Microclimate variability in alpine ecosystems as stepping stones for non-native plant establishment above their current elevational limit

Microclimate variability in alpine ecosystems as stepping stones for non-native plant establishment above their current elevational limit

Jonas J. Lembrechts[1,*], Jonathan Lenoir[2], Martin A. Nuñez[3], Aníbal Pauchard[4,5], Charly Geron[6], Gilles Bussé[1], Ann Milbau[7], Ivan Nijs[1]

1 Centre of Excellence Plant and Vegetation Ecology, University of Antwerp, 2610 Wilrijk, Belgium

2 UR "Ecologie et Dynamique des Systèmes Anthropisés" (EDYSAN, FRE 3498 CNRS-UPJV), Université de Picardie Jules Verne, 1 Rue des Louvels, 80037 Amiens Cedex 1, France

3 Grupo de Ecología de Invasiones, Universidad Nacional del Comahue, INIBIOMA, CONICET, Quintral 1250, C.P. 8400, Bariloche, Argentina

4 Laboratorio de Invasiones Biológicas, Facultad de Ciencias Forestales, Universidad de Concepción, Casilla 160-C, 4030000 Concepción, Chile

5 Institute of Ecology and Biodiversity (IEB), 8320000 Santiago, Chile

6 Faculté des Sciences, Université d'Angers, 2 Boulevard de Lavoisier, 49000 Angers, France

7 Research Institute for Nature and Forest - INBO, Kliniekstraat 25, 1070 Brussels, Belgium

*Corresponding author: jonas.lembrechts@uantwerpen.be, +3232651727.

Orcid ID: orcid.org/0000-0002-1933-0750.

Keywords: Alien plant invaders, climate change, disturbance, invasibility, invasive species, mountain topography, species distributions, subarctic

Abstract

Alpine environments are currently relatively free from non-native plant species, although their presence and abundance have recently been on the rise. It is however still unclear whether the observed low invasion levels in these areas are due to an inherent resistance of the alpine zone to invasions or whether an exponential increase in invasion is just a matter of time. Using a seed-addition experiment on north- and south-facing slopes (cf. microclimatic gradient) on two mountains in subarctic Sweden, we tested the establishment of six non-native species at an elevation above their current distribution limits and under experimentally enhanced anthropogenic pressures (disturbance, added nutrients and increased propagule pressure). We found a large microclimatic variability in cumulative growing degree days (GDD) (range = 500.77 °C, SD = 120.70 °C) due to both physiographic (e.g. aspect) and biophysical (e.g. vegetation cover) features, the latter being altered by the experimental disturbance. Non-native species establishment and biomass production were positively correlated with GDD along the studied microclimatic gradient. However, even though establishment on the north-facing slopes caught up with that on the south-facing slopes throughout the growing season, biomass production was limited on the north-facing slopes due to a shorter growing season. On top of this microclimatic effect, all experimentally imposed anthropogenic factors enhanced non-native species success. The observed microclimatic effect indicates a potential for non-native species to use warm microsites as stepping stones for their establishment towards the cold end of the gradient. Combined with anthropogenic pressures this result suggests an increasing risk for plant invasion in cold ecosystems, as such stepping stones in alpine ecosystems are likely to be more common in a future that will combine a warming climate with persistent anthropogenic pressures.

Introduction

Plant invasions in mountains have been increasing significantly in the last decades, and are expected to expand higher up in elevation towards alpine and nival ecosystems under predicted scenarios of global change (Pauchard et al. 2009, McDougall et al. 2011, Pyšek et al. 2011, Angelo and Daehler 2013, Pauchard et al. 2016). These ecosystems − especially those in cold high-latitude regions − are however currently still relatively free from non-native plant species (Pauchard et al. 2009, Lembrechts et al. 2014, Zefferman et al. 2015), although a recent global review reported a total of 183 distinct non-native species from the alpine areas of 15 mountain regions (Alexander et al. 2016).

Studies of plant invasions in mountains are mostly observational and tend to focus on patterns of non-native species richness and the dynamics of spread along elevation gradients, but these studies often overlook the alpine and nival extremes of the gradient (Seipel et al. 2012, Lembrechts et al. 2017). Much less is therefore known about the potential of non-native plants to establish there (Alexander et al. 2016): does the prevailing harsh climate in alpine environments represent an inherent resistance to invasion as often assumed, or has anthropogenic pressure not yet reached these areas and is invasion just a matter of time (Pauchard et al. 2009)? To shed light on this question, it is fundamental to gather experimental proof on the effects of both anthropogenic influences and the alpine climate (Lembrechts et al. 2016), including the inherent topo- and microclimatic variability, on the performance of non-native species introduced above their current range limits.

Alpine landscapes are characterized by a complex topography, which can cause annual temperatures to vary more than 2 °C within a particular elevation band (Ackerly et al. 2010, Scherrer and Körner 2011, Graae et al. 2012). This variability can even reach up to 6 °C within a given 1 km^2 spatial unit in mountains of northern Europe (Lenoir et al. 2013). Physiographic processes due to topography and geomorphology also affect the snow

distribution and therefore the length of the growing season (Körner 2003). In addition, biophysical processes due to vegetation cover may further decouple upper atmospheric conditions from boundary layer effects (Geiger 1950) and thus provide peculiar microclimatic conditions that may facilitate the establishment of plant invaders. At high latitudes, where solar angles are low, micro-habitats regularly have seasonal mean soil temperatures that are 7 °C warmer than free-air or synoptic temperature (Scherrer and Körner 2010, Lenoir et al. 2013). Thus even when average synoptic temperatures in the alpine zone are outside the climatic niche of non-native species, microclimatic variability might still provide favorable conditions on a local scale. Hitherto, the role of microclimate for non-native species establishment in alpine areas has not yet been examined. The inclusion of microclimatic variability in the assessment of species distributions has however proven critical to solve the mismatch between the resolution of climatic data and the scale at which species experience this climate (Potter et al. 2013, Lenoir et al. 2017).

Several recent studies in mountain regions have determined disturbance as the key anthropogenic driver of the occurrence and success of non-native species (Marini et al. 2009, Alexander et al. 2011, Lembrechts et al. 2016). Both large-scale (e.g. roads) and small-scale (e.g. gaps of a few cm) disturbances have indeed been demonstrated to increase invader establishment in mountains (Seipel et al. 2012, Milbau et al. 2013). However, under extreme environmental conditions such as at high elevations and latitudes, disturbance might disrupt the microclimatic buffering provided by facilitative interactions between plants, thereby potentially hindering invader establishment (Callaway et al. 2002, Cavieres et al. 2007, Cavieres et al. 2008). Experimental studies along elevation gradients from the subalpine to the alpine zone in high-latitudinal mountains recently challenged the generality of this theory on facilitation (Milbau et al. 2013, Lembrechts et al. 2016). They revealed how the net effect of neighbors on invader recruitment can be consistently negative (Milbau et al. 2013), and that

disturbance is the main determinant of plant invader establishment along an entire elevation gradient, including the alpine zone (Lembrechts et al. 2016).

Nutrient addition, often seen in combination with anthropogenic disturbances like construction works, roads or agriculture, can be an additional important driver of plant invader establishment in the alpine zone, where soils are often low in nitrogen content (Körner 2003, Lembrechts et al. 2016). Similarly, poor soil conditions, together with low propagule pressure, served as the main limitation for invasion of *Taraxacum officinale* in dry mountains (Quiroz et al. 2011). Despite the accumulating evidence on the importance of these factors for plant invasion in mountains, few experiments were performed above the current range limits of the invaders, where their relative importance might differ from milder areas, especially in interaction with microclimatic variability.

Here, we performed a seed-addition experiment on north- and south-facing (cf. microclimatic variability) slopes in the alpine zone at approximately 400 meters above the tree line on two subarctic Scandinavian mountains (Northern Scandes). With this experiment, we aim to assess the physiographic and biophysical drivers of microclimate variability within an elevational band, as well as the impact of microclimate variability itself and that of human influences (disturbance, nutrient addition and increased propagule pressure) on the performance of non-native species introduced above their current range limits. We used 6 non-native plant species that are globally common in mountains and have their current distribution limits at lower elevations in the study region (Lembrechts et al. 2014). We hypothesize that microclimatic variability within this elevational band will cause significant variation in non-native species establishment and biomass production. Nutrient addition and increased propagule pressure are expected to have positive effects on establishment success of the non-native species, while disturbance is predicted to induce lower invader success through

the disruption of suitable microclimatic conditions due to facilitative effects from the neighboring plants.

Methods

Study regions and site characteristics

We performed a two-year multifactorial split plot experiment (Fig. 1) in the alpine zone on two mountains in the northern Scandes around Abisko, Sweden (N 68°21' E 18°49'), a region with a subarctic climate with a mean annual air temperature and precipitation of respectively -0.5 °C and 310 mm (Abisko Scientific Research Station, 400 m a.s.l., from 1913 till 2011, www.polar.se/abisko). On each mountain, two study sites were chosen at around 1000 m a.s.l. (approximately 400 m above the tree line), with an aspect of respectively 180° ± 15° (south-facing slope) and 0° ± 20° (north-facing slope). Sites were chosen in (poor) alpine meadows, as meadows in this area are known to have a high invasibility relative to other high-latitude habitat types (Milbau et al. 2013).

Experimental set-up

In early July 2014, we installed six randomly located 120 x 160 cm plots at each of the two sites on each of the two mountains (Fig. 1), with the longest side oriented parallel to the slope. Half of each plot (60 x 160 cm) was experimentally disturbed by removing the vegetation (above and below-ground biomass) as well as the top 3-cm soil layer, to disrupt biotic interactions with neighboring plants, as happens in anthropogenic or natural disturbances like construction works, road- and trail sides, avalanches and trampling or digging by animals. The lowest half of the plot (80 x 120 cm) was fertilized with 50 g Substral Osmocote slow release fertilizer (N-P-K-Mg 19-9-11-2, equaling 45 kg N/ha/y), evenly spread on the soil surface to release plants from any nutrient limitation. This nutrient addition treatment mimics a likely scenario of nitrogen deposition and faster nutrient release from increased

decomposition under a warming climate (Flechard et al. 2011, Portillo-Estrada et al. 2016). Each plot was thus divided in four subplots: control (undisturbed-not fertilized); undisturbed-fertilized; disturbed-not fertilized; and disturbed-fertilized. Within every subplot, six species of non-native forbs and grasses were sown in microplots (1 cm diameter), either at a low (five seeds) or high (30 seeds) propagule pressure, resulting in twelve evenly distributed microplots per subplot, each of them 20 cm apart (total amount of microplots in the experiment: n = 1152). Every combination of species and propagule pressure was randomly assigned to one of the twelve microplots, with each of the microplots equaling one growth place.

We chose two species from each of three distinct plant families characterized by a large number of globally invasive species (Daehler 1998), i.e. from Asteraceae (A), Fabaceae (F) and Poaceae (P): *Achillea millefolium* L. (A); *Agrostis capillaris* L. (P); *Anthoxanthum odoratum* L. (P); *Medicago lupulina* L. (F); *Taraxacum officinale* F.H.Wigg. (A); and *Trifolium repens* L. (F). All chosen species belong to the 50 most widely spread global mountain invaders (Seipel et al. 2012) and are present as non-native species in the northern parts of Scandinavia (Weidema 2000, Lembrechts et al. 2014) but yet had a current distribution limit at or below the tree line (approximately 600 m a.s.l., Lembrechts et al. 2014). They all followed the spread of agriculture to higher latitudes and were recently shown to expand their ranges into the mountains via linear disturbances like roads and trails (Lembrechts et al. 2014). Seeds were bought from a seed distributor (www.cruydthoeck.nl).

Soil temperature

Soil temperature was logged every hour (iButtons DS1922L with 0.0625 °C accuracy, Maxim Integrated, San José, CA, USA). The iButtons were placed at 3 cm below the soil surface, one in both the middle of the disturbed and the undisturbed half of every plot (n = 48). The resulting temperature time series were used to calculate cumulative growing degree days

(GDD with base 0, being the sum of all positive daily averages in °C) for a period of 365 days during the experiment (from August 1st 2014 till July 31st 2015).

Invader establishment success

Invader establishment was recorded as presence/absence per microplot at 6 different times throughout the second growing season (early July 2015 till the beginning of September 2015). At the end of this second growing season (September 2015), aboveground biomass of the sown species was harvested per microplot (all individuals combined), dried and weighed. Biomass of the native vegetation in the different subplots was estimated at the same time by harvesting the aboveground plant parts within a randomly located 400 cm² square, drying this biomass, weighing it and rescaling the result to biomass per 1 m². In the undisturbed subplots, this represents standing biomass per m², while it is a proxy for plot productivity over two growing seasons for the mostly perennial species establishing in the disturbed subplots.

All above- and belowground non-native biomass was removed at the end of the experiment, before non-native plants could flower or produce seeds. Sites were revisited the next year to check for new germinations.

Statistical analyses

To understand the main determinants behind microclimatic variability in our study system, GDD was modelled with a linear model (LM, function *lm* in R (R Core Team 2015)) against aspect (factor variable with two levels: north or south), mountain (factor variable with two levels: 1 or 2), disturbance (factor variable with two levels: yes or no) and their two-way interactions. We used the function *calc.relimp* from the package 'relaimpo' (Grömping 2006), with the *lmg* metric (R² partitioned by averaging over orderings among regressors), to calculate the relative contribution of each variable and interaction to the total variance in the

dataset. Additionally, a post-hoc TukeyHSD test was performed on the outcome of an analysis of variance (ANOVA) to test for two-by-two differences between treatments.

Final invader establishment (presence/absence at the end of the second growing season, n = 1152) and biomass production of the established invaders (n = 287) were analyzed with a multi-model inference approach, comparing the AICc (corrected Akaike Information Criterion) from a series of models containing either GDD, biomass of the native vegetation, experimental anthropogenic factors (disturbance, nutrient addition and propagule pressure) or combinations between those. We follow the framework proposed by Burnham and Anderson (2003), using the function *model.sel* from the R-package 'MuMIn' (Barton 2016). In addition to the possible effects of all single factors, we hypothesized interactive effects between GDD or native biomass and disturbance, and between disturbance and nutrient addition (Lembrechts et al. 2016). When models had a ΔAICc of less than 2, model coefficients were averaged using the function *model.avg* (R-package 'MuMIn', Barton 2016). For invader establishment, we used a generalized linear mixed model (GLMM) with a binomial family and a logit link (function *glmer*, from the package 'lme4' (Bates et al. 2013), following Zuur et al. (2013)). For biomass production, we scaled the natural logarithm of species-specific biomass to standardize the biomass per species (mean 0 and SD 1, function *scale*). The resulting normally-distributed biomass data was analyzed with linear mixed models (LMM) with the function *lme* from the package 'nlme' (Pinheiro et al. 2013). We used ln(x+0.001) to allow log-transformation, with 0.001 g being the lowest measured biomass.

We corrected for random effects with species nested within plot, within site and within mountain as a random intercept. As such, we accounted for other potential drivers that vary between plots, sites and mountains that are not captured by the microclimatic effect in GDD. Species-specific patterns in biomass production for each treatment, as well as species-specific

model outcomes (following the same multi-model inference approach as explained above), are provided as Supplementary material.

Invader establishment throughout the whole growing season (n = 6912) was analyzed with a GLMM with day of the year (DOY) as continuous variable, together with disturbance, nutrient addition, propagule pressure, aspect (north/south) and all two-way interactions. Again and for the same reasons as mentioned above, we corrected for random effects with species nested within plot, within site and within mountain as a random intercept. All data analyses were performed in R version 3.2.3 (R Core Team 2015).

Results

We found that differences in GDD (total range = 500.77 °C, SD = 120.70 °C) across the studied area were due to a mixture of topoclimatic (aspect north *vs*. south), macroclimatic (mountain 1 *vs*. 2) and biophysical (disturbed *vs*. undisturbed) processes (Fig. 2, Table 1 and Supplementary material Table S1). Aspect explained the largest part (50 %) of the variance in GDD, and an additial 27 % in interaction with mountain (Table 1). Disturbance accounted for 6 % of the total explained variance, half of which in interaction with mountain, while mountain on its own explained only 1 % of the total explained variance. Within-site microclimatic differences unexplained by these three predictors (i.e. the residual variance) made up the remaining 16.3 % of the total variance.

Models of final invader establishment against biomass of the native vegetation showed a better fit (lower AICc) than those against GDD (Table 2, Supplementary material Table S2). In the undisturbed plot halves, establishment probability of the non-natives decreased with increasing standing biomass of the native vegetation (Fig. 3b, Table 2), yet in the disturbed plots the relation with plot productivity was exponentially positive. Invader establishment was positively related to GDD in undisturbed plots, yet constant and higher

along the whole gradient in disturbed plots (Fig. 3a). Next to the mostly positive effects of disturbance on invader establishment, nutrient addition had only minor effects, yet a higher propagule pressure increased establishment in all treatments. The patterns in invader establishment however changed surprisingly throughout the second growing season (Fig. 4). At the beginning of spring (DOY 180), non-natives were present in significantly more microplots in the disturbed plot halves on the south-facing than on the north-facing slopes. During the second growing season, however, levels of establishment increased more rapidly on the north-facing slopes, resulting in the same establishment in disturbed plots on both mountain slopes at the moment of the harvest (DOY 240).

Models of invader biomass production against GDD had a lower AICc than those including biomass of the native vegetation (Table 2, Supplementary material Table S2). Invader biomass production was positively correlated with a warm microclimate (high levels of GDD) in all treatments (Fig. 5a, Table 2). Additionally, invader biomass was largest in disturbed and fertilized plots for invaders sown at a high propagule pressure (Supplementary material Fig. S1). In the undisturbed plot halves, biomass production of the non-natives decreased with increasing standing biomass of the native vegetation (Fig. 5b), yet in the disturbed plots the relation with plot productivity was exponentially positive. Added nutrients and higher propagule pressure nevertheless had a positive effect on invader biomass production both in the disturbed and undisturbed plots.

Patterns were largely consistent between all study species, with largest biomass production observed in disturbed plots with added nutrients on south-facing slopes for 8 out of 9 cases with sufficient survival (Supplementary material Fig. S1). We found also a high consistency across species in the direction of the effects of disturbance, propagule pressure and biomass of the native vegetation on invader establishment, and of GDD, disturbance,

nutrient addition, increased propagule pressure and biomass of the native vegetation on invader biomass production (Supplementary material Table S3).

Discussion

In our study focusing on cold ecosystems, locally warmer microclimates significantly enhanced non-native species biomass production at an elevation well above their current range limits, shown by a positive correlation with GDD in all our experimental treatments (Fig. 5). That non-native species are promoted by higher temperatures along elevation gradients and that they can survive above their current range limits in mountain areas has been shown before (Poll et al. 2009, Trtikova et al. 2010, Haider et al. 2011). Yet the importance of microclimatic variability as a determinant of non-native species' performance was till now unproven empirically. These results imply that non-native species could use the present thermal variability available across short spatial distances and within a given elevational band in alpine environments as stepping stones towards higher elevations: patches of suitable habitat in a less-suitable matrix that facilitate propagule migration and thus range expansion.

Observed temperature differences were the result of both physiographic (e.g. slope and aspect) and biophysical (e.g. vegetation cover) features, with the latter being altered by plant removal from disturbances (Fig. 2). The effect size of both features differed between mountains, with lower differences on mountain 2 most likely due to a stronger exposure to wind. We thus argue that the integration of different spatial levels of climatic conditions is required to adequately model and predict the potential of non-native plant species to be invasive, especially in alpine environments. This will be especially relevant for predictions of species distributions in a warming climate, which are currently limited by the use of coarse climate data (Randin et al. 2009, Potter et al. 2013, Hannah et al. 2014, Lenoir et al. 2017).

Our experiment additionally showed that the studied non-native species required a unique combination of conditions to succeed in the alpine zone, even in those sites with a warmer microclimate: their biomass production was the highest in microplots that were disturbed and fertilized and had received a high propagule pressure (Fig. 5). Anthropogenic influences could thus be a significant trigger and even a necessity for non-native species to expand their ranges towards high elevations (Alexander et al. 2009, Lembrechts et al. 2016, Pauchard et al. 2016), by alleviating negative biotic interactions and improving microclimatic conditions in the advantage of the invaders. Indeed, even though the low plot productivity at these elevations does not suggest strong competition, the standing biomass – mostly consisting of mosses and slow growing *Carex* species – does create strong competition that can limit seedling establishment (Milbau et al. 2013), visualised here by the negative correlation between invader performance and biomass of the native vegetation in the undisturbed plot halves (Fig. 3b and 5b, Pollnac et al. 2012, Milbau et al. 2013, Lembrechts et al. 2016). Our results also suggest that this competition with standing biomass is more important than temperature for actual establishment, while temperature played a bigger role in biomass production after establishment. This decisive role of disturbance has been shown before at lower elevations (Lembrechts et al. 2016), yet it was surprising that even this high in the alpine zone facilitation was still found to be subordinate to competition as the main biotic interaction determining invasion (Olofsson et al. 1999, Poll et al. 2009, Klanderud 2010, Milbau et al. 2013).

A key factor explaining this decisive role of disturbance even at this elevation is the positive effect it had on microclimate through an increase in GDD (Fig. 2, Table 2), thus making the growing season longer and maximum soil temperatures higher, whereas intact vegetation in contrast kept soil temperatures buffered and centered around lower temperatures (Körner 2003, Delgado et al. 2007, Lembrechts et al. 2015). A disturbed mountain ecosystem

thus provides a much more elaborate set of stepping stones for plant invaders towards higher elevations (Alexander et al. 2016). The combination of the high competitiveness of the standing biomass with the positive effect of disturbance on GDD adequately explains why we did not find a facilitative effect of the native vegetation in our study system.

The relation of invader succes with the biomass of the native vegetation surprisingly switched from negative in the undisturbed plots to positive in the disturbed plots (Fig. 3b and 5b). This positive correlation in the disturbed plots hints to a similar effect of biomass removal on native and non-native species. Indeed, plots that supported more regrowth of the natural vegetation also promoted the establishment and biomass production of non-native species, suggesting these plots hosted better soil and microclimatic conditions than those with lower native and non-native biomass production. Additionally, the native vegetation regrowing in the disturbed plots mostly consisted of forbs and grasses with limited competitive advantage over the non-native seedlings, compared with the dense cover of mosses and sedges in the undisturbed vegetation. A similar discrepancy has been shown before for native and non-native species richness on different spatial scales (the biotic acceptance hypothesis, predicting more invaders in species rich areas on a large spatial scale, but less invaders in rich plots on a small scale, Stohlgren et al. 2006). Our results add a temporal discrepancy throughout succession to this theory: resource-rich plots will favor both native and non-native species in the early stages of succession, i.e. in disturbed plots, while they will have a denser native vegetation cover and thus limit non-native seedling success when the vegetation is in a climax state.

Nutrient addition did not increase invader establishment, but it did have a strong positive effect on biomass production, especially in combination with disturbance (Fig. 3 and 5). This links to the invasion theory of fluctuating resources: the combination of an increase in resource supply with a decrease in resource uptake serves as a determinant of invasion (Davis

et al. 2000). Propagule pressure also had positive effects on both establishment and biomass production, with a high propagule pressure being a prerequisite for the non-native species to benefit from the warmer microclimate (Fig. 5). Interestingly, while all studied anthropogenic pressures (disturbance, nutrient addition and increased propagule pressure) on their own had a positive effect on invader success, the simultaneous presence of these factors and a favourable microclimate was needed for plant invaders to grow to maturity in this alpine ecosystem (Lembrechts et al. 2015).

While establishment success was significantly lower in disturbed plots on north-facing (colder) slopes at the beginning of spring, it surprisingly showed a faster increase there throughout the second growing season due to the germination of seeds that had remained dormant, until the probability of establishment eventually became unrelated to aspect at the moment of the harvest (Fig. 4). The growing season was however too short in these colder plots to result in the same levels of biomass production at the end of the summer (Fig. 5a, Laube et al. 2015). These surprising results imply that non-native species may establish in disturbed plots everywhere at these elevations, but that they will only grow to maturity when the microclimate is suitable throughout the whole growing season (Greenwood et al. 2015). Non-native plant invaders may thus have higher chances in warmer plots to accomplish a full life cycle, ensuring a succesful flowering and subsequent production of seeds to build up an established population and a subsequent founder effect. While this difference in growing season length explains the large discrepancy between north- and south-facing slopes, it could also be part of the reason why invader success was higher in the disturbed plots compared to the undisturbed ones.

In conclusion, we showed that a vanguard of non-native species can establish in warm microsites at high elevations in cold-climate mountains as soon as propagules are introduced. Such warm microsites may act as stepping stones for non-native plant invaders to expand

their ranges to elevations high above their current limits. These climatic stepping stones suggest that substantial range expansion can be expected in the near future for several non-native, but also native, species currently residing at lower elevations. Yet, the strong correlation of invader establishment with anthropogenic influences in our experiment shows the fundamental role of humans as catalysts of this upward expansion in high-elevation areas. We thus predict an increasing risk for plant invasion in the alpine zone in a future that is likely to combine a warming climate with increasing anthropogenic influences at high elevations (Pauchard et al. 2009). An increase in the average annual temperature of 1 °C (365 GDD) would even be sufficient to make the least favorable plots in our experiment experience the temperature currently measured in the warmest plots. This would boost invader growth significantly, especially in disturbed sites. We thus emphasize the importance of implementing our findings into management plans for non-native species in cold regions. While climate cannot be controlled at a local scale, human disturbances can be greatly reduced by local regulations (Pauchard et al. 2016), which would lower the propagule pressure and limit the creation of climatically suitable microsites.

Acknowledgements

References

Ackerly, D. D. et al. 2010. The geography of climate change: implications for conservation biogeography. — Divers Distrib 16: 476-487.
Alexander, J. M. et al. 2009. Plant invasions along mountain roads: the altitudinal amplitude of alien Asteraceae forbs in their native and introduced ranges. — Ecography 32: 334-344.

Alexander, J. M. et al. 2011. Assembly of nonnative floras along elevational gradients explained by directional ecological filtering. — P Natl Acad Sci USA 108: 656-661.

Alexander, J. M. et al. 2016. Plant invasions into mountains and alpine ecosystems: current status and future challenges. — Alpine Bot 126: 89-103.

Angelo, C. L. and Daehler, C. C. 2013. Upward expansion of fire-adapted grasses along a warming tropical elevation gradient. — Ecography 36: 551-559.

Barton, K. 2016. MuMIn: Multi-Model Inference. R package version 1.15.6.

Bates, D. et al. 2013. lme4: linear mixed-effects models using Eigen and S4.

Burnham, K. P. and Anderson, D. R. 2003. Model selection and multimodel inference: a practical information-theoretic approach. — Springer Science & Business Media.

Callaway, R. M. et al. 2002. Positive interactions among alpine plants increase with stress. — Nature 417: 844-848.

Cavieres, L. A. et al. 2007. Microclimatic modifications of cushion plants and their consequences for seedling survival of native and non-native herbaceous species in the high andes of central Chile. — Arct Antarct Alp Res 39: 229-236.

Cavieres, L. A. et al. 2008. Facilitation of the non-native *Taraxacum officinale* by native nurse cushion species in the high Andes of central Chile: are there differences between nurses? — Funct Ecol 22: 148-156.

Daehler, C. C. 1998. The taxonomic distribution of invasive angiosperm plants: ecological insights and comparison to agricultural weeds. — Biol Conserv 84: 167-180.

Davis, M. A. et al. 2000. Fluctuating resources in plant communities: a general theory of invasibility. — J. Ecol. 88: 528-534.

Delgado, J. D. et al. 2007. Edge effects of roads on temperature, light, canopy cover, and canopy height in laurel and pine forests (Tenerife, Canary Islands). — Landscape Urban Plan 81: 328-340.

Flechard, C. R. et al. 2011. Dry deposition of reactive nitrogen to European ecosystems: a comparison of inferential models across the NitroEurope network. — Atmos Chem Phys 11: 2703-2728.

Geiger, R. 1950. The climate near the ground. — Harvard University Press.

Graae, B. J. et al. 2012. On the use of weather data in ecological studies along altitudinal and latitudinal gradients. — Oikos 121: 3-19.

Greenwood, S. et al. 2015. Temperature and sheltering determine patterns of seedling establishment in an advancing subtropical treeline. — J Veg Sci 26: 711-721.

Grömping, U. 2006. Relative importance for linear regression in R: the package relaimpo. — Journal of statistical software 17: 1-27.

Haider, S. et al. 2011. Elevational distribution limits of non-native species: combining observational and experimental evidence. — Plant Ecology & Diversity 4: 363-371.

Hannah, L. et al. 2014. Fine-grain modeling of species' response to climate change: holdouts, stepping-stones, and microrefugia. — Trends Ecol Evol 29: 390-397.

Hijmans, R. J. et al. 2005. WorldClim, version 1.3.

Klanderud, K. 2010. Species recruitment in alpine plant communities: the role of species interactions and productivity. — J. Ecol. 98: 1128-1133.

Körner, C. 2003. Alpine plant life: functional plant ecology of high mountain ecosystems. — Springer.

Laube, J. et al. 2015. Small differences in seasonal and thermal niches influence elevational limits of native and invasive Balsams. — Biol Conserv 191: 682-691.

Lembrechts, J. et al. 2015. Trade-off between competition and facilitation defines gap colonisation in mountains. — Aob Plants 7: plv128.

Lembrechts, J. J. et al. 2014. Alien roadside species more easily invade alpine than lowland plant communities in a subarctic mountain ecosystem. — Plos One 9(2): e89664.

Lembrechts, J. J. et al. 2016. Disturbance is the key to plant invasions in cold environments. — P Natl Acad Sci USA

Lembrechts, J. J. et al. 2017. Mountain roads shift native and non-native plant species' ranges. — Ecography DOI: 10.1111/ecog.02200.

Lenoir, J. et al. 2013. Local temperatures inferred from plant communities suggest strong spatial buffering of climate warming across Northern Europe. — Global Change Biol 19: 1470-1481.

Lenoir, J. et al. 2017. Climatic microrefugia under anthropogenic climate change: implications for species redistribution. — Ecography 40: 253-266.

Marini, L. et al. 2009. Contrasting response of native and alien plant species richness to environmental energy and human impact along alpine elevation gradients. — Global Ecol Biogeogr 18: 652-661.

McDougall, K. L. et al. 2011. Plant Invasions in Mountains: Global Lessons for Better Management. — Mt Res Dev 31: 380-387.

Milbau, A. et al. 2013. Plant community type and small-scale disturbances, but not altitude, influence the invasibility in subarctic ecosystems. — New Phytol 197: 1002-1011.

Olofsson, J. et al. 1999. On the balance between positive and negative plant interactions in harsh environments. — Oikos 86: 539-543.

Pauchard, A. et al. 2009. Ain't no mountain high enough: plant invasions reaching new elevations. — Front Ecol Environ 7: 479-486.

Pauchard, A. et al. 2016. Non-native and native organisms moving into high elevation and high latitude ecosystems in an era of climate change: new challenges for ecology and conservation. — Biol Invasions 18: 345-353.

Pinheiro, J. et al. 2013. nlme: linear and nonlinear mixed effects models.

Poll, M. et al. 2009. Seedling establishment of Asteraceae forbs along altitudinal gradients: a comparison of transplant experiments in the native and introduced ranges. — Divers Distrib 15: 254-265.

Pollnac, F. et al. 2012. Plant invasion at landscape and local scales along roadways in the mountainous region of the Greater Yellowstone Ecosystem. — Biol Invasions 14: 1753-1763.

Portillo-Estrada, M. et al. 2016. Climatic controls on leaf litter decomposition across European forests and grasslands revealed by reciprocal litter transplantation experiments. — Biogeosciences 13: 1621-1633.

Potter, K. A. et al. 2013. Microclimatic challenges in global change biology. — Global Change Biol 19: 2932-2939.

Pyšek, P. et al. 2011. Colonization of high altitudes by alien plants over the last two centuries. — P Natl Acad Sci USA 108: 439-440.

Quiroz, C. L. et al. 2011. Assessing the importance of disturbance, site conditions, and the biotic barrier for dandelion invasion in an Alpine habitat. — Biol Invasions 13: 2889-2899.

R Core Team 2015. R: a language and environment for statistical computing. R Foundation for Statistical Computing.

Randin, C. F. et al. 2009. Climate change and plant distribution: local models predict high-elevation persistence. — Global Change Biol 15: 1557-1569.

Scherrer, D. and Körner, C. 2010. Infra-red thermometry of alpine landscapes challenges climatic warming projections. — Global Change Biol 16: 2602-2613.

Scherrer, D. and Körner, C. 2011. Topographically controlled thermal-habitat differentiation buffers alpine plant diversity against climate warming. — J Biogeogr 38: 406-416.

Seipel, T. et al. 2012. Processes at multiple scales affect richness and similarity of non-native plant species in mountains around the world. — Global Ecol Biogeogr 21: 236-246.

Stohlgren, T. J. et al. 2006. Scale and plant invasions: a theory of biotic acceptance. — Preslia 78: 405-426.

Trtikova, M. et al. 2010. No adaptation to altitude in the invasive plant Erigeron annuus in the Swiss Alps. — Ecography 33: 556-564.

Weidema, I. R. 2000. Introduced Species in the Nordic Countries. — Nordic Council of Ministers.

Zefferman, E. et al. 2015. Plant communities in harsh sites are less invaded: a summary of observations and proposed explanations. — Aob Plants 7: plv056.

Zuur, A. F. et al. 2013. A beginner's guide to GLM and GLMM with R. A frequentist and Bayesian perspective for ecologists. Highland Statistics Ltd., p 256.
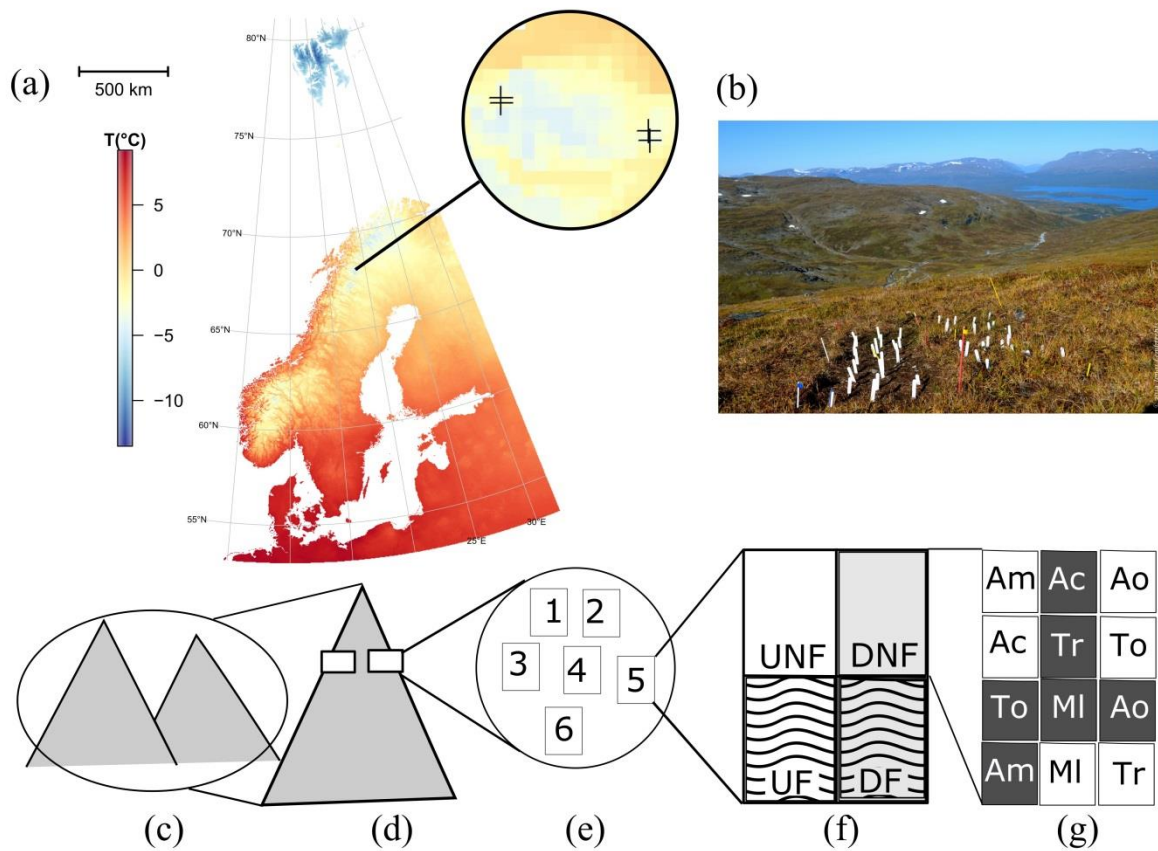
Figure 1. Experimental design. (a) Mean annual temperature (Worldclim, Hijmans et al. 2005) in Scandinavia, in the study region (inset, N 68°21'; E 18°49') and at the experimental sites (crosses on the inset). We chose two sites on each of two mountains (c) at 1000 m a.s.l., one site facing north and the other facing south (d). At each site, we installed six plots (e) with the right half of each plot disturbed (grey, f) and the bottom half with added nutrients (waved lines), resulting in four subplots (UNF – undisturbed-not fertilized, UF – undisturbed-fertilized, DNF – disturbed-not fertilized, DF – disturbed-fertilized). Within each subplot, six species were sown randomly at low (5 seeds, white small squares) and high (30 seeds, darkgrey small squares) propagule pressure, resulting in four times twelve microplots per plot (g). The picture in (b) shows an experimental plot in the alpine tundra. Used species: Achillea millefolium (Am), Agrostis capillaris (Ac), Anthoxanthum odoratum (Ao), Medicago lupulina (Ml), Taraxacum officinale (To) and *Trifolium repens (Tr)*
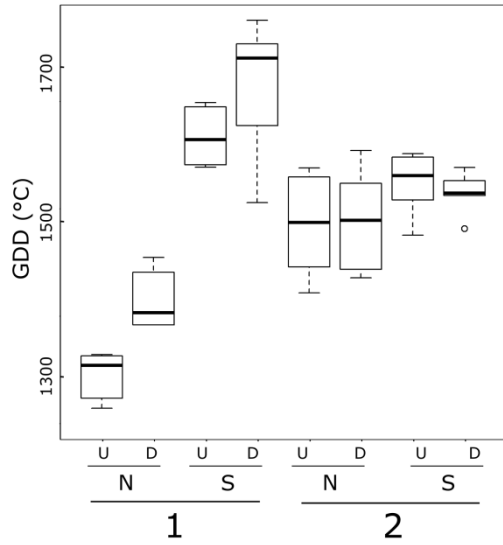
Figure 2. Boxplots of the climatic variation in the experimental plots as measured by cumulative growing degree days (GDD) for the different mountains (1, 2), aspects (N = north, S = south) and disturbance treatments (U = undisturbed, D = disturbed). n = 48, 6 per treatment
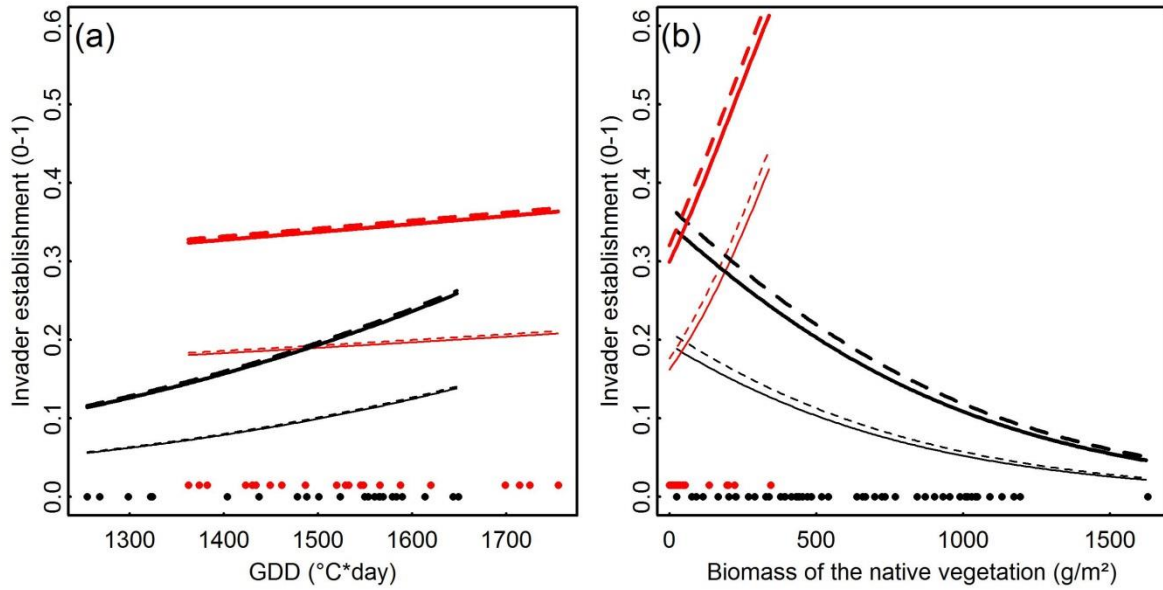
Figure 3. Graphical representation of the average of the best models (ΔAICc < 2) for probability of invader establishment at the end of the second growing season against cumulative growing degree days (GDD) (a) and biomass of the native vegetation (plot productivity or standing biomass) (b). Different lines represent different treatments: disturbed (red) versus undisturbed (black), fertilized (full line) versus unfertilized (dashed line) and high propagule pressure (thick line) versus low propagule pressure (thin line). Dots show observed levels of the respective explanatory variables in the undisturbed (black) and disturbed (red) plot halfs, lines are drawn only over the observed range of the explanatory variables for disturbed and undisturbed plots, respectively. Support for the model against GDD was lower than that for the model against biomass of the native vegetation (ΔAICc = -14.25). n = 1152
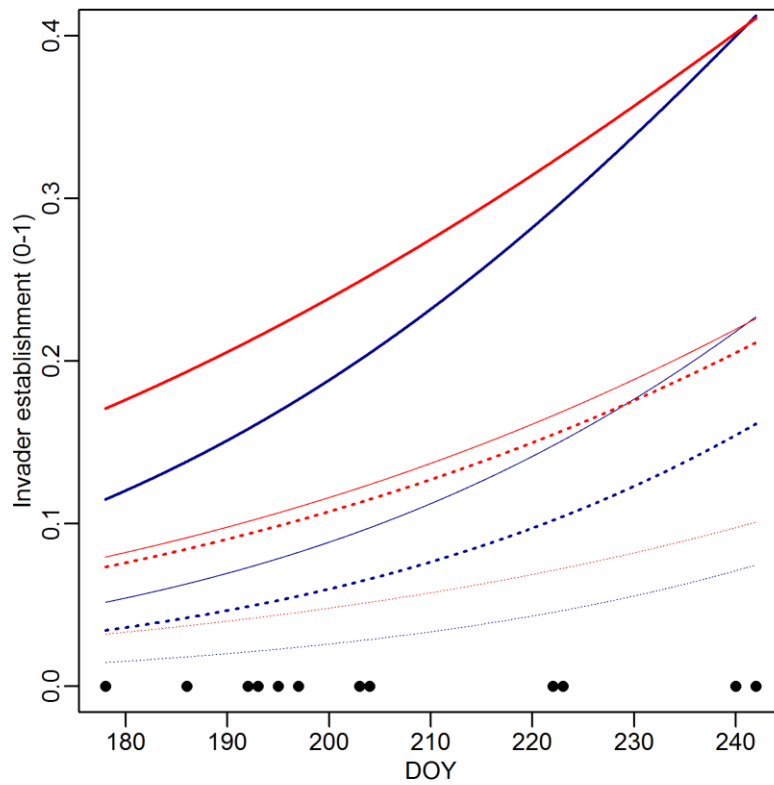
Figure 4. Graphical representation of the best model for probability of invader establishment throughout the second growing season. The x-axis shows the day of the year (DOY) since January 1st 2015 (180 = June 29th 2015). Different lines represent different treatments: north-facing (blue) versus south-facing slopes (red), disturbed (full line) versus undisturbed (dotted line) and high propagule pressure (thick line) versus low propagule pressure (thin line). Effect of nutrient addition was only borderline significant and is not shown. Black dots indicate observation days. n = 6912
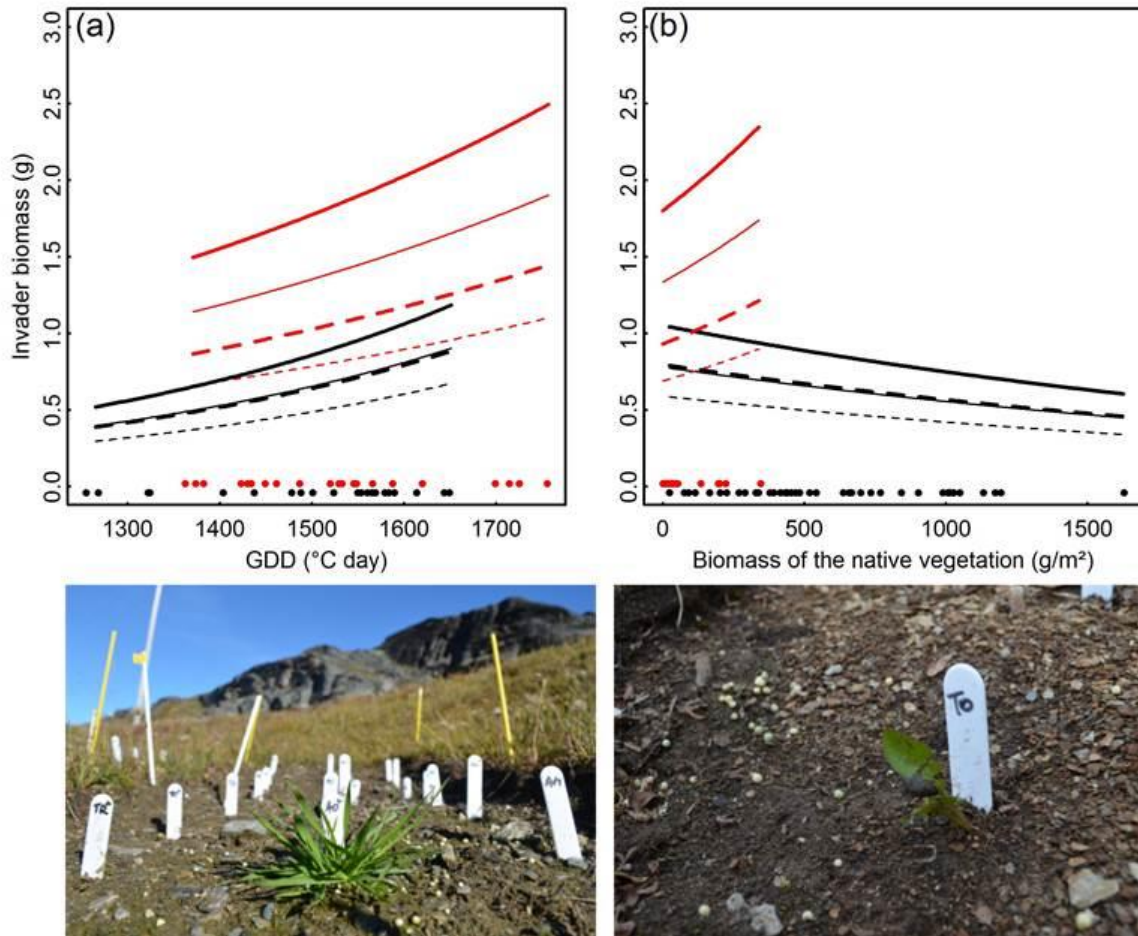
Figure 5. Graphical representation of the average of the best models (△AICc < 2) for invader biomass production per microplot against cumulative growing degree days (GDD) (a) and biomass of the native vegetation (plot productivity or standing biomass) (b). Different lines represent different treatments: disturbed (red) versus undisturbed (black), fertilized (full line) versus unfertilized (dashed line) and high propagule pressure (thick line) versus low propagule pressure (thin line). Dots show observed levels of the respective explanatory variables in the undisturbed (black) and disturbed (red) plot halves, lines drawn only over the observed range of the explanatory variable for disturbed and undisturbed plots, respectively. Raw data not shown for clarity. Pictures (bottom row) show examples of disturbed microplots with a high biomass of A. odoratum (left) and a low biomass of T. officinale (right). Support for the model against GDD was higher than that for the model against biomass of the native vegetation (△AICc = 5.87). n = 287

Table

Table 1. Estimates for, P-values of and percentage of the total variance explained by all factors and significant two-way interactions for the model with the best fit for cumulative growing degree days (GDD). Estimates and P-values from a linear model, percentage of explained variance obtained with calc.relimp(type=lmg) from the R package "relaimpo". Explanatory variables: mountain (1 or 2), aspect (north or south) and disturbance (no or yes). Proportion of variance explained by the model: 83.69 %. n = 48

	Estimate	*P*	*% of variance*
(Intercept)	*1386.59*	*<0.0001*	*-*
Mountain$_2$	*106.17*	*<0.0001*	*1.05*
Aspect$_{South}$	*292.80*	*<0.0001*	*49.90*
Disturbance$_{Yes}$	*81.11*	*<0.0001*	*2.64*
Aspect$_{South}$:mountain$_2$	*-248.10*	*<0.0001*	*26.97*
Disturbance$_{Yes}$:mountain$_2$	*84.65*	*0.0069*	*3.14*

Table 2. Estimates for all experimental factors and interactions for all models for invader establishment (Esta, top, n = 1152) and invader biomass (Biom, bottom, n = 287) along the gradient of cumulative growing degree days (GDD, see Fig. 3a and 5a) and biomass of the native vegetation (plot productivity or standing biomass, NatBiom, see Fig. 3a and 5a). Coefficients defined by averaging all models with ΔAICc < 2 with the best model containing either GDD or biomass of the native vegetation. Shown AICc is that of the best model. D = disturbance, N = nutrients, P = propagule pressure. AICc of the null models for invader establishment and biomass production were 1212.1 and 814.0 respectively. For the full set of models, see Appendix Table S2.

Model	(Int)	D	N	P	D:N	GDD	GDD:D	Biom	Biom:D	AICc
Esta~GDD	-2.165	0.736	-0.019	0.777	-	0.302	-0.249	-	-	1165.9
Esta~NatBiom	-1.819	1.573	-0.010	0.792	-	-	-	-0.580	2.101	1151.7
Biom~GDD	-0.671	0.456	0.292	0.271	0.253	0.244	-0.092	-	-	761.8
Biom~NatBiom	-0.604	0.409	0.278	0.300	0.241	-	-	-0.106	0.126	767.6
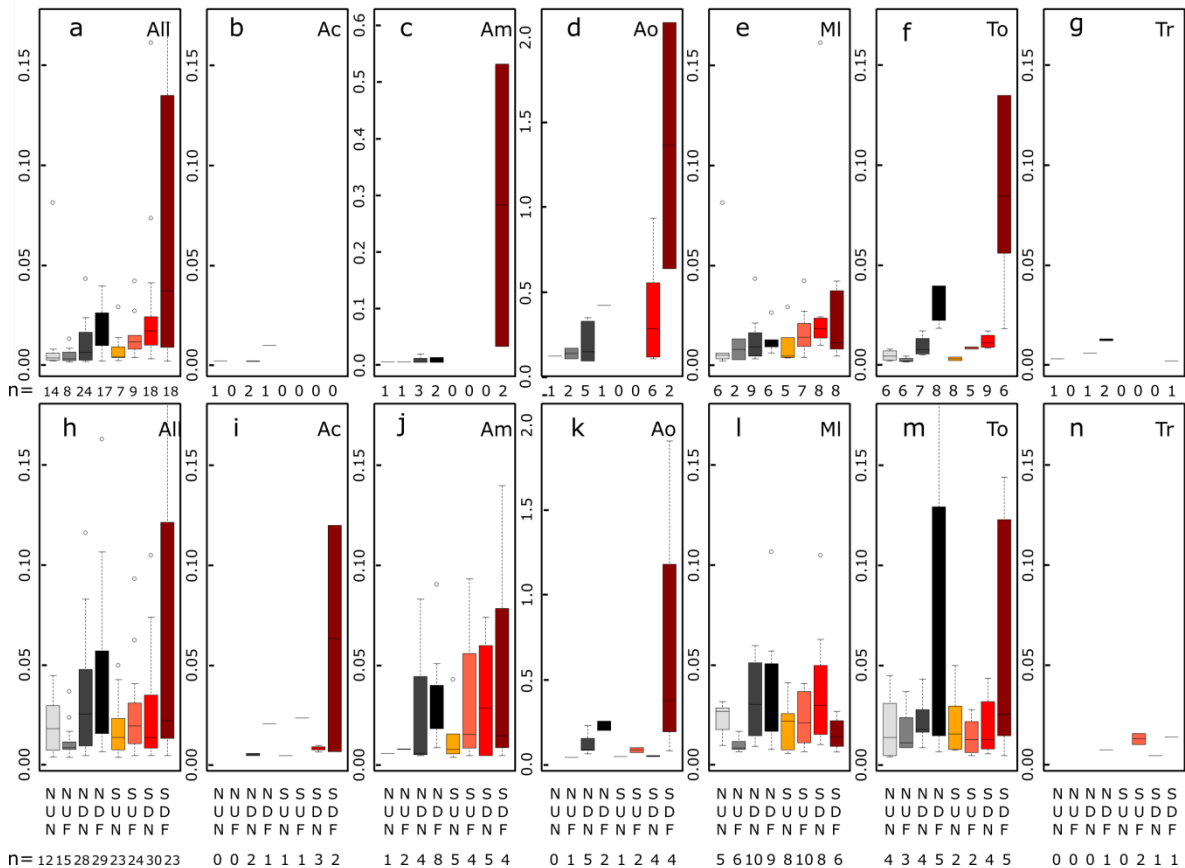
Figure S1. Boxplots of the original biomass data for all species combined (a and h) and every species separately (b-g and i-n) for every treatment: north (N, grey to black) versus south (S, yellow to red); undisturbed(U, two lightest shades) versus disturbed (D, two darkest shades); not fertilised (N, shade 1 and 3) versus fertilised (F, shade 2 and 4); low propagule pressure (top row) versus high propagule pressure (bottom row). The six studied species are: Agrostis capillaris (Ac), Alchemilla millefolia (Am), Anthoxanthum odoratum (Ao), Medicago lupulina (Ml), Taraxacum officinale (To) and Trifolium repens (Tr). Number of subplots with established individuals at the end of the second growing season (n, out of 12 subplots per species per treatment and 72 per treatment in total) underneath each boxplot. Total n=288.

Table S1. P-values from a TukeyHSD post-hoc test on an ANOVA for the differences in cumulative growing degree days (GDD) between the different mountains (1 and 2), aspects (north (N) and south (S)) and disturbance treatments (disturbed (D) and undisturbed (U)), as shown in Fig. 2. n = 48

	1-N-U	1-S-D	1-S-U	2-N-D	2-N-U	2-S-D	2-S-U
1-N-D	0.0560	<0.0001	<0.0001	0.0276	0.0458	0.0010	0.0003
1-N-U	-	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
1-S-D	-	-	0.3655	<0.0001	<0.0001	0.0010	0.0035
1-S-U	-	-	-	0.0198	0.0115	0.2683	0.5159
2-N-D	-	-	-	-	1.0000	0.9388	0.7510
2-N-U	-	-	-	-	-	0.8695	0.6269
2-S-D	-	-	-	-	-	-	0.9998

Table S2. Estimates for all experimental factors and interactions, as well as degrees of freedom (df), AICc, ΔAICc and model weight for all models for invader establishment (top, n = 1152) and invader biomass (bottom, n = 287) along the gradient of cumulative growing degree days (GDD, see Fig. 3 and 5A) and biomass of the native vegetation (plot productivity or standing biomass, Biom, see Fig. 3 and 5B). D = disturbance, N = nutrients, P = propagule pressure

(Int)	GDD	D	N	P	GDD:D	D:N	Biom	Biom:D	Df	AICc	ΔAICc	weight
-1.870	-	1.503	-	0.791	-	-	-0.574	2.003	9	1151.7	0.00	0.445
-1.760	-	1.654	-0.214	0.793	-	-	-0.587	2.216	10	1152.0	0.30	0.384
-1.745	-	1.606	-0.238	0.800	-	0.041	-0.590	2.194	11	1154.1	2.38	0.135
-1.865	-	0.013	-	0.783	-	-	-0.543	-	8	1157.8	6.11	0.021
-1.808	-	0.009	-0.106	0.783	-	-	-0.547	-	9	1159.4	7.69	0.010
-1.752	-	-0.010	-0.209	0.785	-	0.184	-0.558	-	10	1161.1	9.40	0.004
-2.171	0.340	0.739	-	0.777	-0.323	-	-	-	9	1165.9	14.25	0.000
-2.184	0.175	0.721	-	0.774	-	-	-	-	8	1167.6	15.96	0.000
-2.133	0.342	0.747	-0.085	0.780	-0.326	-	-	-	10	1167.7	15.98	0.000
-2.172	-	0.783	-0.079	0.772	-	-	-	-	8	1169.4	17.68	0.000
-2.152	0.174	0.725	-0.069	0.776	-	-	-	-	9	1169.4	17.77	0.000
-2.126	0.340	0.737	-0.092	0.777	-0.326	0.010	-	-	11	1169.7	18.02	0.000
-2.165	-	0.770	-0.093	0.772	-	0.025	-	-	9	1171.4	19.70	0.000
-1.419	-	-	-	-	-	-	-0.531	-	6	1178.5	26.81	0.000
-1.369	0.303	-	-	-	-	-	-	-	6	1207.2	55.56	0.000
-1.355	-	-	-	-	-	-	-	-	5	1212.1	60.38	0.000
-0.625	0.296	0.394	0.212	0.268	-0.169	0.377	-	-	12	761.8	0.00	0.310

-0.639	*0.187*	*0.384*	*0.211*	*0.278*	*-*	*0.397*	*-*	*-*	*11*	*761.9*	*0.09*	*0.295*
-0.735	*0.298*	*0.582*	*0.440*	*0.263*	*-0.183*	*-*	*-*	*-*	*11*	*762.9*	*1.11*	*0.178*
-0.757	*0.181*	*0.583*	*0.452*	*0.274*	*-*	*-*	*-*	*-*	*10*	*763.3*	*1.57*	*0.141*
-0.677	*-*	*0.443*	*0.212*	*0.306*	*-*	*0.360*	*-*	*-*	*10*	*767.0*	*5.28*	*0.022*
-0.558	*-*	*0.252*	*0.195*	*0.303*	*-*	*0.391*	*-0.110*	*-*	*11*	*767.6*	*5.87*	*0.016*
-0.782	*-*	*0.621*	*0.432*	*0.302*	*-*	*-*	*-*	*-*	*9*	*767.8*	*6.08*	*0.015*
-0.690	*-*	*0.475*	*0.433*	*0.298*	*-*	*-*	*-0.092*	*-*	*10*	*768.9*	*7.15*	*0.009*
-0.554	*-*	*0.445*	*0.191*	*0.299*	*-*	*0.363*	*-0.118*	*0.313*	*12*	*769.0*	*7.23*	*0.008*
-0.674	*-*	*0.706*	*0.406*	*0.294*	*-*	*-*	*-0.102*	*0.405*	*11*	*769.7*	*7.96*	*0.006*
0.005	*-*	*-*	*-*	*-*	*-*	*-*	*-0.270*	*-*	*7*	*793.9*	*32.16*	*0.000*
-0.004	*0.217*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*7*	*806.9*	*45.17*	*0.000*
0.004	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*-*	*6*	*814.0*	*52.22*	*0.000*

1 *Table S3. Estimates for all experimental factors and interactions for the average of the best species-*
2 *specific models (ΔAICc < 2) for invader establishment (top) and invader biomass (bottom) along the*
3 *gradient of cumulative growing degree days (GDD) and biomass of the native vegetation (plot*
4 *productivity or standing biomass, NatBiomass). Variables not included in the best model were displayed*
5 *with the "-" symbol. D = disturbance, N = nutrients, P = propagule pressure. The six studied species are:*
6 *Agrostis capillaris (Ac), Alchemilla millefolia (Am), Anthoxanthum odoratum (Ao), Medicago lupulina (Ml),*
7 *Taraxacum officinale (To) and Trifolium repens (Tr).*

Species	(Int)	GDD	D	N	P	GDD:D	D:N	Biom	Biom:D	n
Ml	0.717	-	3.703	-0.154	0.347	-	-	-1.182	6.086	187
To	-0.970	-	3.014	-0.107	1.304	-	-	-0.292	3.948	188
Am	-2.729	0.195	0.493	0.088	1.602	-0.326	0.4367	-0.027	-	188
Ao	-1.908	-	-	-	-	-	-	-1.170	-	188
Ac	-4.801	-0.044	1.884	-4.959	1.418	-	4.564	-0.011	-	188
Tr	-2.850	-	-	-	-	-	-	-0.324	-	188
Ml	-0.538	0.273	0.559	0.021	0.398	-	-	-	-	110
To	-0.429	0.219	0.538	0.090	0.175	-	0.856	-0.291	1.250	81
Am	0.001	-	-	-	-	-	-	-0.327	-	39
Ao	-1.407	-	0.907	0.780	0.475	-	0.462	-	-	34
Ac	0.006	-	-	-	-	-	-	-	-	14
Tr	0.000	-0.608	-	-	-	-	-	-	-	10

8

9