**Title**: Alien plant species that invade high elevations are generalists: support for the directional ecological filtering hypothesis.

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**Questions**:

The richness of invasive alien plant species tends to decrease with increasing elevation. This pattern may be due to alien plant species requiring traits allowing survival at high elevations (the Abiotic Limitation Hypothesis; ALH). In contrast, the more recent Directional Ecological Filtering Hypothesis (DEFH) suggests that only species with broad environmental tolerances will successfully spread from lowlands (where most introductions occur) to high elevations. Here we test the support for the DEFH and the ALH along an elevational gradient by asking: First, are alien species that occur at higher elevations generalists? Second, do alien species occurring at higher elevations exhibit traits that distinguishes them from lowland alien species?

**Location**:

Sani Pass, Maloti-Drakensberg Transfrontier Conservation Area, South Africa

**Methods**:

A nestedness analysis was conducted to test whether alien species were nested along the elevational gradient, and ANOVA and Chi$^2$ tests (supplemented by resampling procedures) were used to determine if functional traits differed between high and low elevation alien species.

**Results**:

Significant nestedness of the alien flora indicates that alien species occurring at high elevations are generalists, being widespread across the elevational gradient. Compared to low elevation aliens, plant height was lower and cold tolerance weaker for high elevation species.

**Conclusion**:

We found support for the DEFH with the majority of high elevation aliens being widespread generalists. Overall only two of the 11 functional traits differed between high and low elevation alien species, with only one trait supporting the ALH: shorter plant stature found at higher elevations. Therefore, complementing nestedness analyses with trait data provides a more nuanced insight into the determinants of alien richness patterns along elevational gradients and highlights how the two contemporary hypotheses might not be mutually exclusive.

**Keywords**: Alien species; Broad environmental tolerance; Elevation; Exotics; Functional traits; Generalists; Gradients; Mountain invasions; Nestedness; Non-native; Specialisation.

**Abbreviations:**

ALH – Abiotic Limitation Hypothesis

DEFH – Directional Ecological Filtering Hypothesis

**Running head**: High elevation invaders are generalists

**Main text**:

## INTRODUCTION

Biological invasions pose the second greatest threat to biodiversity after habitat degradation (Wilcove et al. 1998). While invasions are occurring globally (Lonsdale 1999; Kueffer et al. 2010), the diversity, extent and impacts of invasive plant species vary along several environmental gradients (Gilbert & Lechowicz 2005; MacDougall et al. 2006; Pauchard et al. 2009). The strongest pattern observed is that low elevation areas tend to have greater alien species richness than higher elevations (Pauchard et al. 2009; Vicente et al. 2013). The reasons for this are still disputed, but two main hypotheses have been proposed to explain this trend.

The Directional Ecological Filtering Hypothesis (DEFH, Alexander et al. 2011) proposes that alien species richness patterns along abiotic gradients are a result of the sequential filtering of species with progressively broader climatic niches along a gradient of increasing environmental severity (Alexander et al. 2011). Consequently, the DEFH posits that the alien plant species found at high elevations tend to be generalists (i.e. lacking specific adaptations for high elevation conditions) that have spread upslope from low elevation source populations where anthropogenic propagule pressure is highest (Marini et al. 2009). Thus, only generalist species with broad environmental tolerances are expected to survive both under conditions dominated by competition (lowland source populations) and by abiotic stress (high elevations). The DEFH therefore predicts that alien species composition will show the species that have successfully invaded upslope areas are nested subsets of the larger lowland species pool (Marini et al. 2013). As a result, high elevation sites with low species richness would comprise only widespread species, while lowland sites with high species richness would contain more range-restricted species (Wright & Reeves 1992). Several studies that looked at species richness and community structure of alien species along elevation gradients have, without implicitly testing for it, found the DEFH to play a role in their studies (Pyšek et al. 2011; Pollnac et al. 2012; Seipel et al. 2012; Andersen et al. 2015; Zhang et al. 2015). DEFH has also been identified to be relevant at both small and large spatial scales (Seipel 2011; Dainese et al. 2014; Pollnac & Rew 2014). Some studies have even suggested that ecological filtering might select for genotypes that are phenotypically plastic (Haider et al. 2011). However, in addition to Alexander et al's (2011) original paper, only one other study has specifically tested the generality of the DEFH by evaluating and identifying the drivers of beta-diversity patterns (Marini et al. 2013). They further indicated that the DEFH is not relevant for native species but instead show an opposite pattern.

In contrast, the Abiotic Limitation Hypothesis (ALH) proposes that increased environmental severity at higher elevations, due to, e.g., lower temperatures, stronger winds and higher UV-B radiation, excludes species that lack adaptations to these severe conditions (Johnston & Pickering 2001; Becker et al. 2005; Vilà et al. 2007; McDougall et al. 2011; Carbutt 2012). Under the ALH, alien species that have successfully colonised higher elevations would be expected to display traits related specifically to the tolerance of the abiotic stresses experienced at these elevations (Pyšek et al. 2011). Therefore, invasive species found at high elevations would be poorly adapted to the dissimilar environmental conditions at milder lowlands, where traits related to competitiveness are likely to be more beneficial (see e.g. MacDougall et al. 2006). The ALH thus implies that species invading lowlands and high elevations are adapted to differing environmental conditions, and therefore possess different suites of functional traits, resulting in significant turnover of species between high and low elevations (in line with Kitayama & Mueller-Dombois 1995).

While the ALH posits that invasive species at high elevations exhibit traits associated with survival under abiotically severe conditions, the DEFH implies that these alien species will rather show traits related to invasiveness (e.g. fast growth and germination rates, Rejmánek & Richardson 1996; Van Kleunen et al. 2010; Van Kleunen et al. 2011). Given the growing availability of trait data (Kattge et al. 2011), and the general validation of their use (Kazakou et al. 2014), comparing trait values between high and low elevation alien species provides a powerful (and novel) test of both the ALH and the DEFH, complementing the more frequently used nestedness analyses (see Alexander et al. 2011; Marini et al. 2013). Indeed, species' inherent characteristics (i.e. functional traits) and their distributions (i.e. nestedness or lack thereof) might be expected to show similar patterns along environmental gradients (e.g. Stahl et al. 2014), and, if this is observed, would provide much stronger support for a specific hypothesis than either nestedness or trait analysis in isolation.

Due to low elevations generally being more heavily invaded than higher elevations (Kueffer et al. 2014), there are comparatively fewer studies of invasions in mountainous regions (although see Dietz et al. 2006; Kueffer et al. 2008; Kueffer et al. 2014), potentially limiting our understanding of how biological invasions function across the full range of elevations (MacDougall et al. 2006; Van Kleunen & Johnson 2007). There is growing evidence in favour of the DEFH, but no study has yet concurrently explicitly tested both the DEFH and the ALH, nor have analyses of functional traits been used to complement nestedness analyses when assessing these hypotheses (but see also Andersen et al.

2015). Therefore, the aim of this study was to investigate the support for the DEFH and for the ALH along an elevational gradient using two complementary approaches. The first analysis determined if alien vascular plant species were nested along an elevational gradient; a nested pattern would provide support for the DEFH, with generalist alpine invaders invading across the entire elevational range, while a lack of nestedness would be consistent with the ALH. The second analysis tested if functional traits differed significantly between alien species occurring at high elevation (i.e. exhibiting adaptations to abiotic extremes) and those limited to lower elevations (i.e. adaptations to competition), as predicted by the ALH.

## METHODS

### Study site

To robustly assess the ALH and the DEFH a study system should comprise a long elevational gradient, to ensure trends in alien species richness and functional traits can be reliably identified, and a large lowland alien species pool. Sani Pass (Maloti-Drakensberg Transfrontier Conservation Area, 29°17'–29°26' E; 29°43'–29°35' S, South Africa) meets these criteria, making it a suitable study area where both trait variation and species richness patterns can be quantified along an extended elevational gradient (Fig. 1). The Pass spans a broad range of conditions (altitudes from 1307 to 2873 m a.s.l.) with more densely-inhabited and heavily transformed lower elevations potentially acting as a source of invasive species propagules for higher elevations (Kalwij et al. 2008b). The site floristically forms part of the Drakensberg Alpine Centre (DAC, Carbutt & Edwards 2003), and receives summer rainfall of c. 800 mm p.a. (peaking at mid-elevations but with relatively little variation across elevation: (Nel & Sumner 2008; Bishop et al. 2014). Night frost is a common occurrence and snow cover can be expected at the highest elevations during winter (Killick 1978).

Sani Pass is an important transport link between Lesotho and South Africa, with frequent vehicular and pack animal traffic, contributing to the potential for propagule transport. The total distance along the entire Sani road between the Sani Top village (located at the top of the pass) and Himeville (the nearest town) is 39 km, which in this study was divided into a high and a low elevation section, corresponding to changes in environmental conditions and a steepening of the elevational gradient. High elevation areas are defined as the area along the Sani Pass, i.e. adjacent to the untarred road between the Sani Top village (29°17'E, 29°35'S) to the start of the asphalt road at the foot of the Pass (29°26'E, 29°39'S; a distance of 20 km and 1307 m elevation gradient). The
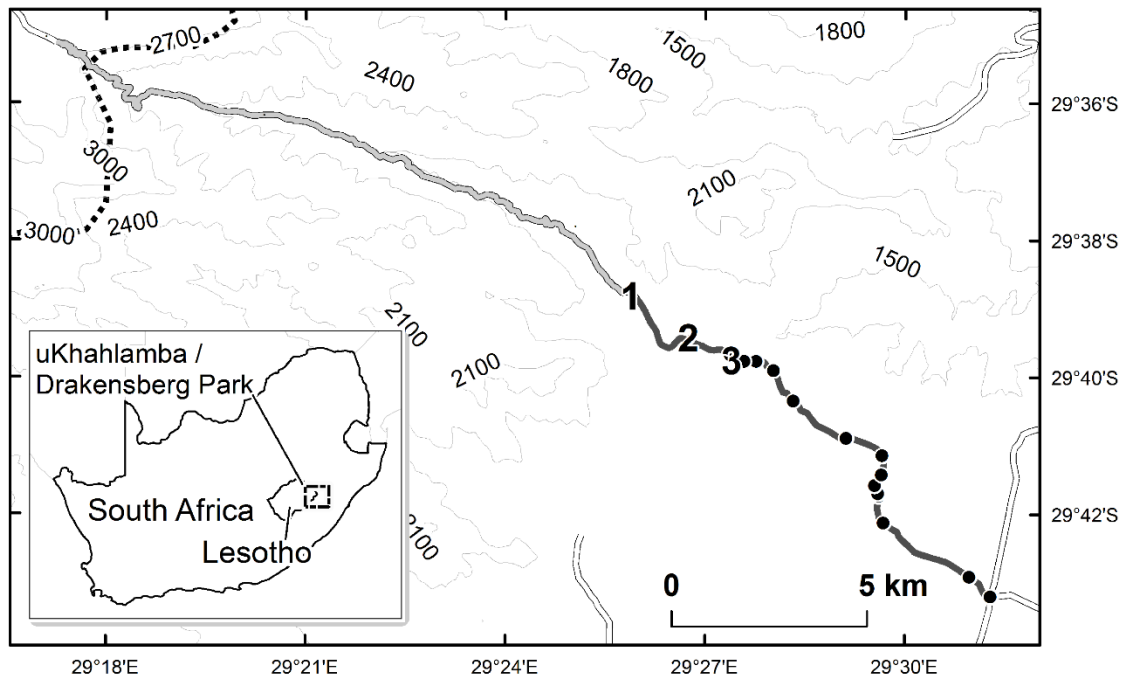
Figure 1: The study area was a cross-border arterial road located in the Maloti-Drakensberg Transfrontier Conservation Area at the border of South Africa and eastern Lesotho. The three additional potential sources of alien species (tourist accommodation; numbered) are located along the Sani Pass lowland road. The gravel section is 20 km long (Pass road; light grey) while the tarred section is 13.4 km long (lowland road; dark grey). The locations of the 17 intersections along the tarred road are depicted with black circles. The international border between Lesotho and South Africa is showed as a dashed line. Projection: Albers equal-area conic.

lowland region was defined as the asphalt roadside from the start of the lower part of Sani Pass to the Himeville intersection (a distance of 13.4 km with a 100 m elevational range).

## Data collection

Road verges are a useful model system for studying patterns of alien species establishment as verges are regularly disturbed and frequently act as introduction sites for human-transported alien species (Arévalo et al. 2010; Pollnac et al. 2012). All species growing along Sani Pass were considered to have successfully established at higher elevations (i.e. to be naturalised, see Appendix S1); these species were identified by annually surveying the occurrence of alien plant species from 2007 to 2014 along the road verges of the Pass (including the first 100 m of the road in Lesotho).The elevation of the three highest individuals of each alien species were recorded while both sides of the road were surveyed by observers walking down the Pass (see Kalwij et al. 2015 for more details). All the alien plant species recorded on the Pass over eight years (Kalwij et al. 2008b, 2015) were considered to comprise the high elevation alien species pool. In the 2014 survey, the Pass was additionally divided into 19 elevational bands within which the presence or absence of all alien species was recorded for nestedness analysis. Elevational bands spanned 150 m of elevation at the steepest parts of the Pass and 50 m in the less steep areas to minimise differences in the horizontal distances surveyed for each band.

The low elevation alien species pool was obtained by surveying the lowlands adjacent to the Pass road in 2014. All alien plant species occurring on the road verges of 17 road intersections in the lowland area were recorded (the majority of which can be classified as naturalised, Appendix S1). Intersections were surveyed instead of random sites along the road due to their greater potential as introduction points for alien species (Kalwij et al. 2008a). Road verges were surveyed for 100 m in either direction of the intersections on both sides of the road, with three observers spending *ca*. 30 minutes per site. The grounds adjacent to hotels within the lowland area were also surveyed for naturalised alien plant species as they were considered a potential additional introduction point of alien species (the Sani Lodge Backpackers (29°27'24" E, 29°39'44" S), the Sani Pass Hotel (29°26'45" E, 29°39'24" S) and the Mkomazana Mountain Cottages (29°25'7" E, 29°37'52" S), Kalwij et al. 2014).

Trait data for alien species were extracted from trait databases (Klotz et al. 2002; Kattge et al. 2011; USDA 2014; see Appendix S2 for TRY contributors and references) and literature (e.g. Pignatti 1982; Elmore & Paul 1983; Friend 1983; Fuentes et al. 2013). Traits previously associated with plant invasiveness or survival in alpine habitats were

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examined (Table 1). A final list of 152 alien species and 11 traits was used for analyses; 16 species were excluded because they had insufficient trait data, and six traits that were not obtainable for most species (or that could potentially generate bias in our dataset, Appendix S3) were excluded.

## Data analysis

To test whether species at higher elevations were subsets of the pool of species at lower elevations, nestedness analysis was conducted using alien species presence-absence data within each elevation band and the lowland species pool. The modified discrepancy metric ($d1$) was used for nestedness analyses. A $d1$ with a value of zero represents a perfectly nested matrix and a value of one indicates a complete lack of nestedness (Greve & Chown 2006). The $d1$ metric was used rather than other more recent metrics such as the nestedness metric based on overlap and decreasing fill (NODF), because the latter assumes that if two (or more) sites have identical species richness their nestedness values decline (Almeida-Neto et al. 2008). As we were interested in outliers in the presence-absence matrix and had no *a priori* expectation that different elevational bands should have different species richness values, $d1$ was considered to be the most appropriate nestedness metric to use. The significance of $d1$ was tested by generating 10 000 randomised presence-absence matrices in which the number of species per site was conserved (using a resampling without replacement procedure). The observed $d1$ was compared to those of the random matrices using a z-test (following Greve et al. 2005).

Two analyses were conducted to test if alien species limited to the lowlands differ in trait values from those occurring at higher elevations. First, analysis of variance (or Chi$^2$) tests were used to compare numeric (or categorical) trait values between species restricted to the lowland (hereafter "lowland restricted species") and those occurring along the Pass (hereafter "high elevation species"). There is substantial overlap between species that occur at high and low elevation. We included species from the lowland in the "high elevation" category if they were also found at higher elevations along the Pass (i.e. "high elevation species" are those not restricted to the lowland), which was necessary for testing our hypotheses. The DEFH assumes that alien species migrate from lowland introduction sites to higher elevations, and should therefore contrast species that have spread to high elevations with those that have not expanded beyond the lowlands. Similarly, this grouping of species is also appropriate to test the ALH since it can distinguish between aliens that have survived at higher elevations and those species that have not. Prior to analyses data were checked to ensure that all assumptions were met, with log transformation utilised to improve normality where necessary.

Second, a resampling procedure was conducted to determine if the observed mean value for each trait in each elevational band differed from what would be expected if species were randomly distributed across elevations. The resampling analysis examines the distribution of trait values within every elevation band, providing a more detailed analysis than an analysis of variance which only compares high and low elevations. This analysis was limited to quantitative data. For each elevation band 10 000 random means were generated for each trait by randomly sampling without replacement *n* species (where *n* is the number of alien species recorded in that elevational band) from the entire pool of species for which values were available for the trait of interest. If the observed mean of a trait fell outside the upper 97.5% or lower 2.5% quantiles of the 10 000 randomised means for that elevational band, the trait was considered significantly different from values expected under the null hypothesis.

Nestedness was calculated using the Nestedness Programme of Greve and Chown (2006), with all other analyses conducted in R 3.2.3(R Core Team 2015).

## RESULTS

For nestedness analysis we analysed all 144 alien species recorded during the 2014 surveys. For these species a significant nested pattern was found along the elevational gradient (d1 = 0.263, z = -10.912, p < 0.001), with higher elevation alien assemblages generally forming subsets of the lowland species pool (Fig. 2).

Out of the 152 alien vascular plant species examined in the trait analysis, 20% were restricted to the Pass, 30% were restricted to the lowland and 50% found both in the lowland and along the Pass. High elevation alien species had a lower stature (F = 8.753, df = 141, p = 0.004) and weaker tolerance to sub-zero temperatures (F = 7.838, df = 37, p = 0.008, Figs 3A-B) than lowland restricted species. Other traits did not differ significantly between widespread and lowland species (Table 1).

The results from the resampling procedure generally matched ANOVA results, albeit with fewer significant patterns (Fig. 4). Plant height was significantly lower than expected by chance at mid- to high elevational bands, but not at lower elevations. SLA was significantly higher than expected by chance at one mid- and one high elevation (Appendix S4, Fig. 1A), but not in low elevation bands. Flowering duration was not significantly different from random except at the highest elevation (longer than expected at 2900 m a.s.l.), however there was a trend for species from higher elevations (2250–2900 m a.s.l.) to exhibit flowering durations longer than the mean duration predicted by resampling

Figure 2: Nestedness matrix of alien species along the elevational bands from the Pass. Data is arranged in the form of a presence/absence matrix, where the black squares represent presence in an elevational band. Within the matrix, species are represented in the columns (arranged to maximize nestedness) and sites in the rows (with the lowest elevation band at the top).
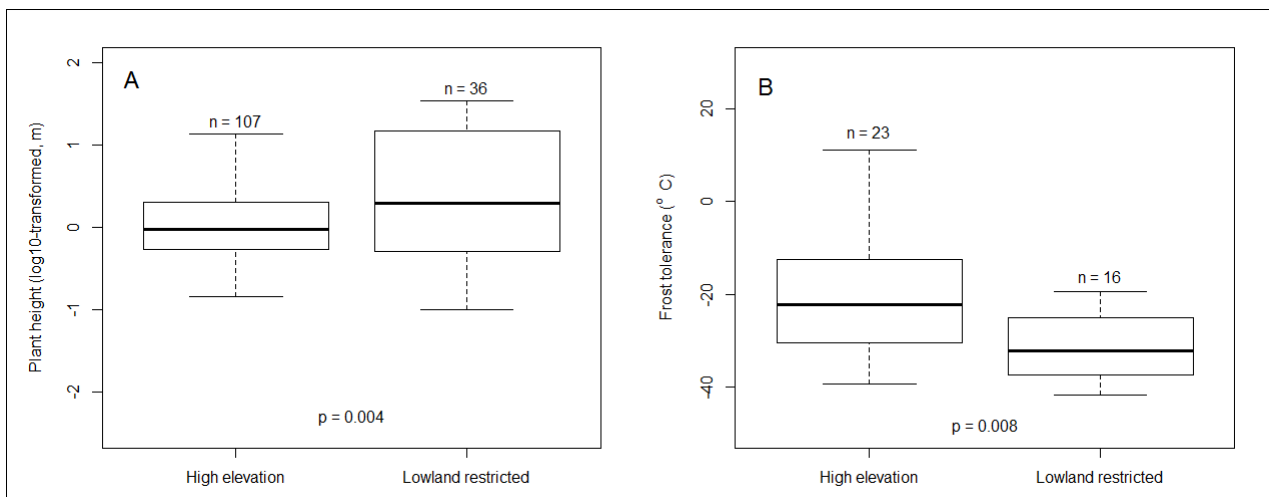


Figure 3: Comparison of trait values between high elevation and lowland restricted alien plant species: plant height (A) and frost tolerance temperature (B). Boxplots represent the range, interquartile range and median values, and p-values indicate results of an ANOVA test comparing high and low elevation species.

**Table 1: Comparison of trait values between high and low elevation alien species.**

| Category | Trait | No. of species with available trait data | Type of analysis | Results |
|---|---|---|---|---|
| **Numeric traits** | Plant height (meters) | 143 | ANOVA | F = 8.753, df = 141, p = 0.004 |
| | Frost tolerance: minimum temperature (°C) | 39 | ANOVA | F = 7.838, df = 37, p = 0.008 |
| | Minimum days without frost (number of days) | 39 | ANOVA | F = 0.205, df = 37, p = 0.654 |
| | Specific leaf area (SLA) | 112 | ANOVA | F = 2.318, df = 110, p = 0.131 |
| **Categorical traits** | Dispersal syndrome (Zoochory, Anemochory, Anthropochory, Hydrochory, Mixed And Unassisted) | 106 | Chi$^2$-test | $X^2$= 0.758, df = 6, p = 0.993 |
| | Number of dispersal vectors (Single or Multiple) | 105 | Chi$^2$-test | $X^2$= 0.051, df = 1, p = 0.8218 |
| | Fruit type (Dry or Fleshy) | 122 | Chi$^2$-test | $X^2$= 0.311, df = 2, p = 0.856 |
| | Growth form (Herbaceous, Grass, Woody, Succulent) | 152 | Chi$^2$-test | $X^2$= 4.073, df = 3, p = 0.254 |
| | Altitude of origin (Montane or not) | 26 | Chi$^2$-test | $X^2$= 0.116, df = 1, p = 0.734 |
| | Climatic region of native distribution (Tropical, Temperate, Mediterranean, Boreal, Continental) | 62 | Chi$^2$-test | $X^2$= 1.904, df = 4, p = 0.753 |
| | Flowers conspicuous (Yes or No) | 107 | Chi$^2$-test | $X^2$= 0.020, df = 1, p = 0.888 |
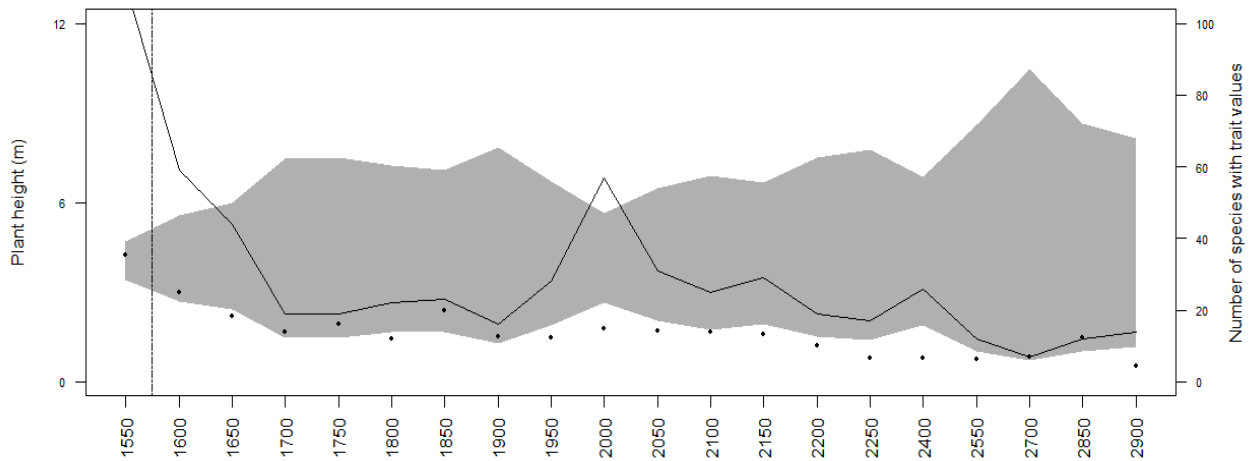
Figure 4: Results from resampling tests for plant height. The black dots represent the observed mean plant traits per elevational band, while the shaded grey area represents median 95% of simulated values. The right-hand axis of the graphs represents the number of species within each altitudinal band for which trait values were available, represented by the black line on the graphs. The vertical line separates the low elevation from the high elevation bands.

(Appendix S4, Fig. 1B). Similarly, tolerance to frost did not differ significantly from the randomly generated means (Appendix S4, Fig. 1C & 1D).

**DISCUSSION**

Complementary nestedness and functional traits analyses revealed that high elevation plant invaders tend to be widely distributed generalists but also display traits related to survival in alpine habitats. Significant nestedness of the alien flora indicates that species at higher elevations tend to have broad tolerances, rather than being specifically adapted to abiotically severe high elevation conditions. This provides strong support for the DEFH which predicts that aliens with broad tolerances are able to survive at both low and high elevations.

The species at higher elevations are nested subsets of the species from the lowland, suggesting that more widespread species occur at higher elevations and more range-restricted species in the lowlands (Wright & Reeves 1992). This is in agreement with the DEFH, although this could, in part, also reflect lag phases in the upslope expansion of alien species introduced to the lowlands (Hengeveld 1989). It appears that the majority of alien species (50% found in both low- and high elevations) have a wide distribution along the Pass and may potentially exhibit broader niches than those limited to the lowlands. Thus most alien plant species that reach the high elevations of the Pass are able to survive both at high (abiotically severe) and low (more biotically severe) elevations, not due to special adaptations, but due to broad environmental tolerances.

While significant nestedness provides good evidence that the majority of high elevation plant invaders are generalists, analyses of alien species functional traits reveal additional, more complex patterns, with two of the 11 traits differing significantly between high elevation and lowland restricted species. However, only one of the traits that differed between high elevation and lowland species showed a pattern solely in line with the predictions of the ALH. Plant height was significantly lower in high elevation aliens than species restricted to the lowlands, showing a survival and performance-related adaptation to higher elevations (i.e. protection against strong winds and energy redirected towards reproduction). Since low stature is a common adaptation in alpine species (Billings 1974; Fabbro & Körner 2004; Peng et al. 2014), lower plant height of alien species occurring at higher elevations is in line with the predictions of the ALH.

Frost tolerance also differed significantly between high elevation and lowland species, despite having smaller sample sizes than several other traits. Tolerance to frost is a trait beneficial for survival at high elevations, particularly in alpine regions (Killick 1978;

Anthelme & Dangles 2012). Therefore, greater tolerance of low temperatures would be expected in species occurring at high elevations if abiotic severity controlled the distribution of alien species along the elevational gradient. However, since the inverse was observed, it suggests that the high elevation alien species are not climatically pre-adapted to cold climates (as is expected, Macdonald et al. 1992; Dukes & Mooney 1999), but instead are generalist species. In combination, these results show that high elevation alien species tend to exhibit broad environmental tolerance, with some pre-adaptations to alpine conditions (low stature). Therefore, while nestedness strongly supports the DEFH, the significant difference in plant height between high elevation and lowland restricted species matches the predictions of the ALH, indicating that the mechanisms underlying both hypotheses are not mutually exclusive and might both contribute to shaping the distribution of alien plant species.

Due to the growing support for the DEFH along elevational gradients, it is now important to test if the predictions of this model also hold across other gradients, or whether the ALH explains the distribution of alien species along, for example, aridity or salinity gradients (especially since environmental filtering plays a significant role along many edaphic gradients, e.g. Moraes et al. 2015). Additionally, it may be instructive to contrast the functional traits of generalists (i.e. widespread across the gradient) and specialists (i.e. localised species) to determine if key traits are necessary for species to survive under abiotic extremes along other gradients where alien species composition follows patterns consistent with the DEFH. For example, in arid areas successful invaders may have broad abiotic tolerances, but might also exhibit some degree of specialisation to cope with extreme moisture stress. Indeed, further comparisons of functional traits may help to elucidate some of the more subtle mechanisms driving patterns of alien species distributions, and should be used in conjunction with nestedness analyses when examining invasion patterns along environmental gradients. In this study the simultaneous use of the two methods showed that for our elevational gradient neither the ALH nor the DEFH completely describe alien species distributions. Our results suggest that these two hypotheses might not be mutually exclusive, and that complementary sets of analyses might be necessary to tease apart the mechanisms driving patterns of species richness in alien plants along environmental gradients.

Our results have practical implications for improving predictions of alien species occurrence at higher elevations. For example, species that exhibit broad environmental tolerances may pose a greater risk for high elevation areas than more specialised (i.e. alpine) species, assuming that the majority of introduction events continue to occur in

owlands. Therefore, invasions into mountain areas may well depend strongly on the ntroduction of generalist alien species into lowlands, and thus, limiting the upslope spread of established lowland aliens may be necessary to prevent invasions into mountains Kueffer et al. 2013). Nevertheless, given the increasing risk of direct introductions between high elevation habitats, due to increased anthropogenic activities related to mountain tourism and the ornamental plant trade (Pauchard et al. 2009), management plans should not ignore the potential risk of specialised alpine invasive plants into high altitude areas.

## ACKNOWLEDGEMENTS

**Author Contribution**: All authors contributed to the design of the study, and the collection and analysis of data. CS led the writing of the manuscript, with input from the other authors.

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**A list of all appendices:**

**Appendix S1**: Species categorisation as naturalised.

**Appendix S2**: Data obtained from the TRY trait database.

**Appendix S3**: Testing for bias in our dataset.

**Appendix S4**: Additional figures.

**Appendix S1**: Species categorisation as naturalised.

Analysis of our data highlight that most of the alien species recorded are probably naturalised (and not casual species). Our data show that 95.9% of high elevation alien species recorded in the first two years of surveys were recorded again in >40% of the following six surveys (with 83.4% recorded again in >4 of the subsequent surveys). This strongly suggests that the overwhelming majority of species from the high elevation bands are naturalised aliens as they have been consistently and repeatedly observed over several years.

Because we sampled the lowland species pool for the first time during this study, we could not conduct similar analyses. However, we found that from the pool of alien species recorded at the three hotels, 65% are common to all sites. Furthermore, 80% of alien species recorded at the first intersect were recorded in ≥15 of the other 16 intersections. Similarly, out of the pool of aliens recorded for the three hotels, 92% are also found in the alien species pool recorded at the intersections. Therefore, there is strong evidence that the overwhelming majority of the species in the lowland species pool are widespread in the lowland (i.e. consistently and repeatedly observed across different sites), and are thus very likely naturalised.

**Appendix S2**: Data obtained from the TRY trait database.

**Table 1: Contributions from the TRY trait database. The table includes the datasets used for obtaining trait values for the majority of our species. The contributor of the dataset and subsequent references are also included.**

| DATASET | LASTNAME | REFERENCES FROM THE DATASETS |
|---|---|---|
| Abisko & Sheffield Database | Cornelissen | Cornelissen, J. H. C., H. M. Quested, D. Gwynn-Jones, R. S. P. Van Logtestijn, M. A. H. De Beus, A. Kondratchuk, T. V. Callaghan, and R. Aerts. 2004. Leaf digestibility and litter decomposability are related in a wide range of subarctic plant species and types. Functional Ecology 18:779-786.<br><br>Cornelissen, J. H. C., M. J. A. Werger, P. CastroDiez, J. W. A. vanRheenen, and A. P. Rowland. 1997. Foliar nutrients in relation to growth, allocation and leaf traits in seedlings of a wide range of woody plant species and types. Oecologia 111:460-469.<br><br>Cornelissen, J. H. C., P. C. Diez, and R. Hunt. 1996. Seedling growth, allocation and leaf attributes in a wide range of woody plant species and types. Journal of Ecology 84:755-765.<br><br>Quested, H. M., J. H. C. Cornelissen, M. C. Press, T. V. Callaghan, R. Aerts, F. Trosien, P. Riemann, D. Gwynn-Jones, A. Kondratchuk, and S. E. Jonasson. 2003. Decomposition of sub-arctic plants with differing nitrogen economies: A functional role for hemiparasites. Ecology 84:3209-3221. |
| ArtDeco Database | Cornwell | Unpubl. |
| Australian Fire Ecology Database | Bradstock | Unpubl. |
| BiolFlor Database | Kühn | Unpubl. |
| BIOME-BGC Parameterization Database | White | White, M. A., P. E. Thornton, S. W. Running, and R. R. Nemani. 2000. Parameterization and sensitivity analysis of the BIOME-BGC terrestrial ecosystem model: Net primary production controls. Earth Interactions 4:1-85. |
| BIOPOP: Functional Traits for Nature Conservation | Poschlod | Unpubl. |
| BROT Plant Trait Database | Pausas | Paula, S. and J. G. Pausas. 2008. Burning seeds: germinative response to heat treatments in relation to resprouting ability. Journal of Ecology 96:543-552.<br><br>Paula, S., M. Arianoutsou, D. Kazanis, Ç. Tavsanoglu, F. Lloret, C. Buhk, F. Ojeda, B. Luna, J. M. Moreno, A. Rodrigo, J. M. Espelta, S. Palacio, B. Fernández-Santos, P. M. Fernandes, and J. G. Pausas. 2009. Fire-related traits for plant species of the Mediterranean Basin. Ecology 90:1420. |
| Catalonian Mediterranean Forest Trait Database | Peñuelas | Unpubl. |
| Categorical Plant Traits Database | Poorter | Unpubl. |

| DATASET | LASTNAME | REFERENCES FROM THE DATASETS |
|---|---|---|
| Categorical Plant Traits Database | Flores | Unpubl. |
| Categorical Plant Traits Database | Wright | Unpubl. |
| Categorical Plant Traits Database | van Bodegom | Unpubl. |
| Categorical Plant Traits Database | Poorter | Unpubl. |
| Causasus Plant Traits Database | Soudzilovskaia | Unpubl. |
| Cedar Creek Savanna SLA, C, N Database | Reich | Unpubl. |
| Chinese Leaf Traits Database | Fang | Han, W. X., J. Y. Fang, D. L. Guo, and Y. Zhang. 2005. Leaf nitrogen and phosphorus stoichiometry across 753 terrestrial plant species in China. New Phytologist 168:377-385.<br><br>He, J. S., L. Wang, D. F. B. Flynn, X. P. Wang, W. H. Ma, and J. Y. Fang. 2008. Leaf nitrogen : phosphorus stoichiometry across Chinese grassland biomes. Oecologia 155:301-310.<br><br>He, J. S., Z. H. Wang, X. P. Wang, B. Schmid, W. Y. Zuo, M. Zhou, C. Y. Zheng, M. F. Wang, and J. Y. Fang. 2006. A test of the generality of leaf trait relationships on the Tibetan Plateau. New Phytologist 170:835-848. |
| Climbing Plants Trait Database | Gallagher | Unpubl. |
| Dispersal Traits Database | Higgins | Unpubl. |
| ECOCRAFT | Medlyn | Medlyn, B. E. and P. G. Jarvis. 1999. Design and use of a database of model parameters from elevated [CO2] experiments. Ecological Modelling 124:69-83.<br><br>Medlyn, B. E., C. V. M. Barton, M. S. J. Broadmeadow, R. Ceulemans, P. DeAngelis, M. Forstreuter, M. Freeman, S. B. Jackson, S. Kellomaeki, E. Laitat, A. Rey, P. Roberntz, B. D. Sigurdsson, J. Strassemeyer, K. Wang, P. S. Curtis, and P. G. Jarvis. 2001. Stomatal Conductance of forest species after long-term exposure to elevated CO2 concentration: a synthesis. New Phytologist 149:247--264.<br><br>Medlyn, B. E., F.-W. Badeck, D. G. G. De Pury, C. V. M. Barton, M. Broadmeadow, R. Ceulemans, P. De Angelis, M. Forstreuter, M. E. Jach, S. Kellomäki, E. Laitat, M. Marek, S. Philippot, A. Rey, J. Strassemeyer, K. Laitinen, R. Liozon, B. Portier, P. Roberntz, K. Wang, and P. G. Jarvis. 1999. Effects of elevated CO2 on photosynthesis in European forest species: a meta-analysis of model parameters. Plant, Cell and Environment 22:1475-1495. |
| ECOQUA South American Plant Traits Database | Pillar | Unpubl. |
| European Mountain Meadows Plant Traits Database | Bahn | Unpubl. |
| Floridian Leaf Traits Database | Cavender-Bares | Unpubl. |

| DATASET | LASTNAME | REFERENCES FROM THE DATASETS |
|---|---|---|
| Fonseca/Wright New South Wales Database | Wright | Fonseca, C. R., J. M. Overton, B. Collins, and M. Westoby. 2000. Shifts in trait-combinations along rainfall and phosphorus gradients. Journal of Ecology 88:964-977.<br><br>McDonald, P. G., C. R. Fonseca, J. M. Overton, and M. Westoby. 2003. Leaf-size divergence along rainfall and soil-nutrient gradients: is the method of size reduction common among clades? Functional Ecology 17:50-57. |
| French Massif Central Grassland Trait Database | Louault | Unpubl. |
| Global 15N Database | Craine | Craine, J. M., A. J. Elmore, M. P. M. Aidar, M. Bustamante, T. E. Dawson, E. A. Hobbie, A. Kahmen, M. C. Mack, K. K. McLauchlan, A. Michelsen, G. B. Nardoto, L. H. Pardo, J. Penuelas, P. B. Reich, E. A. G. Schuur, W. D. Stock, P. H. Templer, R. A. Virginia, J. M. Welker, and I. J. Wright. 2009. Global patterns of foliar nitrogen isotopes and their relationships with climate, mycorrhizal fungi, foliar nutrient concentrations, and nitrogen availability. New Phytologist 183:980-992. |
| Global A, N, P, SLA Database | Reich | Reich, P. B., J. Oleksyn, and I. J. Wright. 2009. Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. Oecologia 160:207-212. |
| Global Leaf Robustness and Physiology Database | Niinemets | Unpubl. |
| Global Respiration Database | Reich | Reich, P. B., M. G. Tjoelker, K. S. Pregitzer, I. J. Wright, J. Oleksyn, and J. L. Machado. 2008. Scaling of respiration to nitrogen in leaves, stems and roots of higher land plants. Ecology Letters 11:793-801. |
| GLOPNET - Global Plant Trait Network Database | Wright | Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P. K. Groom, J. Gulias, K. Hikosaka, B. B. Lamont, T. Lee, W. Lee, C. Lusk, J. J. Midgley, M. L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V. I. Pyankov, C. Roumet, S. C. Thomas, M. G. Tjoelker, E. J. Veneklaas, and R. Villar. 2004. The worldwide leaf economics spectrum. Nature 428:821-827.<br><br>Wright, I. J., P. B. Reich, O. K. Atkin, C. H. Lusk, M. G. Tjoelker, and M. Westoby. 2006. Irradiance, temperature and rainfall influence leaf dark respiration in woody plants: evidence from comparisons across 20 sites. New Phytologist 169:309-319. |
| Herbaceous Leaf Traits Database Old Field New York | Siefert | Unpubl. |
| Herbaceous Traits from the Öland Island Database | Hickler | Hickler, T. 1999. Plant functional types and community characteristics along environmental gradients on Öland's Great Alvar (Sweden) Masters Thesis, University of Lund, Sweden. |
| Jasper Ridge Californian Woody Plants Database | Cornwell | Ackerly, D. D. and W. K. Cornwell. 2007. A trait-based approach to community assembly: partitioning of species trait values into within- and among-community components. Ecology Letters 10:135-145.<br><br>Cornwell, W. K. and D. D. Ackerly. 2009. Community assembly and shifts in plant trait distributions across an environmental gradient in coastal California. Ecological Monographs 79:109-126.<br><br>Cornwell, W. K., D. W. Schwilk, and D. D. Ackerly. 2006. A trait-based test for habitat filtering: Convex hull volume. Ecology 87:1465-1471. |

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| KEW Seed Information Database (SID) | Dickie | Royal Botanical Gardens KEW. 2008. Seed Information Database (SID). Version 7.1. Available from: http://data.kew.org/sid/ (May 2008). Royal Botanical Gardens KEW. 2008. Seed Information Database (SID). Version 7.1. Available from: http://data.kew.org/sid/ (May 2011). |
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| Leaf Biomechanics Database | Onoda | Unpubl. |
| Leaf Physiology Database | Kattge | Kattge, J., W. Knorr, T. Raddatz, and C. Wirth. 2009. Quantifying photosynthetic capacity and its relationship to leaf nitrogen content for global-scale terrestrial biosphere models. Global Change Biology 15:976-991. |
| Midwestern and Southern US Herbaceous Species Trait Database | Weiher | Unpubl. |
| New South Wales Plant Traits Database | Leishman | Unpubl. |
| Overton/Wright New Zealand Database | Wright | Unpubl. |
| Photosynthesis and Leaf Characteristics Database | Blonder | Unpubl. |
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| PLANTSdata USDA | Green | Green, W. 2009. USDA PLANTS Compilation, version 1, 09-02-02. (http://bricol.net/downloads/data/PLANTSdatabase/) NRCS: The PLANTS Database (http://plants.usda.gov, 1 Feb 2009). National Plant Data Center: Baton Rouge, LA 70874-74490 USA. |
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| Reich-Oleksyn Global Leaf N, P Database | Reich | Reich, P. B., J. Oleksyn, and I. J. Wright. 2009. Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. Oecologia 160:207-212. |
| Roots Of the World (ROW) Database | Craine | Craine, J. M., W. G. Lee, W. J. Bond, R. J. Williams, and L. C. Johnson. 2005. Environmental constraints on a global relationship among leaf and root traits of grasses. Ecology 86:12-19. |
| Sheffield & Spain Woody Database | Cornelissen | Castro-Diez, P., J. P. Puyravaud, and J. H. C. Cornelissen. 2000. Leaf structure and anatomy as related to leaf mass per area variation in seedlings of a wide range of woody plant species and types. Oecologia 124:476-486. |

| DATASET | LASTNAME | REFERENCES FROM THE DATASETS |
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**Appendix S3**: Testing for bias in our dataset.

Values for all traits were not available for all species and this could generate bias in our results if species for which traits were available were not randomly distributed along the elevation gradient. To evaluate this we tested if the proportion of species with trait data available for each trait varied significantly with elevation (using linear regression; Figs 1 and 2). The majority of traits showed no significant relationship (e.g. Fig. 1), however, the proportion of species with data available for photosynthetic system, pollination syndrome, mode of fertilisation and flowering duration show significant decrease with elevation (Fig. 2).These traits were removed from subsequent analyses to eliminate this potential source of bias from our dataset.
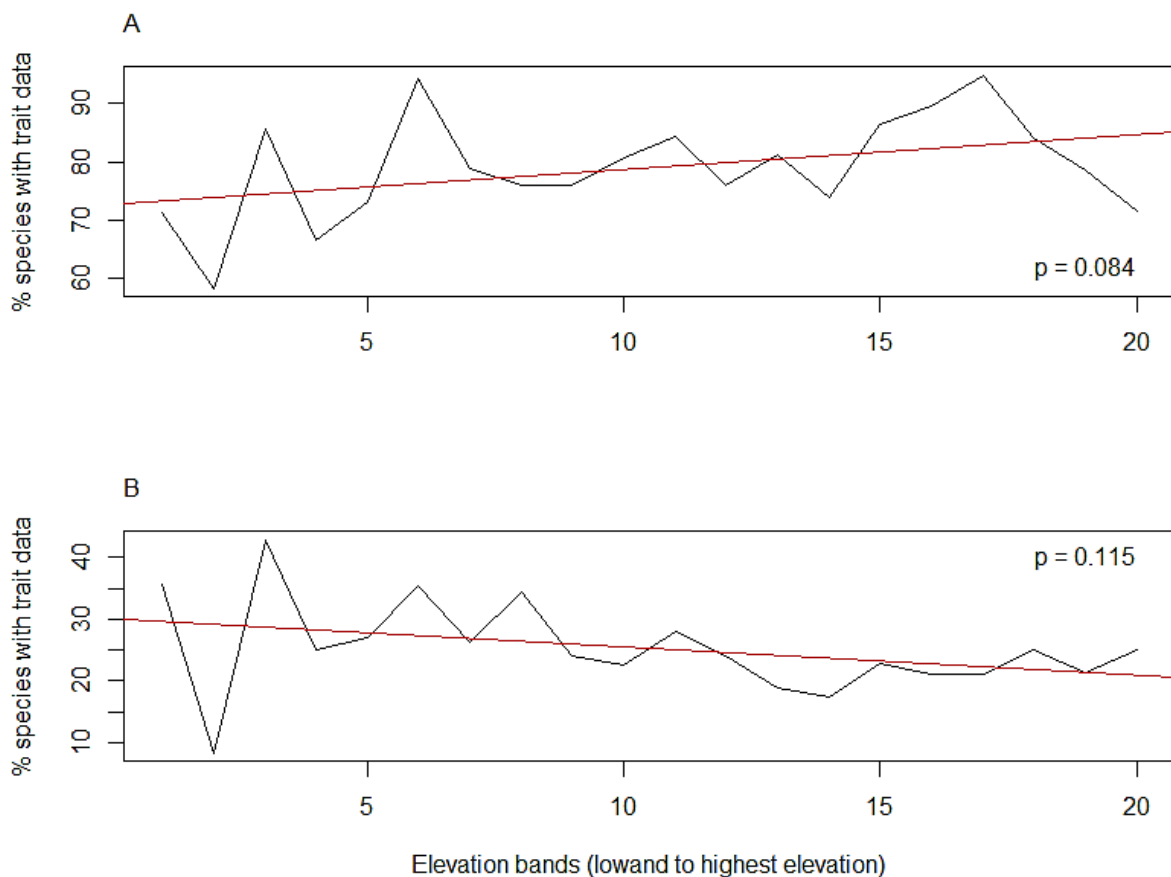


*Figure 1: Percentage species with data available for a) flower conspicuousness and b) frost tolerance do not show significant changes along an elevation gradient (x-axis, low to high elevation). The red line indicates the best fit linear regression with the p-value indicated on the graph.*
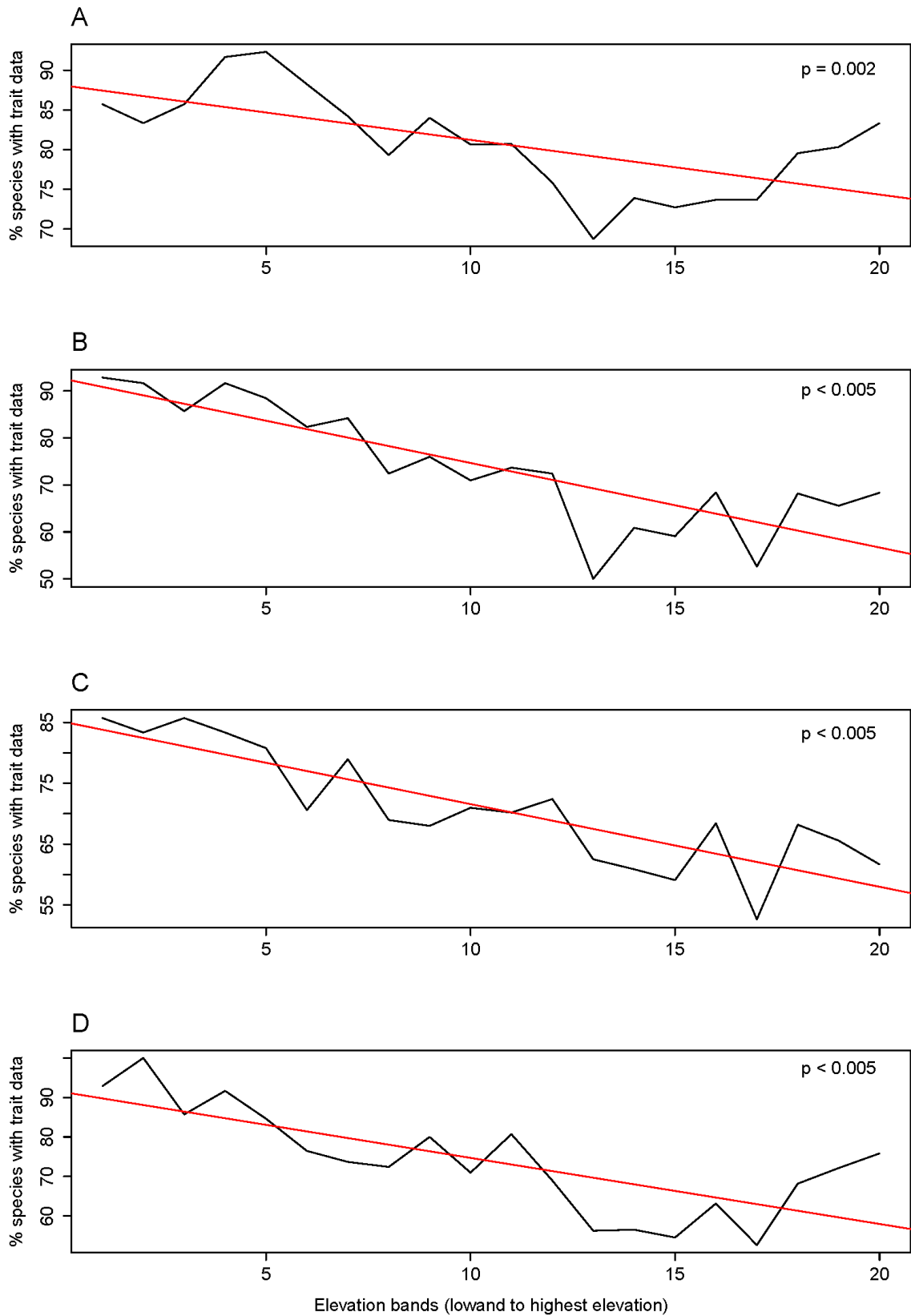
*Figure 2: Percentage species with data available for a) photosynthetic system, b) pollination, c) mode of fertilization, and d) flowering duration significantly decreases along an elevation gradient. The red line indicates the best fit linear regression, and the p-value indicates the significance of the linear fit.*
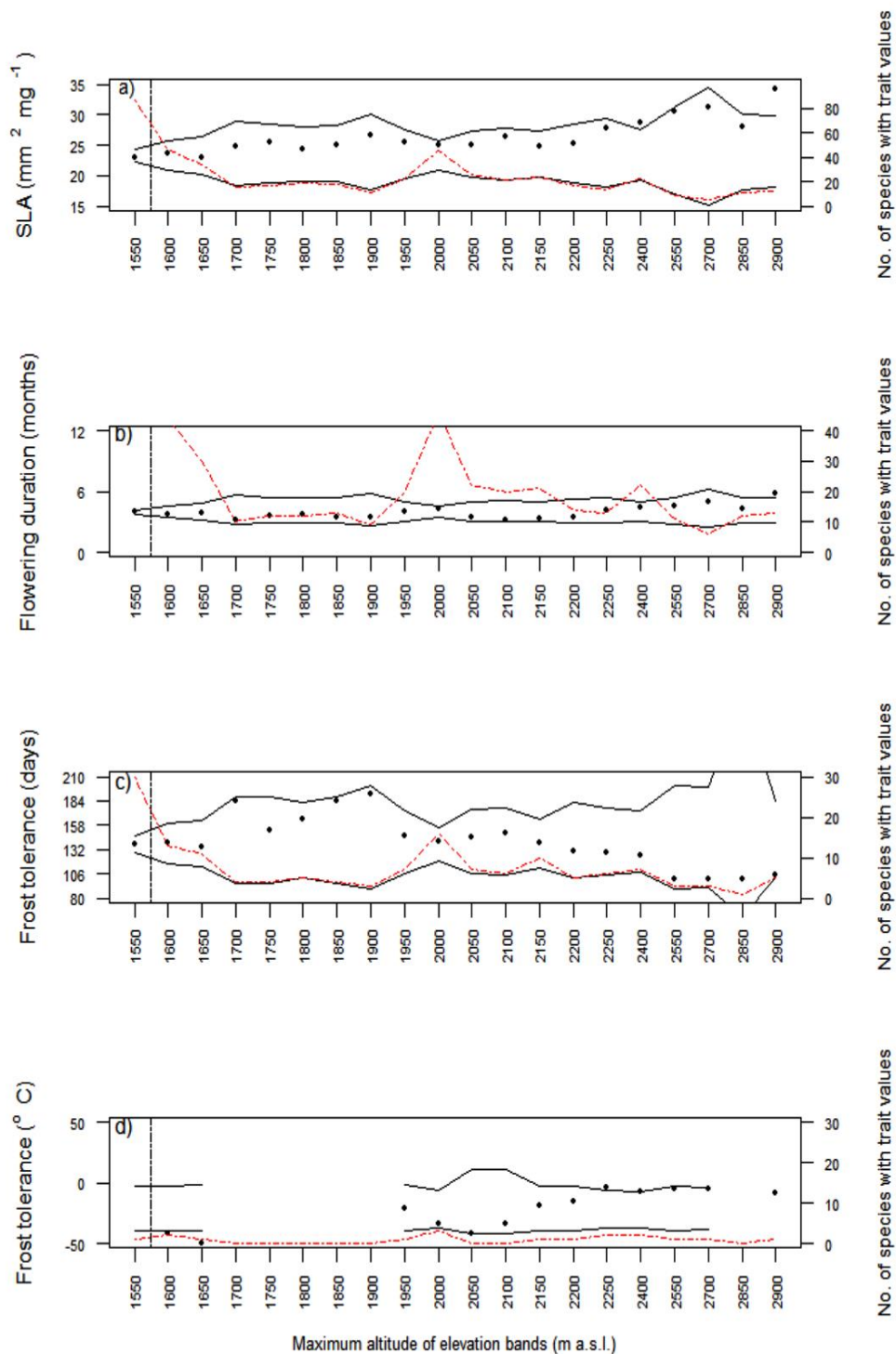
**Figure 1: Results from resampling tests for a) specific leaf area, b) flowering duration, c) duration of tolerance to frost, and d) minimum temperature of frost tolerance. The black dots represent the observed mean plant traits per elevational band (bottom x-axis), while the solid lines represents the area between the 2.5 and 97.5 percentiles of mean trait values obtained from 10 000 random resampling procedures. The right axis of the graphs represents the number of species within each altitudinal band for which trait values were available, shown by the dashed line. If the black dots fall outside the solid lines, the observed trait values are significantly different from random expectation. The vertical line separates the low elevation from the high elevation bands.**