	36 (9/29/18)	38 (9/31/18)
Wahlenbergia ceracea	Low (1610–1720m)	6	35 (9/31/17)	31 (9/27/16)
	High (1875–2128m)	5	34 (9/28/17)	35 (9/31/18)

Numbers within brackets indicate sample sizes for various trait analyses, in order: individuals used for tissue chemistry analysis, individuals used for specific leaf area, individuals used for physiological trait analysis

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triggered when pre-set VPD targets were reached. High water plants received ~160 ml per watering (enough to saturate the soil); low water plants received ~80 ml per watering. Watering occurred roughly once per week for low water plants and every 2–3 days for high water plants, depending on VPD in the glasshouse. Under this regime, low water plants received ~25% of that which the high water plants received.

Physiological and leaf chemistry measurements

In order to understand how the soil moisture treatments affected plant physiology, we measured changes in gas exchange rates, leaf morphology, and tissue chemistry on a representative subset of plants, the number of which varied among species (Table 1). Gas exchange was measured using a LiCor 6400 portable photosynthesis system (LiCor, Lincoln, Neb) after 6 months of growth (26/11/2011 to 16/12/2011). On each plant, a young fully expanded leaf was selected for measurement. Measurements were taken at two time points; on the morning following a watering event (wet cycle) when the plants were at their least water-limited, and on the final day of a watering cycle (dry cycle) when the low water plants were at the driest point in the cycle. Block temperature was set at ~20 °C, CO_2 was 400 ppm, relative humidity was maintained around 60% and light levels were 500 µmol m^2 s. Preliminary assessment indicated that this light level was saturating. Photosynthetic measurements were done between 8:00 till 13:00 h, before stomatal closure.

Leaf samples were taken to measure leaf size and specific leaf area (SLA, cm^{-2} g^{-1}) at the time of gas exchange measurements. Collected leaves were placed with petioles positioned in saturated florist foam and kept at 4 °C in the dark overnight before being weighed to determine saturated weight, scanned on a flatbed scanner, and dried at 60 °C for 72 h before being weighed again to determine dry weight [34]. Leaf size was calculated as an area (cm^2/per leaf), and specific leaf area was calculated as area/dry mass (cm^{-2} g^{-1}).

For each species by elevation combination that had been measured for photosynthesis, replicates from three to four maternal lines were selected for isotope analysis. From these lines, three replicates from each of the high and low water treatments were chosen to be measured from each block ($n = 112$). Carbon isotopic ($\delta^{13}C$) and elemental analysis (%N) were performed on an isochrome continuous flow stable isotope ratio mass spectrometer (Micromass, Middlewich, England).

Harvest measurements

In November 2011 (late-spring) all plants were harvested. The total number of leaves on each plant was counted. Plant leaves, stems and roots were separated

and dried in an oven at 60 °C for 72 h before weighing using a Mettler-Toledo AB304-S Analytical Balance (Mettler-Toledo, Switzerland). For the few plants that flowered, total flower number was counted and reproductive biomass weighed. Above-ground biomass was calculated as the sum of leaves and stem dry weight (g), with reproductive biomass included for those that flowered.

Statistical analysis

Trait means were compared using a restricted maximum likelihood (REML) model that included elevation (categorical), treatment, and their interaction as fixed factors. Random factors were block and maternal line. Individual replicates were nested within maternal line, which itself was nested within elevation. For traits where there was not sufficient replication at a given hierarchical level (eg. block or maternal line) to fit the model, the term was excluded.. Each analysis was conducted independently for each of the three species. Comparisons between wet and dry cycle traits revealed no significant difference, so only dry cycle results are presented henceforth.

Traits were examined for conformity to the assumptions of normality, and transformed as required. Data outliers were excluded when associated with data entry, or where for example drippers had failed to deliver the allocated water dose for the treatments [35].

To calculate plasticity across watering treatments, plants were paired within maternal line x treatment combinations within each block. This was performed per each species. Then, we calculated the plasticity index (PI) as: (largest trait value − smallest trait value)/largest trait value. Not all plants could be paired, e.g. if the individual in the other treatment had died. Where more than one pair was possible within a maternal line*block, plants were paired by proximity on the glasshouse bench to minimize differences in conditions. As a result of the study design the number of pairs varied across the three species (*A. glacialis* = 25, *O. eriopoda* = 62 and *W. ceracea* = 58).

The potential for adaptive, maladaptive, or neutral plasticity was analyzed using selection gradient analysis, where the average fitness of plants across environments (or a proxy thereof) is regressed against the index of trait plasticity [15]. We standardized plasticity indices and trait values both to a mean of zero. We used total biomass as a fitness proxy and calculated relative fitness as the mean total biomass for a pair of plants divided by the mean of all pairs, and then subsequently log (e) transformed [24, 36]. Selection differentials were assessed separately for each species. Using a REML model (lmer, package lme4, R) [37], the selection gradient model assesses relative fitness regressed against trait plasticity as

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a fixed factor, with maternal line nested within elevation as a random factor. We also assessed plasticity against relative fitness for each water treatment individually to investigate which treatment, if any, may be driving adaptive plasticity. Here, relative fitness was again regressed against plasticity, using the maternal line as a random factor. Using the same models as above, we also investigated how trait values regressed against fitness to see if there was selection upon the trait mean itself.

Results

Responses to water availability

Reducing water availability to 25% of saturated conditions affected many morphological and physiological traits across the three species. As predicted, total biomass was significantly lower under water limitation for *O. eriopoda* and *W. ceraceae* whilst in *A. glacialis* this was found only to be true for the high elevation plants (Fig. 1a–c). In conjunction with reduced growth, a greater investment into water-sourcing root relative to shoot biomass was observed in all species under water limitation and was significant for both *A. glacialis* and *W. ceracea* (Fig. 1d, f). Total leaf number was lower under water limitation in all but the *A. glacialis* low elevation plants (Fig. 1g–i). Reductions in leaf size were also observed under water limitation and were significant for *A. glacialis* (Fig. 1j). We predicted that SLA would also be lower under water limitation, but changes in SLA were varied across the three species, with no clear main treatment response observed (Fig. 1 m–o; Table 2).

Physiological and tissue chemistry responses to water limitation were more varied. As predicted, all three species had significantly lower stomatal conductance (*gs*) under water-limiting conditions (Fig. 2a–c). Rates of photosynthesis were also lower under water-limiting conditions, though these differences were not statistically significant (Fig. 2d–f). Consequently, instantaneous water use efficiency (WUEi), being the ratio of photosynthesis to stomatal conductance, while slightly greater under water limitation was not significantly so (Fig. 2g–i). Similarly, this pattern was also observed across species using the longer term indicator of water use efficiency, $\delta^{13}C$, although less negative values, indicating increased water use efficiency, were observed for water-limited plants within *W. ceracea* (Fig. 2l). Leaf nitrogen content was lower under water limitation for *A. glacialis* (Fig. 2m).

Elevation effects and interactions

We found little evidence of intra-specific variation in traits or response to water limitation along the elevation gradient (Table 2), in keeping with our alternative hypothesis. *Wahlenbergia ceracea* plants sourced from low elevation produced fewer leaves throughout the experiment (Fig. 1i), and in general, these leaves had higher levels of stomatal conductance (Fig. 2c), and correspondingly more negative $\delta^{13}C$ (higher water use efficiency) (Fig. 2l). Low elevation sourced *O. eriopoda* plants also displayed more negative $\delta^{13}C$ values (Fig. 2k).

We predicted that low elevation sourced plants would show greater plasticity than high elevation plants but found few significant interaction terms (Table 2). For *A. glacialis*, low elevation sourced plants consistently maintained homeostasis in total biomass across treatments, whereas high elevation plants showed a decline in biomass under water-limiting conditions, our proxy for fitness (see below) (Fig. 1a, g). For SLA, high elevation sourced plants maintained homeostasis between treatments (Fig. 1m). The same pattern was also observed for $\delta^{13}C$, whereby low elevation sourced plants displayed a more negative $\delta^{13}C$ and a lower water use efficiency across treatments in comparison to high elevation plants (Fig. 2j). Finally, for *O. eriopoda* we found a significant treatment by elevation (TxE) interaction for SLA, whereby high elevation sourced plants increased SLA under low water conditions, while low elevation sourced plants decreased SLA under low water conditions (Fig. 1n).

Selection gradient analyses

For traits that showed significant plasticity in response to water treatment and/or elevation effects, we examined whether the plastic response was associated with increased fitness. However, despite widespread treatment effects few of these plastic shifts were shown to have adaptive value. The single instance of selection for adaptive plasticity observed was total leaf count in *O. eriopoda* (Table 3). In contrast, selection towards homeostasis was observed for more traits: stomatal conductance in *A. glacialis*, percentage nitrogen in *O. eriopoda*, and total leaf count in *W. ceracea*, the latter for high elevation sourced plants only (Table 3). Instances whereby plasticity significantly influenced fitness were primarily observed where selection differentials were in opposing directions within high and low water treatments (Additional file 1: Table S1).

Significant directional selection gradients on trait means were also observed in some cases, but again varied among species, treatment and elevation (Table 4). For *A. glacialis* larger leaf size was significantly correlated with increased fitness (Table 4), and this was primarily driven by variation among high water treatment plants (Additional file 1: Table S1). For *O. eriopoda*, increased percentage nitrogen was negatively correlated with fitness, and also primarily driven by variation among plants in the high water, rather than low water treatment (Additional file 1: Table S1). For *W. ceracea*, where significant elevation effects on trait means were more common, increased total leaf count was positively correlated with fitness, and
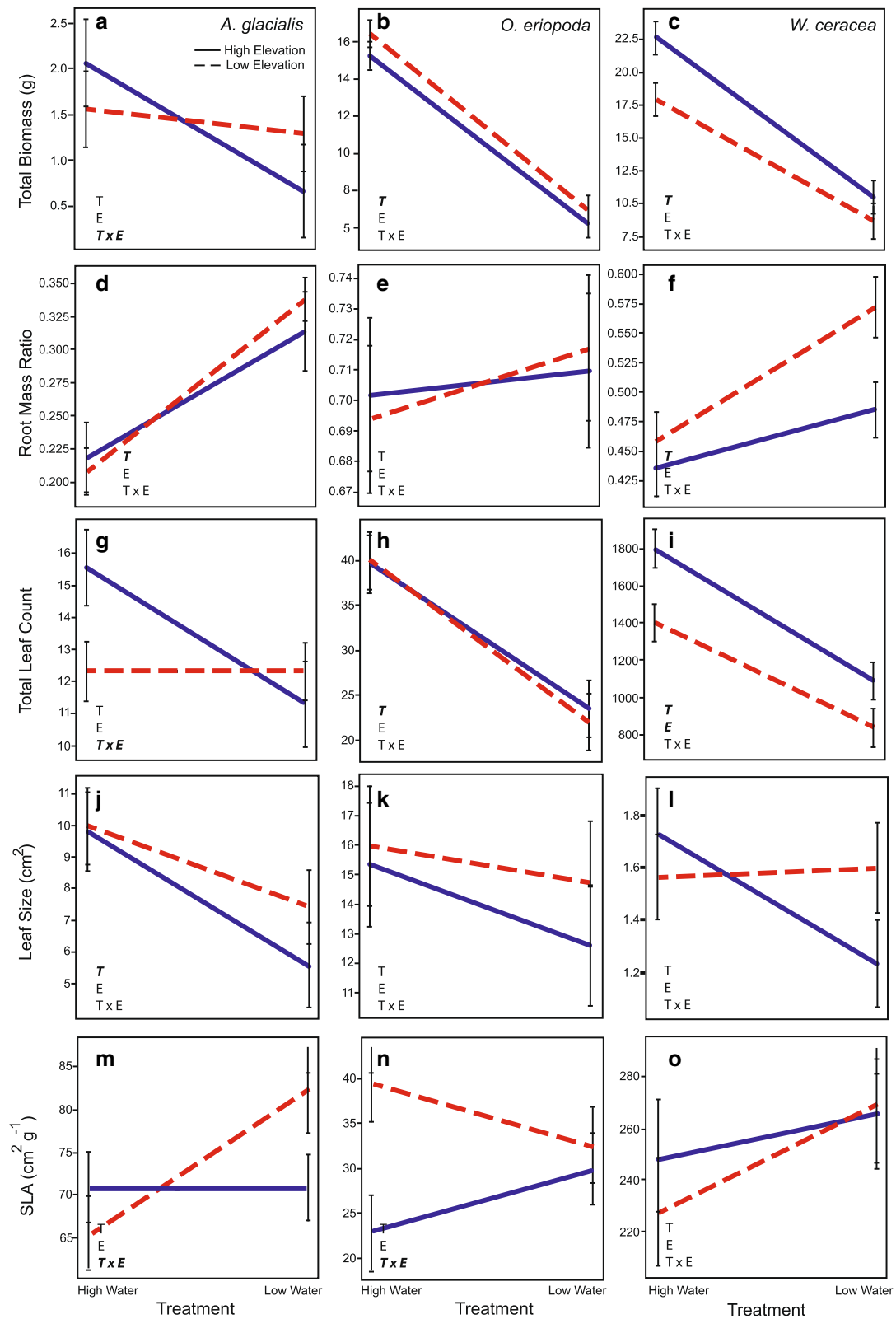
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Fig. 1 (See legend on next page.)

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(See figure on previous page.)
Fig. 1 Comparison of morphological traits comparing responses of low (dashed) and high elevation (solid lines) across high and low water treatments for each species. Species trait responses by column are: a) *Aciphylla glacialis* b) *Oreomyrrhis eriopoda* and c) *Wahlenbergia ceracea*. Significance bars are mean differences. Significant results are represented for treatment (T), elevation (E) and treatment by elevation interactions (TxE) by bolded and italic font

this was driven by variation among the high elevation sourced plant responses (Table 4). Finally, less negative $\delta^{13}C$ (higher water use efficiency) was associated with increased fitness (Table 4) and was driven by variation in the response of high elevation sourced plant grown under high water treatment in *W. ceracea* (Additional file 1: Table S1).

While slightly more cases of selection upon plasticity than would be expected by chance alone, likely arising from false significant effects due to multiple testing, the 5/22 traits displaying significant results provide only very weak evidence that water response traits and plasticity therein are under selection in these species.

Discussion

In this study we examined the effects of reduced water availability on morphological and physiological trait means and plasticity in three Australian alpine herb species; *Aciphylla glacialis*, *Oreomyrrhis eriopoda*, and *Wahlenbergia ceracea*. Specifically, we asked whether there was evidence of adaptive plasticity in water response and/or intra-specific variation between low and high elevation populations in trait plasticity to water availability. We found widespread evidence of plasticity in morphological and physiological traits in response to water limitation in all three species. As expected, under water limitation, plants accumulated less biomass, had

an increased investment into root structures, and consequently a reduction in both the number and size of leaves (Fig. 1). In addition, reduced stomatal conductance, and in some instances higher integrated water use efficiency, measured according to $\delta^{13}C$, were observed under low water availability (Fig. 2). Despite this, we observed little evidence that the phenotypic plasticity in response to water availability was adaptive, nor did we detect much variation in either trait means or trait plasticity as a function of elevation (Tables 3, 4). Here we discuss why, despite eliciting a significant water treatment, little evidence of adaptive plasticity or elevation based differentiation in trait values or plasticity was observed within our study.

Why elevation doesn't affect water response patterns?

The use of elevation gradients within ecological studies is increasingly common, particularly as a method of assessing responses to future climate change conditions. Our first elevation based hypothesis predicted that low elevation sourced plants would display greater variation in trait mean, and therefore increased plasticity as a result of occupying a more heterogeneous environment [12, 38]. For the high elevation populations it was predicted that little evidence of plasticity would be observed [27, 39], though see Frei et al. [40] and Pohlman et al. [41] for opposing perspectives. We found relatively few

Table 2 Summary of significant trait responses to treatment, elevation or treatment by elevation interaction for the three species *Aciphylla glacialis* (Ag), *Oreomyrrhis eriopoda* (Oe) and *Wahlenbergia ceracea* (Wc)

Plant Functional Traits	Treatment			Elevation			Treatment x Elevation		
	Ag	*Oe*	*Wc*	*Ag*	*Oe*	*Wc*	*Ag*	*Oe*	*Wc*
Morphological									
Total Biomass		•	•				•		
Root Mass Ratio	•		•			•			
Total Leaf Count	•	•	•			•			
Leaf Area	•	•							
Specific Leaf Area							•		
Physiological									
Photosynthesis									
Conductance	•	•	•			•			
Water Use Efficiency									
Tissue Chemistry									
% Nitrogen	•	•							
δ13			•		•	•	•	•	

Significant responses are shown by black dots ($p < 0.05$)

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Fig. 2 (See legend on next page.)

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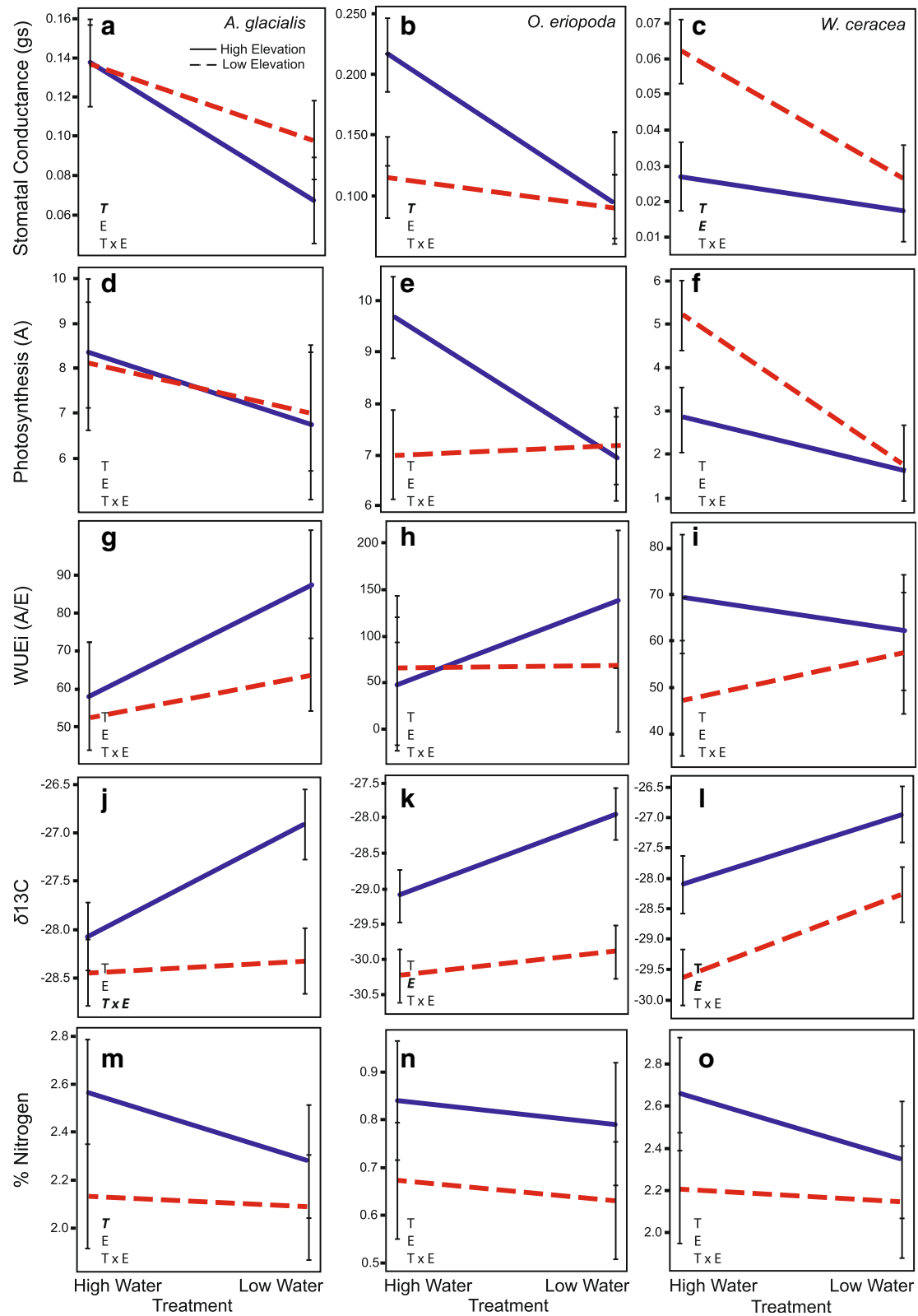Page 9 of 12

> (See figure on previous page.)
> **Fig. 2** Comparison of physiological traits comparing responses of low (dashed) and high elevation (solid lines) across high and low water treatments for each species. Species trait responses by column are; a) *Aciphylla glacialis,* b) *Oreomyrrhis eriopoda* and c) *Wahlenbergia ceracea.* Significance bars are means. Significant results are represented for treatment (T), elevation (E) and treatment by elevation interactions (TxE) by bolded and italic font

significant elevation responses, and, in many instances within our study low elevation sourced plants displayed a greater degree of trait homeostasis across our water treatment (Figs. 1 and 2). In contrast, previous studies have demonstrated differences in plastic responses along elevation gradients and associated these with differing selection pressures at each elevation, such as increased temperature [24, 25, 42], increased frost risk due to

earlier melting snow [43–45], and competition from upward shift in low elevation alpine plants [46]. Likewise, past research has assessed elevation based changes in traits such as; growth rate, biomass [29], leaf size [20], stomatal conductance [30] and carbon isotope discrimination [47].

Generally within alpine regions, decreasing soil depth and vegetation cover with increasing elevation may

Table 3 Selection gradient analysis on the paired dataset of trait plasticity and relative fitness on traits which displayed significant treatment responses for; A) *Aciphylla glacialis,* B) *Oreomyrrhis eriopoda* and C) *Wahlenbergia ceracea*

Species	Trait	β	*P* value	*N*
A) *A. glacialis*				
	Root Mass Ratio	0.01	0.940	23
	Leaf Size	−0.08	0.532	21
	Stomatal Conductance	−0.25	**0.031**	22
	% N	0.92	0.477	11
B) *O. eriopoda*				
	Leaf Mass Ratio	−0.05	0.420	53
	Leaf Size	0.18	0.157	51
	% N	−0.18	**0.016**	16
	Total Leaf Count	0.12	**0.036**	63
	Water Use Efficiency	0.11	0.247	30
C) *W. ceracea*				
	Photosynthesis	0.11	0.063	34
	Root Mass Ratio	0.00	0.996	51
	High Elev	0.12	0.234	29
	Low Elev	−0.06	0.592	22
	Stomatal Conductance	0.03	0.650	28
	High Elev	0.03	0.527	16
	Low Elev	0.03	0.838	12
	Total Leaf Count	−0.06	0.126	58
	High Elev	−0.09	**0.023**	29
	Low Elev	−0.03	0.639	29
	δ ^{13}c	−0.13	0.654	16
	High Elev	−0.18	0.966	8
	Low Elev	−0.20	0.443	8

Selection differentials were calculated combining data from plants of different elevations. In cases where elevation effects were significant (Figs. 1 and 2), additional analyses were performed for data from plants of the different elevations separately. Positive selection differentials indicate adaptive responses, while negative values indicate maladaptive responses. Significant responses are indicated by bolded *p*-value. n = number of pairs within each analysis

Table 4 Selection gradient analysis on trait value and relative fitness of functional traits which displayed significant treatment responses for; A) *Aciphylla glacialis,* B) *Oreomyrrhis eriopoda* and C) *Wahlenbergia ceracea*

Species	β	*P* value	*N*
A) *A. glacialis*			
Root Mass Ratio	−0.20	0.074	23
Leaf Size	0.51	**<0.001**	20
Stomatal Conductance	0.20	0.090	22
% N	0.01	0.940	11
B) *O. eriopoda*			
Leaf Mass Ratio	0.02	0.707	63
Leaf Size	0.08	0.207	48
% N	−0.23	**0.025**	16
Total Leaf Count	−0.06	0.248	63
Water Use Efficiency	0.11	0.245	30
C) *W. ceracea*			
Photosynthesis	−0.01	0.000	34
Root Mass Ratio	−0.05	0.813	53
High Elev	−0.03	0.455	29
Low Elev	−0.05	0.473	24
Stomatal Conductance	−0.04	0.544	29
High Elev	−0.02	0.756	16
Low Elev	−0.05	0.627	13
Total Leaf Count	0.14	**0.001**	57
High Elev	0.19	**<0.001**	29
Low Elev	0.09	0.228	28
δ ^{13}c	0.22	**0.007**	17
High Elev	−0.18	0.232	9
Low Elev	0.21	0.067	8

Selection differentials were calculated combining data from plants of different elevations. In cases where elevation effect were significant (Figs. 1 and 2), additional analyses were performed for data from plants of the different elevations separately. Positive selection differentials indicate selection for larger values, while negative values indicate selection for smaller values of the trait. Significant responses are indicated by bolded *p*-value. n = number of pairs within each analysis

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mean that soil moisture deficits, particularly during summer, may be higher at high elevations [18, 48]. However, others suggest that because water availability within an alpine landscape may be more likely to vary over micro-scales than across an elevation gradient per se we considered an alternative hypothesis: that patterns of plasticity in response to water availability might be consistent across elevations, and in this differ from plasticity patterns in response to temperature. Thus, we would predict plenty of plasticity in response to water availability, but not differentiation in the pattern of that plasticity across elevations. Water availability within alpine ecosystems is not only a function of precipitation, or evapotranspiration, but may be determined by prevailing weather conditions, snow melt, localised topography, aspect, soil structure, and/or vegetation cover [18, 49]. In our tall alpine herb field, heavy vegetation cover may act to minimize evaporative moisture loss during the summer period such that variation with elevation is moderated [18]. Thus, it appears that these alpine plants have plasticity in response to water in expected directions, but that there has been no selective pressure for differentiation in that plasticity across elevation [23, 49].

Water availability is just one component of microhabitat heterogeneity that may influence the evolution of adaptive plasticity in physiological and morphological traits, consequently conflict in selective pressures are likely to arise. Shifts in abiotic factors may co-occur and act synergistically, or alternatively antagonistically, such that the fitness landscape may be highly complex [27]. Given the significant differentiation in distribution of adaptive plasticity in response to water between populations of different elevation found by Nicotra et al. [24] and Briceno et al. [25], we expected to detect adaptive plasticity in water response, and potentially elevation differences as well. The paucity of such differentiated responses and limited evidence of adaptive plasticity in response to water thus could reflect such conflicts [26, 50]. This finding highlights the fact that adaptive plasticity in response to a given trait may reflect a neutral or even maladaptive response for another, thereby limiting the development of a phenotypic optimum in response to any one environmental trait [26, 51–53]. Furthermore, given that phenotypic plasticity is predicted to evolve when environmental cues are reliable [54], it is important to consider both spatial and temporal scales of different environmental parameters [51]. Follow-up experiments could therefore investigate the simultaneous effects of water and temperature, perhaps incorporating elements of frequency and duration of deficit events to fully understand whether and how selective conflicts and trade-offs exist within this system.

We found little evidence that the observed plasticity in water-use traits was adaptive (Tables 3, 4), and this may reflect both evolutionary history and the different ways phenotypic plasticity may influence fitness. If selection for plasticity in water response has been strong and consistent across the landscape, inability to detect an adaptive signal may simple reflect loss of variation in that plasticity, not that the plastic response is not adaptive per se. Observations of positive correlations between fitness and plasticity may indicate an ability to capitalize upon favourable conditions, or alternatively, observations of high plasticity under unfavourable, or stressful conditions may result in the maintenance of fitness homeostasis [38, 55]. Furthermore, such responses are likely to be trait and species specific. It is also important to note that plasticity may not always be advantageous [13, 26]. There are costs and limits associated with plasticity that may limit not only its development, but also its extent and effectiveness. It is also the case that costs and adaptive value of plasticity are not always easy to assess under experimental settings, or on naturally occurring genotypes [15, 26, 51, 56–58]. Finally, plasticity may operate in a non-linear manner and it is possible that our study represents a smaller portion of a much larger and complex reaction norm [59]. Thus, further research to identify the genetic architecture of the observed plastic response to water availability might improve our understanding of its history and adaptive role.

Conclusions

As we increasingly strive to predict species' responses to changing climatic conditions, the use of space-for-time substitution, including the use of elevation gradients, is becoming more common. While these methods may in certain cases inform us about the influence of particular abiotic factors such as temperature [24, 25], we caution against broad generalizations as responses to differing abiotic factors vary. Future investigation into climate change responses within alpine ecosystems should seek to understand how shifts in co-occurring abiotic factors may act synergistically or antagonistically upon fitness, but may also vary as a function of time and space. Moreover, investigations into not only the mean changes in abiotic variables, but also the periodicity and frequency of extreme events, will be of increasing importance [59, 60]. Finally, when considering the capacity for adaptive phenotypic plasticity to buffer climate change, it is also important to assess how observed trait changes may translate into population-level responses [61]. An increased understanding of variability in phenotypic plasticity, in particular, adaptive plasticity, may allow us to better assess the evolutionary potential of species. Knowledge of how plasticity varies as a function of differing abiotic pressures will aid in developing informative models of more complex, realistic climate change scenarios.

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Additional file

Abbreviations
REML: Restricted Maximum Likelihood Model; SLA: Specific Leaf Area; TxE: Treatment by elevation interaction; VPD: Vapour Pressure Deficit

Availability of data and materials
The dataset supporting the conclusions of this article will be made availability in Dryad.

Authors' contribution
JAR-V and ABN formulated the ideas for the study, established the design and developed the hypotheses. ABN, VFBR, NCA and SRG collected the data. SRG completed the statistical analysis and drafted the manuscript. ABN and M-MH-P contributed substantial conceptual and editorial advice. All authors contributed to writing and approved the final manuscript.

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