Title: Foliar phosphorus content predicts species relative abundance in P-limited Tibetan alpine meadows

Authors: Kechang Niu^{1, 2, 3 *}, Jin-Sheng He² & Martin J. Lechowicz³

1. Department of Biology, Nanjing University, Nanjing, 210093, China

2. Department of Ecology, Peking University, Beijing, 100871, China.

3. Department of Biology, McGill University, Montréal, Québec, H3A 1B1, Canada

*Corresponding author. E-mail: kechangniu@nju.edu.cn

Running head: *Functional traits associated with greater species abundance*

Abstract

Traits that favor rapid plant growth are expected to have an important influence on species relative abundance (SRA) in semi-natural grasslands where competitive exclusion is reduced by grazing. However, which traits are more strongly associated with variation in SRA remains largely unknown, especially in P-limited but species-rich grasslands. We assessed the relative importance of traits predicting SRA in P-limited Tibetan alpine meadows subject to long term grazing. We assessed abundance in terms of both aboveground biomass and ramet numbers in relation to seven traits (specific leaf area, LDMC: leaf dry matter content, leaf carbon concentration, leaf nitrogen concentration, LPC: leaf phosphorus concentration, mature height, and seed size) for all component species in grazed and ungrazed plots in each of three contrasting sites on the Tibetan Plateau. We used CATS regression (*i.e.* regression for Community Assembly through Trait Selection) to quantify the relative importance of different traits in predicting SRA, and tested dependence of trait importance values on both environmental context and alternative measures of abundance.

Species were primarily differentiated along a trade-off axis involving traits promoting nutrient acquisition for fast growth vs resource conservation and competitive ability. A higher LPC consistently was the most important trait predicting species abundance in grazed plots, while species with low LPC but higher LDMC held an advantage in ungrazed plots. This suggests that in competition-released communities, species with a high ability to uptake soil available P tend to become abundant, while in competition-dominated communities, species using fixed P efficiently do better. The relative importance of traits in ungrazed plots did not change across sites, but in grazed plots species that have traits associated with nutrient conservation were favored in colder and drier conditions. The results highlight the importance of traits affecting acquisition of soil available P in these Tibetan alpine meadow communities. Species tradeoffs in nutrient acquisition versus conservation are a primary determinant of SRA, but the relative importance of traits depended on both environmental context and whether SRA was estimated as above-ground biomass or as number of ramets.

Key words: community assembly; competition; functional trait; species relative abundance; trade-offs;

1. Introduction

Plant community ecologists have a reasonably comprehensive and well-established conceptual framework in which to address the mechanistic basis for the assembly of plant communities (Weiher et al. 2011, Kraft et al. 2015a). The idea of limiting similarity that traces back to Darwin (1859) combines well with a multivariate view of the niche (Hutchinson 1957) to allow evaluation of the likelihood that species similarity limits

co-occurrence (Holt 2009, Leimar et al. 2013). That likelihood, which lies at the heart of the concept of species coexistence (Kraft et al. 2015b), is considered to be affected by traits that are essential if a species is to successfully establish in given environmental regime. Contemporary theory to explain the coexistence of species focuses on the influence of stabilizing niche differences and relative fitness differences (Chesson 2000, Hille Ris Lambers et al. 2012) that can be tied to variation in functional traits (Kraft et al. 2015b). In turn, the origin of interspecific trait variation lies in adaptive evolution, raising the question of the degree to which phylogenetic relatedness constrains the likelihood that two species occur together (Cadotte et al. 2013, Godoy et al. 2014). While this conceptual framework governing coexistence and community assembly can help us make *post hoc* sense of a great deal of the voluminous observational records for plant distribution and abundance, two seemingly intractable problems make it difficult to operationalize current theory in a way that yields robust predictions of community assembly and species relative abundance (SRA) in a particular environmental context (Lawton 1999, Cadotte et al. 2015).

The difficulties arise in uncertainties at the interface between the processes of environmental filtering and dispersal. Dispersal, the necessary prerequisite to establishment in a community, is an intrinsically stochastic, distance-weighted process characterized by probability distributions in which slight variations in infrequent events weigh heavily in determining successful outcomes (Clobert et al. 2012). Since the shape of these dispersal functions varies among species, the probability distribution for arrival of propagules at a site is a complex function of the geospatial distribution of species populations around the site (Ricklefs 2015). By comparison environmental filtering can be fairly straightforward at

spatial scales from biomes to habitat, but at the scale of a local community, where interactions among neighboring plants are in play, stochasticity driven by fine-scale spatiotemporal heterogeneity predominates (Morin and Lechowicz 2008, Kraft et al. 2015a). Hence it is difficult to discern what trait or combination of traits determines the success or failure of a propagule in the face of both biotic and abiotic environmental factors in a locality, a challenge made all the more difficult by interactions between the trait dependencies and the spatiotemporal heterogeneity. Given the compounded uncertainty that a propagule will both disperse to a site and have the requisite traits to successfully establish, one might well despair of there ever being a predictive theory for community assembly (Lawton 1999).

In this paper we explore the utility of an approach called “community assembly through trait selection” (CATS: Shipley 2010, Shipley et al. 2012), which aims to identify traits that best predict species relative abundance. The CATS approach has proven effective in identifying traits that affect changes in community structure in an experimental study of rangeland management strategies on a decadal time scale in New Zealand (Laliberte et al. 2012). The experiment reported in Laliberte et al. (2012) involved sowing 25 grass and legume species into a degraded tussock grassland, which was then broken into experimental plots subjected to treatments involving various combinations of grazing and, for the first 19 years, annual fertilization with sulphur-superphosphate. A CATS regression of species relative abundance (i.e. % cover) on 12 functional traits in this experimental system showed that after 27 years species with traits promoting rapid growth were favored in high fertility treatments, and those with traits promoting plant nutrient conservation were favored under nutrient limiting treatments (Laliberte et al. 2012). Considering the dynamic interplay

between competition for light versus soil resources under grazing that is typical in grasslands (Suding et al. 2005, Hautier et al. 2009, Borer et al. 2014, Niu et al. 2015a), these outcomes after 27 years will have arisen in a potentially complex series of changes over time that may have favored different traits during successional change in each treatment regime. In the present study, to extend this promising line of inquiry, we evaluate the utility of the CATS approach with an emphasis on identifying traits affecting short-term changes in species relative abundance under release from grazing.

To do so, we conducted a short-term (i.e. 3 to 5 years) grazing exclusion experiment in three contrasting Tibetan alpine meadows (Niu et al. 2015a) that have been subjected to grazing by wild and domesticated animals for millennia (Miehe et al. 2009). Previous studies show that plant competition for limited soil available P controls plant diversity (Hong et al. 2014, Yang et al. 2014a, Niu et al. 2015a) and niche differentiation among co-occurring species in these Tibetan meadows (Niu et al. 2015b). Using trait values determined at both grazed and ungrazed plots to better allow for the effects of trait plasticity (Albert et al. 2011, Violle et al. 2012) in response to grazing, we considered seven functional traits (SLA: specific leaf area, LDMC: leaf dry matter content, LCC: leaf carbon concentration, LNC: leaf nitrogen concentration, LPC: leaf phosphorus concentration, mHeight: mature height, and seed size) that might influence species relative abundance in these alpine meadows. To better differentiate the effects of the establishment and growth of individual plants versus the effects of vegetative spread of individuals, we assessed species relative abundance on the basis of both harvested above-ground biomass and number of ramets. To further strengthen our ability to detect the influence of traits that affect species relative abundance we used a recently

developed CATS regression model (Warton *et al.* 2015), which generalized the CATS approach by relaxing the constraints to facilitate analyses over multiple sites along environmental gradients. In summary, we assessed the influence of traits on species relative abundance in grazed as well as ungrazed plots in each of three contrasting Tibetan alpine meadows using a generalized CATS approach.

In grazing exclusion plots where competition for limited nutrients drives community assembly, succession and fitness difference drive SRA, and lack of competition promotes species abundance (Hille Ris Lambers *et al.* 2012, Niu *et al.* 2015a), we expected species with higher leaf dry matter content (Wright *et al.* 2004, Laughlin 2014) and lower foliar phosphorus (Hidaka and Kitayama 2013, Maire *et al.* 2015) should be favored. In grazed plots where competition is reduced by removal of aboveground biomass (Tilman 1988, Collins and Glenn 1990, Gibson 2009), niche differences drive SRA, and fast (re)growth underpins species abundance (Hille Ris Lambers *et al.* 2012, Niu *et al.* 2015b), we expected species with high foliar nutrient content and high specific leaf area (Rivas-Ubach *et al.* 2012, Hidaka and Kitayama 2013, Maire *et al.* 2015) would be favored. Because species that spread by rhizomes or stolons can quickly command open space under grazing, we also anticipated that SRA would be better related to traits if measured in terms of aboveground biomass in ungrazed plots and in terms of ramet numbers in grazed plots. Finally, we anticipated that the relative importance of traits in determining species fitness and SRA would depend on environmental context (Shipley *et al.* 2016), hence varying across our three study sites. To test all these expectations we combined generalized CATS regression (Warton *et al.* 2015) with an automated model selection procedure (Calcagno and de Mazancourt 2010) to

quantify the relative importance of different traits in predicting SRA across the environmental regimes represented by our three study sites.

2. Material and methods

2.1 Study sites

We carried out this field experiment in alpine meadows distributed from east to north on the Tibetan Plateau: HY site in Hongyuan County of Sichuan province (see Zhao et al. 2013), AZ sites (see Zhang et al. 2014) and QH site in Mengyuan County of Qinghai province (see Wang et al. 2012), China. The sites ranged in elevation from 3178 m at the QH site ($37^{\circ} 37' N$, $101^{\circ} 12' E$) to 3484 m at the HY site ($32^{\circ} 53' N$, $102^{\circ} 36' E$) and 3508 m at the AZ site ($33^{\circ} 39' N$, $101^{\circ} 52' E$). Climate in all three sites is cold, continental and characterized by a short and cool spring, summer and autumn and a long winter. The soils have a high organic content and low available N and P (cf. Niu et al. 2015a). The three sites fall along gradients of moisture, temperature and productivity: AZ is a wet alpine meadow with relatively high daytime temperatures and growing season precipitation, deep soil, and high productivity; HY is a typical alpine meadow with intermediate productivity and QH is a dry, cold alpine meadow with a short growing season, shallow soil and low productivity (see Niu et al. 2015a).

There is a long history of grazing by both wild and domesticated species in the Tibetan plateau, but grazing by domesticated yaks and Tibetan sheep has increased markedly at all sites in the last three decades (Miehe et al 2009). Grazed meadows are dominated by *Carex rigescens* at the AZ site, by *Kobresia capillifolia* at the HY site and by *Stipa aliena* at the QH site; subdominants, which also vary from site to site, include sedges such as *C. atrofusca*,

grasses such as *Elymus nutans* and *Deschampsia cespitosa*, and forbs such as *Potentilla anserina*, *P. saundersiana* and *Gueldenstaedtia diversifolia*. Nitrogen-fixing legumes are only a minor component of these alpine meadows. Complete species abundance and trait data for both grazed and ungrazed meadows at the three study sites are in the supplemental material. Total vegetation cover in midsummer exceeds 80%; vegetation height in ungrazed meadows is 70~100 cm at the AZ site, 50-80 cm at the HY site and 40-60 at the QH site (see. Niu *et al.* 2015b).

2.2 Experimental design

In a 20 to 30 ha area of reasonably uniform alpine meadow at each site, we built exclosures (1 to 5 ha) in 2005-2006 that were designed to exclude grazing by both small and large mammals. At the time of this study the alpine meadow within the exclosure had been protected from grazing for 3-5 years. Grazing was excluded throughout the year at HY and QH but occasionally allowed within the exclosures during the non-productive winter months at AZ. Outside of the exclosure, the meadows were moderately grazed by yaks and sheep during all months except for 40-60 days between June and mid-August when herds were moved to high-elevation pasture (Niu *et al.* 2010, Niu *et al.* 2012).

In June 2009-2010, at each site eight 5×8 m plots were randomly established within the exclosures and another eight in the meadow surrounding the exclosures. The grazed plots outside the exclosures were separated by 5-15 m, while ungrazed plots within the exclosures were separated by 2-5 m. This should ensure that spatial autocorrelation effects (e. g., any issue of pseudo-replication) among the sampled communities (0.25m^2) will be negligible considering the low β diversity and the fact that the species-area curve in these meadows

179 saturates at 0.15-0.22 m² (Niu et al. 2009, Niu et al. 2010, Niu et al. 2015b). There were
180 300~400 meters between grazed and ungrazed plots. Each replicate plot was divided into two
181 parts: a 5 × 5 m subplot for measurement of plant traits and a 5 × 3 m subplot for community
182 monitoring, counting of individual ramets, biomass harvest and soil sampling.

183 2.3 Trait and abundance measurements

184 In July 2009 and 2010, we randomly sampled a mature leaf from 7-10 individuals at fruiting
185 time for all but a few very infrequent species in the 5×5 m subplots in grazed and ungrazed
186 plots at each site. We measured specific leaf area (SLA, mm²·mg⁻¹), leaf dry matter content
187 (LDMC, mg·g⁻¹), leaf carbon concentration (LCC, mg·g⁻¹), leaf nitrogen concentration (LNC,
188 mg·g⁻¹), and leaf phosphorus concentration (LPC, mg·g⁻¹) using these leaves and measured
189 mature height (mHeight, cm) and seed size (mg) on the same individuals (Niu et al. 2015a).
190 The sampled species accounted for 90-98% of the aboveground biomass and 95-99 % of
191 vegetation cover in the meadow community. All measurements of functional traits followed
192 standardized methods (He et al. 2010, Pérez-Harguindeguy et al. 2013).

193 From mid-August to early September 2009 and 2010, we harvested plants in the 0.5 ×
194 0.5 m quadrat within each of the grazed and ungrazed 5 × 3 m subplots at each site.
195 Individual ramets were counted and harvested by species, and the oven-dried biomass of each
196 species determined (Niu et al. 2010). These data were used to calculate species relative
197 abundance (SRA) as described subsequently.

198 2.5 Data analysis

199 2.5.1 SRA and functional traits

200 In each 0.5 x 0.5 m² quadrat in grazed and ungrazed plots, we calculated the relative

abundance of each species as the percentage of 1) the total aboveground biomass and 2) the ramet numbers represented by each species in the quadrat. This provides eight replicate estimates for two complementary measures of SRA in grazed and ungrazed plots in the meadow community at each site (Niu et al. 2010). We calculated the means of SLA, LDMC and mHeight (7-10 replicates) as well as LCC, LNC, LPC and seed size (3 replicates) for each species in grazed and ungrazed plots in each of three sites.

We used principal component analysis (PCA) to visualize the interrelationships among the seven plant functional traits for the component species in grazed and ungrazed plots at the three sites. The angles between trait vectors in the PCA biplot (using type-II scaling) reflect correlations among traits, with a small angle indicating a high correlation. Statistical significance of the correlations among traits was tested by Pearson correlation and differences between treatments (grazed vs. ungrazed nested in sites) were tested by ANOVA. These analyses as well as those described subsequently were conducted in the R environment (R Development Core Team 2014)

2.5.2 CATS regression

Since there is no theory to suggest *a priori* a particular model predicting SRA from functional traits under alternative grazing regimes, we used CATS regression to explore a number of alternative models for predicting SRA as a function of our species-specific data on foliar traits, plant stature and seed size for grazed plots and ungrazed plots separately. These CATS analyses use the mean of the eight quadrats in each site-treatment combination. As is the case for λ -values in the CATS model (Shipley 2010), β -values in CATS regression can be directly compared to estimate the relative importance of different traits in determining SRA

(Warton et al. 2015). A positive β -value for a given trait indicates that when other traits are held constant, species with greater values for this trait are relatively more abundant in the local community than could be expected by chance, while negative β -values indicate the opposite. A β -value of zero suggests that a trait does not influence SRA (Shipley 2010, Warton et al. 2015).

In a first round of analyses, we regressed all seven traits on SRA estimated either as aboveground biomass or ramet numbers to estimate relative importance of each trait in predicting SRA over our three sites (Warton et al. 2015). We fitted this model using Poisson regression for both biomass and ramet estimates of SRA; we also tried a negative binomial regression for SRA based on ramet counts because count data are often over-dispersed compared to the Poisson distribution (O'Hara and Kotze 2010). Since we did not know SRA for the regional species pool, we used a uniform prior in the CATS regression; this may influence the accuracy of estimates for trait importance in determining SRA but will not significantly impact comparison of the relative importance of different traits (Shipley 2010). We evaluated the significance of model and regression coefficients (β) in the CATS regression using a Wald-test in resampling-based inference ($n=999$; Warton *et al.* 2015). The interaction between site and trait variables was tested by an ANOVA on the matrix of bootstrap values ($n=999$) in the CATS regression (Warton et al. 2015).

We then explored the statistical structure of the relationships among the seven traits using the 'glmulti' package (Calcagno and de Mazancourt 2010) to identify a minimal combination of the traits that could provide robust predictions of SRA. Although the seven traits we studied are well established components of plant function (Wright *et al.* 2004, Díaz

et al. 2016), their relative importance in affecting SRA will depend on both environmental context and life history variation in the species that comprise the local community (Laughlin and Messier 2015, Shipley *et al.* 2016). Since there will be some degree of functional coordination and hence statistical collinearity among the traits (Wright *et al.* 2004, Díaz *et al.* 2016) the best model for predicting SRA may not be immediately apparent (Shipley 2010, Laliberte *et al.* 2012). Hence we considered generalized linear model (GLM) regressions using both Poisson and negative binomial approaches to predict SRA, and decided among the many alternative models based on comparison of the Akaike Information Criteria (AIC); the model with the minimum AIC was selected. Using trait(s) in the best GLM model, we re-analysed the trait-SRA relation with the multi-site analysis of CATS regression mentioned above, and re-estimated β -values as well as tested the significance of the model and β -values.

Finally, since there were few significant interactions between sites and trait variables in the multi-site analysis, we performed single-site analyses using CATS regression to quantify β -values for each of seven traits at each of the three study sites using both Poisson and negative binomial regression. We report the results from Poisson regression and present the results from negative binomial regression in Supplemental Materials Figure S1; the β -values were consistent in both cases, although significance levels differed slightly due to inevitable variation inherent in the pit.trap resampling for the Wald-test.

3. Results:

3.1 Relationship among traits

Not surprisingly, plant height declined significantly following grazing and also was lower at the cold, dry QH site ($P < 0.05$, Fig. 1), but due to the large variance component associated with species ($P > 0.05$, Fig. 1) neither the grazing nor site factors significantly influenced any other traits or the main axis of the PCA. Although trait correlations often are weak among species in local communities, there are significant negative relationships between traits associated with nutrient conservation and traits involved in nutrient acquisition. For example, LDMC is negatively correlated with SLA ($r = 0.41$, $P < 0.05$, Fig. 1) and with LPC ($r = 0.25$, $P < 0.05$, Fig. 1), a contrast that organizes the first axis of the PCA, which accounts for 23.1 % of the variance in these data (Fig. 1). Similarly, the second axis of the PCA, accounting for 19.3 % of the variance, is organized by the negative correlation ($r = 0.16$, $P < 0.05$) between seed size and LNC (Fig. 1). It is noteworthy that the positive correlation between SLA and LPC ($r = 0.24$, $P < 0.05$, Fig. 1) is stronger than that of SLA and LNC ($r = 0.13$, $P < 0.05$). Seed size is positively correlated with mature height ($r = 0.25$, $P < 0.05$, Fig. 1).

3.2 Relative importance of traits in grazed plots.

Although the prediction of SRA from traits in grazed plots is relatively weak (pseudo R^2 ranges from 0.32 to 0.57), in all models a higher LPC is the trait most strongly associated with species abundance (Fig. 2a-b and Table 1). Indeed, in comparisons among alternative models, the two best models used only LPC or LPC and mature height to predict SRA (Table 1). Species with low SLA and larger seed size also tended to be more abundant in grazed plots, more significantly when SRA was measured as aboveground biomass (Table 1, Fig.

2a).

In all the variants of multi-site CATS regression for grazed plots, no models found a significant site effect. There were some significant interactions between site and traits (Table S1), suggesting that the relative importance of traits can change from site to site (Fig. 2). Overall, however, the relative importance of traits in grazed plots differs only slightly across sites (Table S1). Species with low SLA and larger seed tend to be abundant at the QH site relative to the wetter AZ site (Fig. 2a-b and Table S1). When SRA is measured as ramet numbers, more abundant species not only have a higher LPC but also are higher in LDMC at the harsh QH site relative to the AZ and HY sites (Fig. 2b and Table S1).

3.3 Relative importance of traits in ungrazed plots.

Although the prediction of SRA from traits is also relatively weak (pseudo $R^2=0.35-0.52$) in ungrazed plots, alternative models in the CATS regressions show a consistent pattern of relative importance for the different traits (Table 1 and Figure 2c-d). In contrast to grazed plots, SRA in ungrazed plots is positively associated with LDMC but negatively correlated with leaf nutrients, most notably LPC (Table 1 and Fig. 2c-d). These patterns hold whether the CATS regression is based on all seven traits or any subset of traits selected in comparisons among alternative models (Table 1). Neither regression approach (Poisson or negative binomial) nor alternative measures of SRA (aboveground biomass vs. ramet numbers) significantly changed the relative values of regression coefficients (Table 1 and Fig. 2c-d). Low LPC and high LDMC, respectively, were the first and second most important traits associated with species abundance in ungrazed plots; when abundance was measured with biomass, low SLA also was significantly, but more weakly, associated with species

abundance (Table 1 and Fig. 2c-d). There was no significant trait x site interaction (Table S1), indicating no significant change in the relative importance of traits in ungrazed plots among sites (Fig. 2), but larger SLA and seed size tended to be more important for species abundance at the HY site (Table S1, Fig. 2c-d).

4. Discussion

4.1 Foliar phosphorus content indicates species position along a gradient from exploitive nutrient-acquisition to nutrient-use efficiency in Tibetan alpine meadows

Studies at broad spatial scales suggest there is a tradeoff between nutrient acquisition vs. conservation that is reflected in foliar traits (Wright et al. 2004, He et al. 2009, Reich 2014). Some species have foliar characteristics associated with high rates of resource uptake (*e.g.* larger SLA and leaf nutrient concentrations) in contrast to traits involved in nutrient-use efficiency (*e.g.* larger LDMC and long lifespan). Specifically, in light or nitrogen limited ecosystems, higher SLA and LNC, which are associated with higher uptake of the limiting resource and greater growth rate, are strongly negatively correlated with LDMC at the species level (Wright et al. 2004, Raouda et al. 2005, Ordo et al. 2009, Hodgson et al. 2011, Dwyer et al. 2014). Similarly, in a P limited ecosystem, LPC should increase with ability to acquire soil available P (Ordo et al. 2009, Maire et al. 2015) and should also be strongly negatively correlated with LDMC at the species level (Rivas-Ubach et al. 2012, Hidaka and Kitayama 2013).

Studies reporting a negative LPC-LDMC relationship among species, however, are surprisingly few. Our results show that LPC, rather than either LNC or SLA, is negatively

correlated with LDMC at the species level in Tibetan alpine meadow communities (Fig. 1), and that the correlation becomes stronger when we focus on common species or community level patterns ($R^2 = 0.46-0.68$). Domínguez et al. (2012) reported similar results at the community level in Mediterranean woodlands. These results and the fact that root phosphorus content is strongly correlated to LPC (Geng et al. 2014) suggest that LPC indicates species position along a gradient trading off nutrient-acquisition against nutrient-use efficiency in these P-limited alpine meadows. Species with fast (re)growth and weak competitive ability have high LPC but lower LDMC compared to slower-growing species with efficient use and conservation of fixed P and strong competitive ability.

4.2 Foliar phosphorus content is associated with greater species relative abundance

Overall, our results show that in P-limited Tibetan alpine meadows abundant species are those with greater ability to uptake soil available P and/or to efficiently use fixed P. In the grazed community, we assumed that uptake of soil P is the most important factor determining plant growth and species abundance when size-dependent competition for soil nutrients and light are reduced by removal of aboveground biomass (Borer et al. 2014, Niu et al. 2015a). In grazed communities higher LPC indicates that acquiring limited soil P for fast (re)growth is the most important trait associated with greater species abundance (Table 1 and Fig. 2a-b). Interestingly, we found that LPC, LDMC and seed size also are important for predicting SRA (Fig. 2d) at the dry and cold site QH. This indicates that not only nutrient acquisition but also the ability to conserve and efficiently use fixed P can become important in harsh environments. In contrast, our result shows that higher LDMC (and LCC) but lower LPC are the most important traits affecting species abundance in ungrazed communities (Fig. 2c-d).

This supports our previous assumption that traits promote competition for limited soil available P as well as conservation and that efficient use of fixed P are more important in a competition dominated community when grazing is excluded. Although neither height, LCC, LNC or SLA were important influences on species abundance under grazing, there is evidence that these traits involved competition for light and soil N do influence species abundance in ungrazed alpine meadows (Hille Ris Lambers et al. 2012, Niu et al. 2015b).

To summarize, these results suggest that traits promoting resource acquisition for fast (re)growth are critical for affecting high species abundance in disturbed communities, while traits associated with nutrient conservation are more important in a competition dominated community. This result supports ideas that SRA in a local community is organized by interspecific variation along a gradient in nutrient conservation versus acquisition (Tilman 1988, Grime 2001). Additionally, the importance of seed size, LDMC and height at our dry and cold study site suggests that species with these traits hold an advantage in seed dispersal and seedling establishment in this relatively harsh alpine environment. This supports the prediction that plant regeneration and recruitment by asexual reproduction will predominate in favorable environments but reproduction by seed in more stressful environments (Bazzaz 1996). In this regard, by considering ramet numbers as a measure of SRA we were able to better discern the contribution of traits to variation in SRA under grazing. Species relative abundance is usually assessed in terms of biomass, but the relative importance of traits depends not only on environmental context but also on the measure of abundance, especially in grazed communities (Fig. 2a-b and Table S1). Site by trait interactions are more significant when abundance is measured by ramet numbers, which especially for graminoids provides a

measure better allied with the potential for regrowth in response to grazing (Niu et al. 2009).

Acknowledgments

We thank Prof. Marc W. Cadotte and Prof. Shucun Sun for valuable discussion and constructive comments on earlier versions of this manuscript. We are especially grateful to Prof. Guozhen Du and Dr. Xianhui Zhou from the Research Station of Alpine Meadow and Wetland Ecosystems at Lanzhou University and Zhenong Jin and Lin Ma from Peking University for providing invaluable field and laboratory assistance. The study was supported by the National Science Foundation of China (grant no 31370008) and National Basic Research Program of China (no 2013CB956304).

References

- Albert, C. H., F. Grassein, F. M. Schurr, G. Vieilledent, and C. Violle. 2011. When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics* **13**:217-225.
- Bazzaz, F. A. 1996. *Plants in changing environments: Linking physiological, population, and community ecology*. Cambridge University Press, Australia.
- Borer, E. T., E. W. Seabloom, D. S. Gruner, W. S. Harpole, H. Hillebrand, E. M. Lind, P. B. Adler, J. Alberti, T. M. Anderson, and J. D. Bakker. 2014. Herbivores and nutrients control grassland plant diversity via light limitation. *Nature* **508**:517-520.
- Butterfield, B. J., and K. N. Suding. 2013. Single-trait functional indices outperform multi-trait indices in linking environmental gradients and ecosystem services in a

396 complex landscape. *Journal of Ecology* **101**:9-17.

397 Cadotte, M., C. H. Albert, and S. C. Walker. 2013. The ecology of differences: assessing
398 community assembly with trait and evolutionary distances. *Ecology Letters*
399 **16**:1234-1244.

400 Cadotte, M. W., C. A. Arnillas, S. W. Livingstone, and S.-L. E. Yasui. 2015. Predicting
401 communities from functional traits. *Trends in Ecology & Evolution* **30**:510-511.

402 Calcagno, V., and C. de Mazancourt. 2010. glmulti: an R package for easy automated model
403 selection with (generalized) linear models. *Journal of Statistical Software* **34**:1-29.

404 Chesson, P. 2000. Mechanisms of maintenance of species diversity. *Annual Review of*
405 *Ecology and Systematics* **31**:343-366.

406 Clobert, J., M. Baguette, T. G. Benton, J. M. Bullock, and S. Ducatez. 2012. Dispersal
407 ecology and evolution. Oxford University Press.

408 Collins, S. L., and S. M. Glenn. 1990. A hierarchical analysis of species' abundance patterns
409 in grassland vegetation. *American Naturalist* **135**:633-648.

410 Domínguez, M. T., C. Aponte, I. M. Pérez-Ramos, L. V. García, R. Villar, and T. Mara09ón.
411 2012. Relationships between leaf morphological traits, nutrient concentrations and
412 isotopic signatures for Mediterranean woody plant species and communities. *Plant &*
413 *Soil* **357**:407-424.

414 Díaz, S., J. Kattge, J. H. C. Cornelissen, I. J. Wright, S. Lavorel, S. Dray, B. Reu, C. Wirth, I.
415 C. Prentice, E. Garnier, G. Bönsch, M. Westoby, H. Poorter, P. B. Reich, A. T. Moles,
416 J. Dickie, A. N. Gillison, A. E. Zanne, J. Chave, S. J. Wright, S. N. Sheremet'ev, H.
417 Jactel, C. Baraloto, B. Cerabolini, S. Pierce, B. Shipley, D. Kirkup, F. Casanoves, J. S.

- Joswig, A. Günther, V. Falczuk, N. Rüger, M. D. Mahecha, and L. D. Gorné. 2016. The global spectrum of plant form and function. *Nature* 529:167-171.
- Dwyer, J. M., R. J. Hobbs, and M. M. Mayfield. 2014. Specific leaf area responses to environmental gradients through space and time. *Ecology* **95**:399-410.
- Geng, Y., L. Wang, D. Jin, H. Liu, and J.-S. He. 2014. Alpine climate alters the relationships between leaf and root morphological traits but not chemical traits. *Oecologia* **175**:445-455.
- Gibson, D. J. 2009. Grasses and grassland ecology. Oxford University Press Inc., New York.
- Godoy, O., N. J. Kraft, and J. M. Levine. 2014. Phylogenetic relatedness and the determinants of competitive outcomes. *Ecology Letters* **17**:836-844.
- Grime, J. P. 2001. Plant Strategies, Vegetation Processes and Ecosystem Properties., John Wiley, Sons Chichester.
- Hautier, Y., P. A. Niklaus, and A. Hector. 2009. Competition for light causes plant biodiversity loss after eutrophication. *Science* **324**:636-638.
- Hayes, P., B. L. Turner, H. Lambers, and E. Laliberté. 2014. Foliar nutrient concentrations and resorption efficiency in plants of contrasting nutrient - acquisition strategies along a 2 - million - year dune chronosequence. *Journal of Ecology* **102**:396-410.
- He, J.-S., X. Wang, D. F. Flynn, L. Wang, B. Schmid, and J. Fang. 2009. Taxonomic, phylogenetic, and environmental trade-offs between leaf productivity and persistence. *Ecology* **90**:2779-2791.
- He, J.-S., X. Wang, B. Schmid, D. F. Flynn, X. Li, P. B. Reich, and J. Fang. 2010. Taxonomic identity, phylogeny, climate and soil fertility as drivers of leaf traits across Chinese

grassland biomes. *Journal of plant research* **123**:551-561.

Hidaka, A., and K. Kitayama. 2013. Relationship between photosynthetic phosphorus-use efficiency and foliar phosphorus fractions in tropical tree species. *Ecology & Evolution* **3**:4872–4880.

Hille Ris Lambers, J., P. B. Adler, W. S. Harpole, J. Levine, and M. Mayfield. 2012. Rethinking community assembly through the lens of coexistence theory. *Annual Review of Ecology, Evolution, and Systematics* **43**:227-248.

Hodgson, J., G. Montserrat-Martí, M. Charles, G. Jones, P. Wilson, B. Shipley, M. Sharafi, B. Cerabolini, J. Cornelissen, and S. Band. 2011. Is leaf dry matter content a better predictor of soil fertility than specific leaf area? *Annals of Botany* **108**:1337-1345.

Holt, R. D. 2009. Bringing the Hutchinsonian niche into the 21st century: ecological and evolutionary perspectives. *Proceedings of the National Academy of Sciences* **106**:19659-19665.

Hong, J., X. Wang, and J. Wu. 2014. Stoichiometry of Root and Leaf Nitrogen and Phosphorus in a Dry Alpine Steppe on the Northern Tibetan Plateau. *PLoS ONE* **9**:e109052.

Hutchinson, G. E. 1957. Cold spring harbor symposium on quantitative biology. Concluding remarks **22**:415-427.

Kraft, N. J. B., P. B. Adler, O. Godoy, E. James, S. Fuller, and J. M. Levine. 2015a. Community assembly, coexistence, and the environmental filtering metaphor. *Functional Ecology* **29**:592–599.

Kraft, N.J.B., O. Godoy & J.M. Levine. 2015b. Plant functional traits and the

multidimensional nature of species coexistence. *Proceedings of the National Academy of Science, USA*, 112, 797-802.

Laliberte, E., B. Shipley, D. A. Norton, and D. Scott. 2012. Which plant traits determine abundance under long - term shifts in soil resource availability and grazing intensity? *Journal of Ecology* **100**:662-677.

Laughlin, D. C. 2014. The intrinsic dimensionality of plant traits and its relevance to community assembly. *Journal of Ecology* **102**:186-193.

Laughlin, D. C., and J. Messier. 2015. Fitness of multidimensional phenotypes in dynamic adaptive landscapes. *Trends in Ecology & Evolution* **30**:487-496.

Lawton, J.H. 1999. Are there general laws in ecology? *Oikos*, 84, 177-192.

Leimar, O., A. Sasaki, M. Doebeli, and U. Dieckmann. 2013. Limiting similarity, species packing, and the shape of competition kernels. *Journal of Theoretical Biology* **339**:3-13.

Maire, V., I. J. Wright, I. C. Prentice, N. H. Batjes, R. Bhaskar, P. M. Bodegom, W. K. Cornwell, D. Ellsworth, I. Niinemets, and A. Ordóñez. 2015. Global effects of soil and climate on leaf photosynthetic traits and rates. *Global Ecology & Biogeography*.

Miehe, G., S. Miehe, K. Kaiser, C. Reudenbach, L. Behrendes, and F. Schlütz. 2009. How old is pastoralism in Tibet? An ecological approach to the making of a Tibetan landscape. *Palaeogeography, Palaeoclimatology, Palaeoecology* **276**:130-147.

Morin, X., and M. J. Lechowicz. 2008. Contemporary perspectives on the niche that can improve models of species range shifts under climate change. *Biology Letters* **4**:573-576.

484 Niu, K. C., P. Choler, B. B. Zhao, and G. Z. Du. 2009. The allometry of reproductive biomass
 485 in response to land use in Tibetan alpine grasslands. *Functional Ecology* **23**:274-283.

486 Niu, K. C., J.-S. He, S. T. Zhang, and M. J. Lechowicz. 2015a. Grazing increases functional
 487 richness but not functional ivergence in Tibetan alpine meadow plant communities.
 488 *Biodiversity and Conservation*. DOI 10.1007/s10531-015-0960-2.

489 Niu, K. C., J. Messier, J.-S. He, and M. J. Lechowicz. 2015b. The effects of grazing on trait
 490 diversity and niche differentiation in Tibetan alpine meadows. *Ecosphere* **6**:Art150.

491 Niu, K. C., B. Schmid, P. Choler, and G. Z. Du. 2012. Relationship between reproductive
 492 allocation and relative abundance among 32 species of a Tibetan Alpine Meadow:
 493 effects of fertilization and grazing. *PLoS ONE* **7**:e35448.

494 Niu, K. C., S. T. Zhang, B. B. Zhao, and G. Z. Du. 2010. Linking grazing response of species
 495 abundance to functional traits in the Tibetan alpine meadow. *Plant and Soil*
 496 **330**:215-223.

497 O'Hara, R. B., and D. J. Kotze. 2010. Do not log - transform count data. *Methods in Ecology*
 498 *and Evolution* **1**:118-122.

499 Ordo, ntilde, J. C. ez, P. M. Van Bodegom, J. P. M. Witte, I. J. Wright, P. B. Reich, and R.
 500 Aerts. 2009. A global study of relationships between leaf traits, climate and soil
 501 measures of nutrient fertility. *Global Ecology and Biogeography* **18**:137–149.

502 Pérez-Harguindeguy, N., S. Díaz, E. Garnier, S. Lavorel, H. Poorter, P. Jaureguiberry, M.
 503 Bret-Harte, W. Cornwell, J. Craine, and D. Gurvich. 2013. New handbook for
 504 standardised measurement of plant functional traits worldwide. *Australian Journal of*
 505 *Botany* **61**:167-234.

506 Raouda, A. H. K., M. Duru, J. P. Theau, S. Plantureux, and P. Cruz. 2005. Variation in leaf
507 traits through seasons and N-availability levels and its consequences for ranking
508 grassland species. *Journal of Vegetation Science* **16**:391–398.

509 Reich, P. B. 2014. The world - wide ‘fast–slow’ plant economics spectrum: a traits manifesto.
510 *Journal of Ecology* **102**:275-301.

511 Ricklefs, R. E. 2015. Intrinsic dynamics of the regional community. *Ecology Letters*
512 **18**:497-503.

513 Rivas-Ubach, A., J. Sardans, M. Pérez-Trujillo, M. Estiarte, and J. Peñuelas. 2012. Strong
514 relationship between elemental stoichiometry and metabolome in plants. *Proceedings*
515 *of the National Academy of Sciences* **109**:4181-4186.

516 Shipley, B. 2010. From plant traits to vegetation structure: chance and selection in the
517 assembly of ecological communities. Cambridge University Press.

518 Shipley, B., C. T. Paine, and C. Baraloto. 2012. Quantifying the importance of local
519 niche-based and stochastic processes to tropical tree community assembly. *Ecology*
520 **93**:760-769.

521 Shipley, B., F. De Bello, J. H. C. Cornelissen, E. Laliberte, D. C. Laughlin, and P. B. Reich.
522 2016. Reinforcing loose foundation stones in trait-based plant ecology. *Oecologia*
523 **180**:923-931.

524 Suding, K. N., S. L. Collins, L. Gough, C. Clark, E. E. Cleand, and K. L. Gross. 2005.
525 Functional-and abundance-based mechanisms explain diversity loss due to N
526 fertilization. *Proceedings of the National Academy of Sciences* **102**:4387-4392.

527 Tilman, D. 1988. Plant strategies and the dynamics and structure of plant communities.

Princeton University Press, Princeton, New Jersey.

Violle, C., B. J. Enquist, B. J. McGill, L. Jiang, C. H. Albert, C. Hulshof, V. Jung, and J. Messier. 2012. The return of the variance: intraspecific variability in community ecology. *Trends in Ecology & Evolution* **27**:244-252.

Wang, S. P., J. C. Duan, G. P. Xu, Y. F. Wang, Z. H. Zhang, Y. C. Rui, C. Y. Luo, B. Y. Xu, X. X. Zhu, and X. F. Chang. 2012. Effects of warming and grazing on soil N availability, species composition, and ANPP in an alpine meadow. *Ecology* **93**:2365-2376.

Warton, D. I., B. Shipley, and T. Hastie. 2015. CATS regression—a model - based approach to studying trait - based community assembly. *Methods in Ecology and Evolution* **6**:389-398.

Weiher, E., D. Freund, T. Bunton, A. Stefanski, T. Lee, S. Bentivenga, E. Weiher, D. Freund, T. Bunton, and A. Stefanski. 2011. Advances, challenges and a developing synthesis of ecological community assembly theory. *Philosophical Transactions of the Royal Society B: Biological Sciences* **366**:2403-2413.

Wright, I. J., P. B. Reich, M. Westoby, D. D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J. H. C. Cornelissen, and M. Diemer. 2004. The worldwide leaf economics spectrum. *Nature* **428**:821-827.

Yang, X. X., F. Ren, H. K. Zhou, and J. S. He. 2014a. Responses of plant community biomass to nitrogen and phosphorus additions in an alpine meadow on the Qinghai-Xizang Plateau. *Chinese Journal of Plant Ecology* **38**:159-166.

Yang, Y. H., J. Y. Fang, C. J. Ji, A. Datta, P. Li, W. H. Ma, A. Mohammat, H. H. Shen, H. F. Hu, and B. O. Knapp. 2014b. Stoichiometric shifts in surface soils over broad

550 geographical scales: evidence from China's grasslands. *Global Ecology and*
551 *Biogeography* **23**:947-955.

552 Zhang, Z. L., K. C. Niu, X. D. Liu, P. Jia, and G. Z. Du. 2014. Linking flowering and
553 reproductive allocation in response to nitrogen addition in an alpine meadow. *Journal*
554 *of Plant Ecology* **7**:231-239.

555 Zhao, C., J. N. Griffin, X. W. Wu, and S. C. Sun. 2013. Predatory beetles facilitate plant
556 growth by driving earthworms to lower soil layers. *Journal of Animal Ecology*
557 **82**:749-758.

558

559

Figure Legends

Fig. 1. Principal component analysis correlation biplot showing the relationships among seven traits for all component species in grazed and ungrazed communities at all three sites. LCC: leaf carbon concentration, LNC: leaf nitrogen concentration, LPC: leaf phosphorous concentration, SLA: specific leaf area, LDMC: leaf dry matter content, mHeight: mature height. Apart from a decline in mHeight with grazing and from AZ to QH, there are no significant changes in traits among sites or between grazed vs. ungrazed plots in a site.

Fig. 2. Results from CATS regressions at single sites using a Poisson distribution to show the relative importance of individual traits in predicting species relative abundance (SRA) in grazed (a-b) and ungrazed plots (c-d) in term of both biomass (a, c) and ramet numbers (b, d) at each of the three study sites. Color indicates positive (red) and negative (blue) trait association with SRA; the magnitude of values indicates the relative importance of traits in predicting SRA. *P*-values indicate the significance of regression coefficients used to gauge the relative importance of traits as determined by a Wald-test using 999 resampling iterations in the CATS regression. LCC: leaf carbon concentration, LNC: leaf nitrogen concentration, LPC: leaf phosphorous concentration, SLA: specific leaf area, LDMC: leaf dry matter content, mHeight: mature height.

Figure 1

Figure 2

Table 1 The relative importance of traits in predicting species relative abundance (SRA) in grazed and ungrazed plots estimated in a multi-site CATS regression using both Poisson and negative binomial (NegBin) approaches. The SRA was estimated either in terms of both ramet numbers and aboveground biomass. Regression coefficients (proportional to relative importance) for different traits are estimated either from CATS regression with all seven traits or with fewer traits selected in alternative models assessed using the Akaike information criterion (AIC). The tabulated values are the regression coefficients of individual traits in predicting SRA. The magnitude of the regression coefficient indicates the relative importance of traits in predicting SRA and the sign positive or negative association with SRA. The significance of models and regression coefficients was assessed using a Wald-*test* based on 999 iterations in pit.trap resampling with bold entries indicating $P < 0.10$, *indicating $P < 0.05$. LCC: leaf carbon concentration, LNC: leaf nitrogen concentration, LPC: leaf phosphorous concentration, SLA: specific leaf area, LDMC: leaf dry matter content, mHeight: mature height. Note that we tabulate the trait $\times$ site interactions in Table S1, as few are significant.

	Ramets		Biomass		Ramets		Biomass	
	Poisson	NegBin	Poisson	NegBin	Poisson	NegBin	Poisson	NegBin
<i>Abundance regressed on all five key traits</i>								
AIC	603.171	509.457	617.456	477.215	555.275	477.690	476.558	409.071
Significance (P)	0.024*	0.433	0.001*	0.063	0.001**	0.014*	0.001**	0.164
<i>Trait effect</i>								
LCC	-0.196	-0.196	0.067	0.065	0.179	0.317	0.247	0.323
LNC	-0.380	-0.128	-0.424	-0.144	-0.598	-0.785*	-0.572	-0.726
LPC	8.986	8.834	22.089*	11.909	-24.902	-30.93*	-17.305	-21.538
SLA	0.002	0.003	0.001	-0.002	-0.005	-0.006	-0.009	-0.011
LDMC	0.298	0.017	1.124	-0.573	1.044	1.926	0.229	0.240
mHeight	0.019	0.020	0.068**	0.073	0.002	-0.008	0.028*	0.029
Seed size	-0.245	-0.092	-0.417	-0.24	-0.183	0.011	-0.330*	-0.087
<i>Abundance regressed on traits selected from model comparison</i>								
<i>Selected trait(s)</i>	LPC		LPC+mHeight		LCC+LNC		LCC+LPC+SLA	
<i>in best model</i>					+LPC +LDMC		+LDMC+Seed size	
AIC	664.176	500.068	736.34	472.959	573.834	470.455	533.495	421.892
Significance (P)	0.254	0.36	0.001**	0.014	0.001**	0.001**	0.001**	0.011*
<i>Trait effect</i>								
LCC					0.195	0.334	0.254	0.333
LNC					-0.729*	-0.874		
LPC	11.71	13.474	24.785*	15.800	-24.546	-33.651	-7.495	-12.231
SLA							-0.014*	-0.016*
LDMC					2.516**	3.062	0.520	0.268
mHeight			0.0659*	0.071				
Seed size							-0.217	0.049