

# The allometry of reproductive biomass in response to land use in Tibetan alpine grasslands

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## Summary

1. Reproductive biomass (RB) is a key component of plant life history. Because RB is allometric, it remains unclear whether the observed variations of RB in response to land use are driven only by a size dependency effect or if the partitioning of plant biomass may also shift independently of plant size. This question has not been addressed for all the main component species of a given plant community.

2. Here, we report the results of a 3-year experimental study conducted in Tibetan alpine grasslands. The above-ground vegetative biomass (VB) and the RB of 32 species were measured during 2 years in control, grazed and fertilized plots. We estimated the allometric slope and intercept for 301 VB–RB bivariate relationships, using type II regression analyses in order to disentangle size-dependent and size-independent effects of treatment on RB.

3. Our results indicate significant changes in the VB–RB allometric slope for 26 out of 102 cases in grazed plots, and 16 out of 88 cases in fertilized plots. Moreover, in cases where slopes were not affected by treatment, a significant change in the allometric intercept was found in 50% of the models. For these cases, intercepts – which are indicative of the biomass invested in reproductive parts for a given VB – generally increased in grazed plots and decreased in fertilized plots.

4. Grazing and fertilization triggered significant decreases or increases in the plant size for species, especially graminoids. However, we found no relationship between changes in allometric coefficients and magnitude of the biomass response.

5. At the interspecific level, the intercept, but not the slope, of the allometric VB–RB relationships was significantly affected by land use. Graminoids and forbs responded similarly to treatment.

6. *Synthesis.* We conclude that (i) size-dependent effects did not explain all the changes observed in RB following grazing and fertilization in alpine grasslands, and that (ii) changes in allometric intercept explained most of the size-independent variations both at the intra- and interspecific level. We discuss why size-independent changes in RB could be important for plant performance in changing environments.

**Key-words:** allometric strategy, alpine grasslands, biomass partitioning, land use changes, plant functional trait, reproductive allocation, Tibetan Plateau

## Introduction

Plant reproductive output is a key component of plant performance, along with vegetative biomass (VB) and survival

(Violle *et al.* 2007). Reproductive output is a function of reproductive allocation (RA) – the proportion of biomass invested in flowers and fruits – and individual size (Bazzaz & Grace 1997). Because of allometric constraints on plant growth, any factor which affects plant VB will also affect the reproductive biomass (RB) (Samson & Werk 1986; Bazzaz, Ackerly & Reekie 2001). This ‘size-dependent effect’ (Bazzaz *et al.* 2001) has been reported in many studies, especially those on annual plants (Ackerly & Jasienski 1990; Schmid & Weiner 1993; Sugiyama & Bazzaz 1998; Müller, Schmid &

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Weiner 2000; Cheplick 2005). The term 'apparent plasticity' coined by (McConnaughay & Coleman 1999) describes the same phenomenon, though the term 'plasticity' should be restricted to studies performed on a given genotype placed in different environments (Weiner 2004).

Several studies have shown that the allometric coefficients of the VB–RB relationship might change in response to environmental factors (reviewed in Weiner 2004; Cheplick 2005). These shifts in RA, which are not exclusively driven by plant size variations, have been called 'true plasticity' (Weiner 2004), but can also be viewed as accommodation in biomass partitioning when genotypes of individuals are not controlled. It has long been suggested that this accommodation could be instrumental for maintaining plant fitness in a changing environment. However, most of the empirical evidence in favour of it has resulted from studies on a few focal species which were grown in controlled conditions (Reekie & Bazzaz 2005). In natural vegetation, however, the relative importance of size-dependent vs. size-independent responses of RB remains largely unknown (Weiner 2004; Cheplick 2005). A better understanding of plant biomass partitioning between vegetative and reproductive parts is essential in any study addressing plant community dynamics in response to environmental changes (Tilman 1988; Bazzaz 1996).

Fertilization and grazing are the two most common land uses in grasslands (Lavorel *et al.* 2007). A large number of empirical and theoretical studies have examined the impact of these two treatments on plant composition and ecosystem function (Vesk & Westoby 2001; Hooper *et al.* 2005; Diaz *et al.* 2007; Harpole & Tilman 2007). However, very few have compared the biomass allocation response of all component species of a given plant community to long-term grazing and fertilization. Optimal allocation theory predicts that plants should invest more in the compartment where resources are limited (Bloom, Chapin & Mooney 1985), and this has consequences for RA (Bazzaz & Grace 1997). For example, preferential allocation to the root system in low-fertility habitats would be associated with a lower RA (Reekie & Bazzaz 2005). Overgrazed plants should exhibit a smaller size and a higher RA (Bazzaz *et al.* 1987), whereas, in fertilized habitats, plants might allocate more biomass to vegetative growth to cope with increased light competition, at the expense of reproduction (Niu *et al.* 2008).

Tibetan alpine grasslands have experienced a long history of grazing (Du, Zhang & Wu 2000). Recent land use changes include overgrazing and fertilization, with impacts on biodiversity and productivity (Du *et al.* 2000). The meadows are dominated by perennial forbs and graminoids, for which the biomass responses to fertilization and grazing have been recently documented (Yang *et al.* 2004; Luo, Qin & Du 2006). In this study, we focused on the allometry of RB in response to land use changes. We took advantage of a large-scale experimental design conducted in the eastern part of the Tibetan Plateau, in which grazing and fertilization have been controlled for several years. We measured the biomass allocation of 32 species representing 90% of the total biomass of the grasslands. We addressed the following questions:

- (i) What is the effect of grazing and fertilization on plant vegetative and plant RB?
- (ii) What is the relative importance of size-dependent vs. size-independent effects on RB changes?
- and (iii) Do forbs and graminoids respond similarly to the experimental treatments?

## Methods

### STUDY SITE

The experiment was on a large, flat area dominated by alpine grasslands in MaQu (N33°59', E102°00'), Gansu, China. This site is located in the eastern part of the Tibetan Plateau, at 3500 m a.s.l. The yearly average temperature is 1.2 °C, ranging from –10 °C in January to 11.7 °C in July, with about 270 days of frost. Annual precipitation, measured over the last 35 years, is 620 mm, occurring mainly during the short, cool summer. During the years of the study, the annual precipitation was 584 mm (2004), 672 mm (2005) and 643 mm (2006). The annual duration of cloud-free solar radiation is about 2580 h.

### EXPERIMENTAL DESIGN

A 13-ha flat area was fenced in October 1999. Grazing was limited to the non-productive winter months. Outside of the enclosure (45 ha), vegetation was moderately grazed by ungulates, with 110 yaks and 2200 sheep during all months except for 40 days between July and mid-August when the animals were moved to higher elevation pastures.

In late May 2004, twenty 5 × 8 m plots, separated by 2 m, were established within the fenced site. We randomly allocated a fertilization treatment to half of the plots. A slow-release, pelletized fertilizer (30 g m<sup>-2</sup> of (NH<sub>4</sub>)<sub>2</sub>HPO<sub>4</sub>, 18% N and 46% P, manufactured by Tianjin International Trading Company, Tianjin, China) was hand-broadcast once annually (26 May 2004, 29 May 2005 and 26 May 2006). Fertilizer was applied during drizzly days to avoid the need for watering. Nitrogen supply corresponded to 10.8 g N m<sup>-2</sup> year<sup>-1</sup> (Luo *et al.* 2006; Niu *et al.* 2008). Outside the enclosure, at a distance of 300 m from the fertilized plots, we selected ten 5 × 8 m plots for the grazing treatments. Plots were separated by 15-m strips.

All the plots were separated into two parts: a 5 × 5 m subplot for individual plant sampling, and a 5 × 3 m subplot for vegetation monitoring. Vegetation monitoring was conducted twice yearly (July and September) within two evenly spaced 0.5 × 0.5 m squares.

### COMMUNITY MEASUREMENTS

In the middle of July and September 2005 and 2006, one 0.5 × 0.5 m quadrat was harvested from the 5 × 3 m subplot of each plot. Harvested quadrats were located at different places each harvesting time. The above-ground parts of all plant individuals were collected. Green parts (stem and leaves) were sorted by species. The number of ramets per species was recorded, and then clipped and brought to the laboratory. In each transect we counted the number of species and the number of individuals. We estimated the total below- and above-ground biomass. For above-ground biomass, we distinguished forbs and graminoids. Above-ground biomass was partitioned into leaf, stem and reproductive biomass (RB).

Three soil cores (3.8 cm diameter × 10 cm deep) were collected from each plot in mid-July and September. Soils were brought to

laboratory in sealed bags and passed through a 1 × 1 mm sieve. Roots were washed out and dried for weighing biomass. Soils were extracted and the nitrate-N and ammonia-N content were measured using an Alpkem RFA 300 auto-analyzer.

In each plot, we measured light with a Decagon Sunfleck Septometer (Decagon, Pullman, Washington, DC) at the time of vegetation monitoring. Photosynthetic active radiation (PAR) was recorded at 10 cm above the soil surface and at the top of canopy. The ratio between these two measurements was taken as a proxy for PAR extinction within the canopy.

#### PLANT BIOMASS MEASUREMENTS

Based on previous studies, we chose 32 species for measuring biomass and biomass allocation (see Table S1 in Supporting Information). These species accounted for 85–95% of the above-ground biomass and 80–90% of the vegetation cover. The species were split into two functional groups: forbs (including two legume species) and graminoids. Individuals were sampled from July through the end of September in 2005 and 2006. The harvesting schedule took into account the differing phenology of the species, that is, species were sampled at their flowering and fruiting time. Only above-ground parts were collected because the sampling of individual root systems was deemed impossible in this dense meadow. We randomly sampled 2–3 individuals of each species in the 5 × 5 m subplot of each plot, so as to obtain nearly 25 individuals for each treatment. In grazed plots, we selected individuals that were not injured by grazing. For clonal plants, we sampled ramets and treated them as individuals (Cheplick 1998; Luo *et al.* 2006; Niu *et al.* 2008). Each individual was brought to laboratory and split into stems, leaves and reproductive parts (including flowers and fruits). Samples were dried at 80 °C, and weighed to the nearest 10<sup>-4</sup> g. Vegetative biomass (VB) corresponds to the above-ground biomass and was the sum of stem biomass and leaf biomass.

#### DATA ANALYSIS

For each species and each harvesting time, we compared VB in control vs. grazed and in control vs. fertilized treatment using a *t*-test.

We tested four different models of size-dependent RB:

- (1)  $RB = a VB + \epsilon$
- (2)  $RB = a (VB - b) + \epsilon$
- (3)  $RB = a VB^c \epsilon$
- (4)  $RB = a (VB - b)^c \epsilon$

where  $\epsilon$  is an error term with mean zero and variance  $\sigma^2$ .

Models (1) and (2) assume that the relationship between RB and VB is linear. Model (2) allows one to test whether the minimum vegetative size for flower production (parameter *b*) differs significantly from zero or not. Model (3) is an allometric model. It assumes that the minimum size for reproduction is zero, but it allows one to test whether the allometric exponent (parameter *c*) is significantly different from 1, that is, to test the degree of nonlinearity of the VB–RB relationship. Model (4) is a three-parameter model introduced by Klinkhamer *et al.* (1992). It overcomes limitations of models (2) and (3) by permitting test for nonlinearity and for the minimum size for reproduction. To be biologically meaningful, parameter *b* should be positive. While fitting model (4), *b* should also be smaller than the minimum value of VB, that is,  $(V_i - b) > 0$  for  $i = 1$  to  $N$  (with  $N$  the number of observations). A linear, or nonlinear, least squares method was used to find the best estimates for parameters *a*, *b* or *c* in models (1) and (2), or (3) and (4) respectively. This meant implementing

an optimization procedure to find the combination of parameters which minimizes the sum of squared residuals:

$$S = \sum_i r_i^2 \text{ for } i = 1 \text{ to } N, \text{ with } r_i = RB_{\text{obs},i} - RB_{\text{fitted},i}$$

where  $RB_{\text{obs}}$  is the observed RB and  $RB_{\text{fitted}}$  is the fitted RB with one of the four models.

For nonlinear least squares, we used the algorithm developed by Byrd *et al.* (1995) because it allows one to set a lower and upper bound for a given parameter. This flexibility was required for a proper estimate of parameter *b* as explained above. Comparing models by using the sum of squared residuals as an index of fit is only applicable when the model equations have the same dependent variables (Hafley 1969). Therefore, no log-transformation of the variables was performed at this stage.

As noted by Klinkhamer *et al.* (1992), the two following sequences, (1) → (2) → (4) and (1) → (3) → (4), correspond to two possible streams of explorative data analysis. In this study, we favoured the second sequence for two main reasons: (i) in almost one case out of three, model (2) led to negative *b* estimates; and (ii) for a majority of species, the sum of square residuals of model (2) was above that of model (3) (see Fig. S1 in Supporting Information).

In attempting to decide which model structure provides the best fit, we estimated the likelihood ratio:

$$LL = N \log(S_{i,\min}/S_{j,\min})$$

where  $S_{i(j),\min}$  is the minimum sum of squared residuals for model  $i(j)$ .

The likelihood ratio significance test was based on the assumption that LL follows a  $\chi^2$  distribution with 1 df.

Along the sequence (1) → (3) → (4), one first tests the null hypothesis  $H_0: c = 1$  (under the assumption  $b = 0$ ) by comparing models (1) and (3), and then tests the null hypothesis  $H_0: b = 0$  by comparing models (3) and (4). We had a total of 111 comparisons corresponding to one species measured in control and grazed plots and a total of 88 comparisons corresponding to one species measured in control and fertilized plots. For each comparison, we examined whether the null hypothesis  $H_0: c = 1$  or  $H_0: b = 0$  was rejected in at least one data set.

Given the constraints on parameter *b* (see above), we found only a few significant cases, 9 out of 102 for control vs. grazed and 8 out of 88 for control vs. fertilized, where the null hypothesis  $H_0: b = 0$  was rejected when fitting model (4). This was probably due to the fact that (i) our data sets did not include enough small values of VB, and (ii) the number of replicates was too small for a proper estimate of *b*. For these reasons, we considered that the three-parameter model (4) was not the most appropriate for our data, and that a proper estimate of the minimum size for reproduction was not achievable with our data.

There was significant support to reject the null hypothesis  $H_0: c = 1$  in more than 40% of the comparisons between control and grazed (48%), and of the comparisons between control and fertilized (47%). Because we were interested in comparing within-species and between-species response to treatments, we had to choose a common model structure for all the data sets analysed. Therefore, the two-parameter allometric model (3) was the only one considered for further analyses.

We transformed model (3) in a log–log linear relation and estimated  $a' = \log(a)$  and *c* using a model II regression (Standardized Major Axis) (Warton *et al.* 2006). Parameters *a* and *c* of model (3) will be referred as the allometric intercept and the allometric exponent (Samson & Werk 1986; Niklas & Enquist 2002). For each species, we

tested whether the allometric exponent was significantly different from 1, and then compared allometric coefficients between control and grazed plots, and between control and fertilized plots. If no significant differences were found for  $c$ , we tested for an effect of treatment on the allometric intercept. Providing the same allometric slope, a significant change in the allometric intercept indicates a change in the amount of biomass invested in reproductive parts for a given VB.

In order to examine VB–RB relationships across species, we used the same allometric equations and statistical procedures. In this case, each species was represented by the mean biomass of all the individuals measured in a given condition (treatment  $\times$  year  $\times$  harvesting time). Various correction factors have been proposed when attempting to back transform log–log linear estimates into the original scale (Hayes & Shonkwiler 2006). However, there is still no consensus whether one should or not attempt to use these methods for bias correction, as they do not account for all sources of uncertainty (D. Warton pers. comm.). In this study, we did not apply correction factors.

All statistical analyses and figures were made using the software R (R Development Core Team 2008). The optimization procedure for nonlinear least squares was conducted with function *optim*. Allometric analyses were performed with the SMATR R library (Warton & Ormerod 2007).

## Results

### COMMUNITY RESPONSE

The treatments had a marked effect on above-ground biomass, but not on below-ground biomass (Table 1). Above-ground biomass significantly increased in fertilized plots and significantly decreased in grazing plots relative to controls (Table 1). Community biomass changes were mainly driven by the response of graminoids in our results (Table 1). Forbs may exhibit an opposite response to the community response: for example, the total biomass of forbs decreased significantly in fertilized plots (Table 1). As expected, fertilization induced increased soil nitrogen content (Table 1). Increased biomass in fertilized plots was associated with strong light extinction, a decrease in forb species and an over dominance of graminoids (Table 1). In contrast, there was a significant increase in forb species and slight decrease in graminoids species in grazing plots. This trend was more noticeable in September of each year (Table 1).

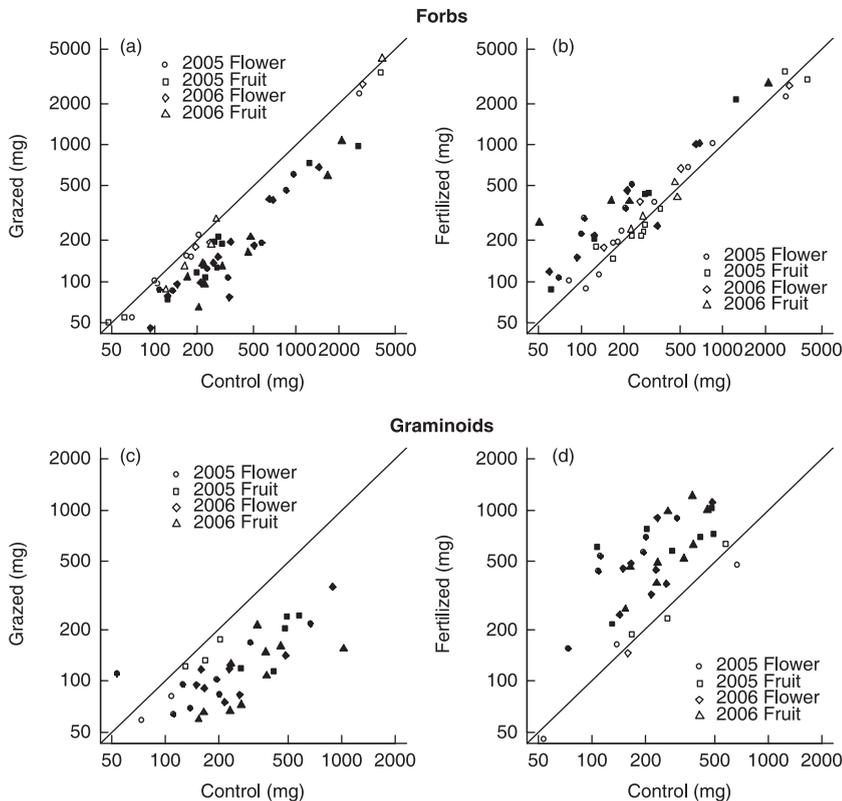
### BIOMASS RESPONSE AND RA AT THE WITHIN-SPECIES LEVEL

A significant decrease, or increase, in total above-ground biomass was the most common response to grazing, or fertilization, respectively (Fig. 1). The magnitude of the response was generally much more pronounced for graminoids as compared to forbs (Fig. 1). Many forbs did not benefit from fertilization (Fig. 1b). The effects of both treatments were more pronounced in 2006 compared to 2005, especially for graminoids (Fig. 1c,d).

Details of the 301 VB–RB allometric relationships are given in Table S2 in the Supporting Information. All but one species (*Euphorbia micractina*) exhibited a highly significant

**Table 1.** Plant community response to experimental treatments. Grazed vs. control, and fertilized vs. control differences were tested with ANOVA and used Tukey's test for post-hoc comparisons ( $P$  value adjusted with the Bonferroni method) (small letters are for  $P < 0.05$  and capital letters for  $P < 0.01$ ). PAR<sub>10</sub> means the ratio between PAR measured at 10 cm above-ground and PAR measured at the top of the canopy under full sunlight conditions

	Vegetative biomass (g)	Forb biomass (g)	Graminoid biomass (g)	Root biomass (g)	Forb species ( $n$ )	Graminoid species ( $n$ )	Available nitrogen (mg kg <sup>-1</sup> )	PAR <sub>10</sub> (%)	Canopy height (cm)
July 2005									
Grazed	46.4 ± 5.8 <sup>A</sup>	15.7 ± 1.8 <sup>A</sup>	36.8 ± 4.8 <sup>A</sup>	420.5 ± 23.8 <sup>b</sup>	18.5 ± 0.7 <sup>B</sup>	10.6 ± 0.9	42.7 ± 3.6	94.5 ± 1.8 <sup>C</sup>	26 ± 1.2 <sup>A</sup>
Control	83.1 ± 10.9 <sup>B</sup>	32.5 ± 2.5 <sup>B</sup>	55.7 ± 10.0 <sup>B</sup>	314.9 ± 33.8 <sup>b</sup>	18 ± 0.8 <sup>B</sup>	11.6 ± 0.6	54.8 ± 3.4	72.1 ± 2.9 <sup>B</sup>	48 ± 1.5 <sup>B</sup>
Fertilized	117.9 ± 3.1 <sup>C</sup>	16.7 ± 1.4 <sup>A</sup>	101.5 ± 6.7 <sup>C</sup>	375.2 ± 25.4 <sup>ab</sup>	12.9 ± 0.6 <sup>A</sup>	12.6 ± 0.8	51.9 ± 5.3	32.0 ± 4.3 <sup>A</sup>	70 ± 0.6 <sup>C</sup>
September 2005									
Grazed	62.1 ± 7.3 <sup>A</sup>	21.4 ± 1.1 <sup>B</sup>	41.1 ± 3.8 <sup>A</sup>	553.7 ± 72.7	18.1 ± 0.8 <sup>C</sup>	10.3 ± 1.1	47.2 ± 1.7 <sup>b</sup>	61.3 ± 5.9 <sup>C</sup>	40 ± 1.4 <sup>A</sup>
Control	105.1 ± 3.8 <sup>B</sup>	22.5 ± 1.9 <sup>B</sup>	85.1 ± 7.4 <sup>B</sup>	623.9 ± 37.6	14.3 ± 0.6 <sup>B</sup>	12.2 ± 1.2	33.6 ± 2.1 <sup>a</sup>	41.5 ± 5.7 <sup>B</sup>	57 ± 2.2 <sup>B</sup>
Fertilized	141.5 ± 8.8 <sup>C</sup>	12.2 ± 2.5 <sup>A</sup>	126.2 ± 9.9 <sup>C</sup>	600.3 ± 53.4	9.5 ± 1.5 <sup>A</sup>	9 ± 1.0	45.2 ± 5.1 <sup>b</sup>	12.6 ± 3.1 <sup>A</sup>	76 ± 2.5 <sup>C</sup>
July 2006									
Grazed	73.8 ± 7.2 <sup>A</sup>			542.1 ± 23.8	21.5 ± 0.8 <sup>B</sup>	11.6 ± 1.3	10.7 ± 0.9 <sup>A</sup>	81.7 ± 1.8 <sup>C</sup>	31 ± 2.3 <sup>A</sup>
Control	131.1 ± 5.0 <sup>B</sup>			507.1 ± 42.9	18.5 ± 0.9 <sup>B</sup>	13.4 ± 0.9	8.7 ± 0.7 <sup>A</sup>	37.7 ± 2.7 <sup>B</sup>	53 ± 1.7 <sup>B</sup>
Fertilized	159.9 ± 7.5 <sup>C</sup>			486.1 ± 34.5	11.8 ± 1.2 <sup>A</sup>	13.7 ± 0.8	64.3 ± 3.6 <sup>B</sup>	12.9 ± 3.0 <sup>A</sup>	70 ± 2.8 <sup>C</sup>
September 2006									
Grazed	96.2 ± 6.0 <sup>A</sup>			578.2 ± 48.7 <sup>b</sup>	20.1 ± 1.6 <sup>C</sup>	10.8 ± 1.2	20.3 ± 1.2 <sup>b</sup>	71.2 ± 4.0 <sup>C</sup>	37 ± 2.8 <sup>A</sup>
Control	147.8 ± 7.8 <sup>B</sup>			565.6 ± 50.9 <sup>b</sup>	14.2 ± 0.9 <sup>B</sup>	12.1 ± 0.8	15.9 ± 0.8 <sup>a</sup>	25.1 ± 2.2 <sup>B</sup>	54 ± 2.3 <sup>B</sup>
Fertilized	199.0 ± 15.8 <sup>C</sup>			396.1 ± 38.7 <sup>a</sup>	9.6 ± 0.7 <sup>A</sup>	10.7 ± 1.1	50.0 ± 2.8 <sup>C</sup>	7.9 ± 1.9 <sup>A</sup>	71 ± 1.5 <sup>C</sup>



**Fig. 1.** Changes in mean vegetative biomass (VB) in response to grazing (a and c) and fertilization (b and d) for forbs (a and b) and graminoids (c and d). Symbols represent the mean VB of each species in each harvesting times, that is, at the flowering or fruiting time of years 2005 and 2006. Black symbols indicate a significant effect of treatment on VB at  $P < 0.05$ .

log–log linear relationship between VB and RB, whatever the treatment (Table S2). The allometric exponent (c) was generally above one, indicating an increase of RA with plant size (Table S2).

Significant effects of the treatment on the allometric exponent were found for 26 out of 102 cases in grazed plots, and 16 out of 88 cases in fertilized plots (Figs 2 and 4a,b). There was no difference between the number of forb and graminoid species that responded significantly (results from Fisher exact test). However, a decreased slope in grazed plots corresponded to a main trend for the graminoid species (Fig. 2c). We did not find a correlation between the magnitude of change in slope and the biomass response (data not shown).

Significant effects of the treatments on the allometric intercept were found for 57 out of 76 cases in grazed plots, and 36 out of 72 cases in fertilized plots (Figs 3 and 4c,d). The intercept increased in the grazed plots (Fig. 3a,c), whereas it decreased in the fertilized plots (Fig. 3b,d). The same trend held for both forbs and graminoids. Because these results were obtained in the case of non-significant differences in allometric slopes, they indicated that the amount of biomass invested in reproductive parts for a given VB changed consistently with experimental treatment.

#### RA AT THE BETWEEN-SPECIES LEVEL

Interspecific relationships between RB and VB were well fitted by the same allometric models (Fig. 5 and Table 2). The slope was not affected by the treatments. Forbs and graminoids responded similarly. In contrast, changes in the

allometric intercept were significant and followed the trend described for the within-species level (Fig. 5).

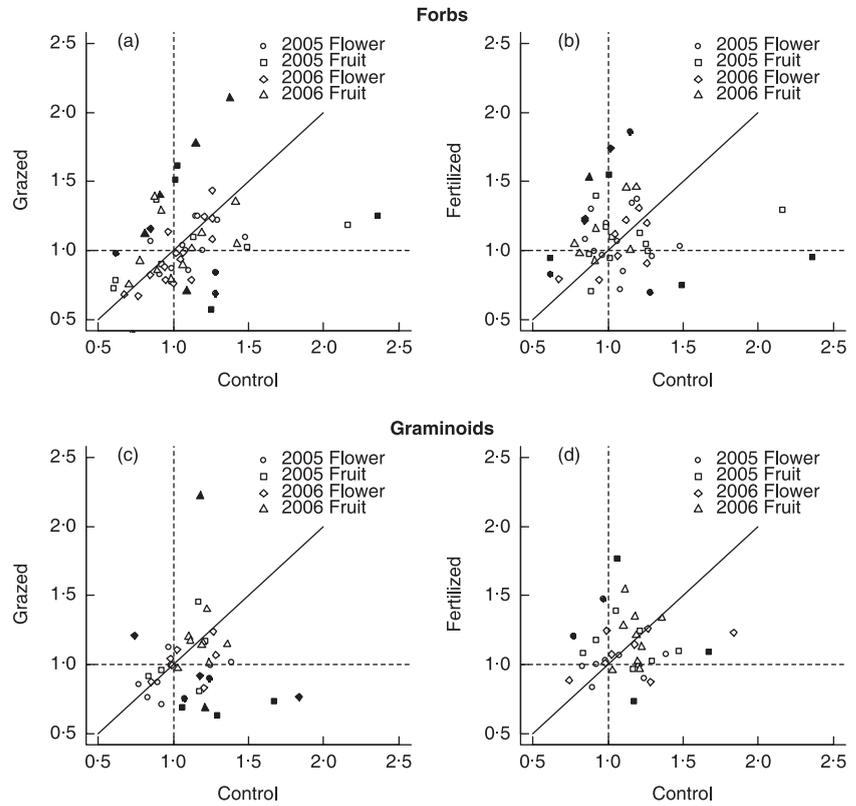
#### Discussion

This study examined to what extent allometric constraints are responsible for changes in RA in response to grazing and fertilization in alpine grasslands. Our results indicated that the allometric coefficients of numerous species were significantly affected by the treatments, but with no marked contrast between forbs and graminoids. Changes in allometric intercept are much more common than changes in allometric slope. The same conclusions hold for within species VB–RB relationships and between species VB–RB relationships.

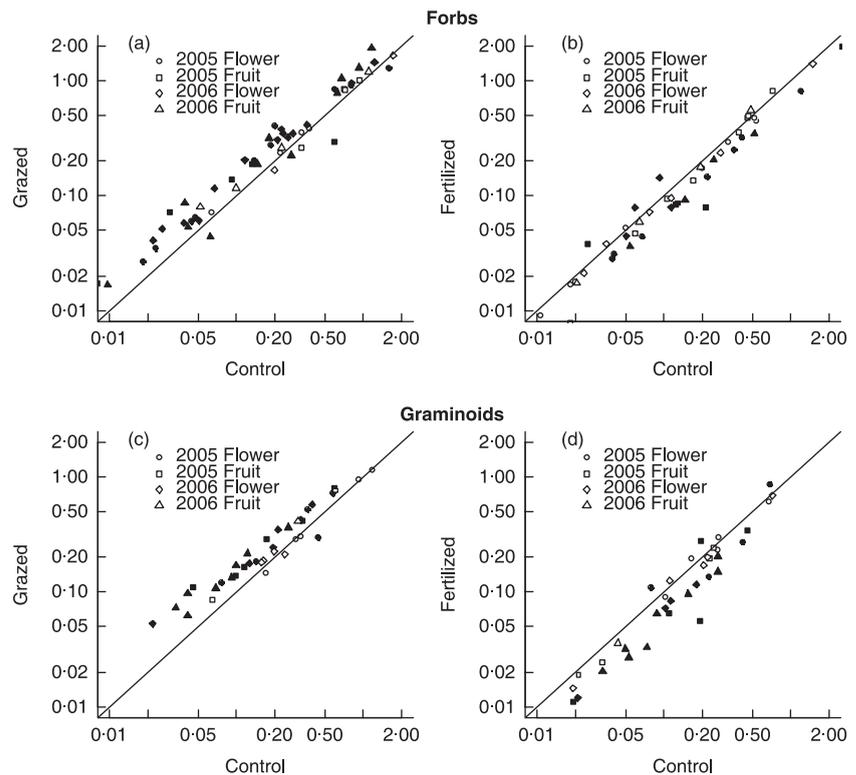
Because our data include only two harvesting times during the growing season, we do not address the question of RA patterns throughout plant development. These issues have been analysed extensively on a few species, usually grown in controlled conditions (reviewed in Samson & Werk 1986; Klinkhamer *et al.* 1992; Cheplick 2005). By contrast, our aim was to investigate a large number of species co-occurring in a natural community. This allowed us to examine the gross trends of RA changes across different species and to draw conclusions at the community level.

#### RA IN RESPONSE TO GRAZING AND FERTILIZATION

How plant traits respond to land use is a key issue in ecology (McGill *et al.* 2006; Westoby & Wright 2006). Several plant traits behave allometrically, that is, the trait value is a function



**Fig. 2.** Changes of the exponent of the allometric VB–RB relationship in response to grazing (a and c) and fertilization (b and d) for forbs (a and b) and graminoids (c and d). The allometric exponent  $c$  of the log–log linear relationship,  $\log(\text{RB}) = a' + c \log(\text{VB})$ , was estimated using regression type II analyses. Symbols represent the allometric exponent of each species in each harvesting time. Black symbols indicate a significant effect of treatment on the exponent.



**Fig. 3.** Changes of the intercept of the allometric VB–RB relationship in response to grazing (a and c) and fertilization (b and d) for forbs (a and b) and graminoids (c and d). The allometric intercept is  $\text{anti-log}(a')$  with  $a'$  estimated from the log–log linear relationship,  $\log(\text{RB}) = a' + c \log(\text{VB})$ , using regression type II analyses. Symbols represent the allometric intercept of each species in each harvesting time. Black symbols indicate effect of treatment on the intercept.

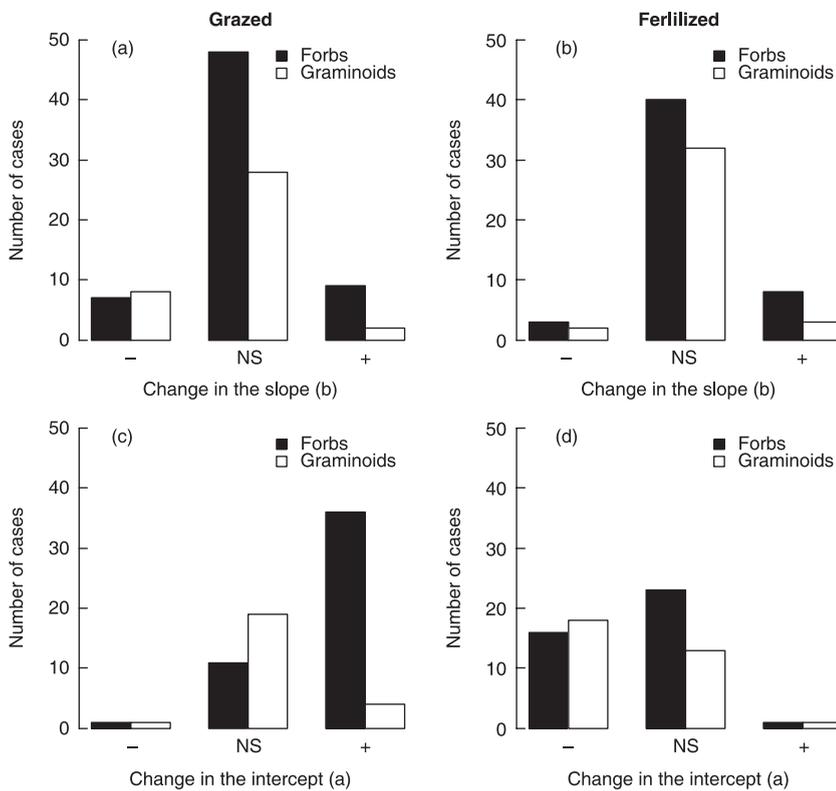
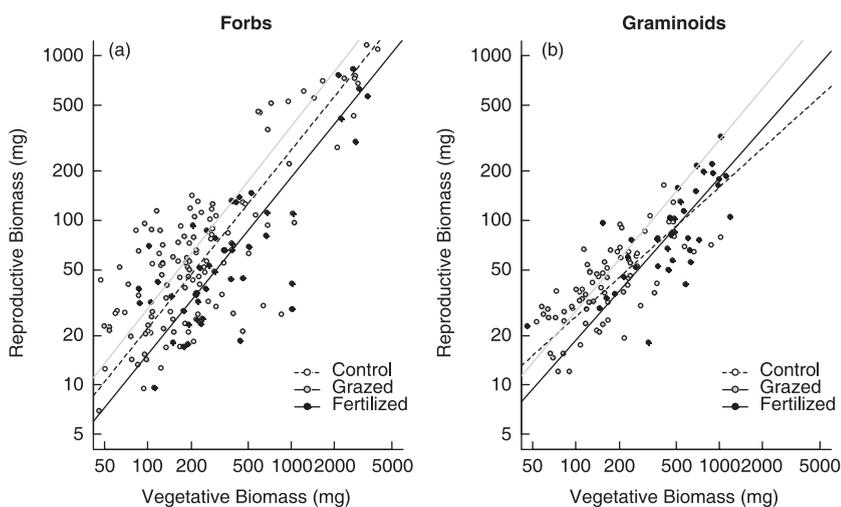
of plant size. Recently, there has been a renewed interest in using allometric approaches to study plant functional traits in relation to environmental gradients (McCarthy & Enquist 2007). For this purpose, a better knowledge of plant allometric

constraints offers a promising way to scale up from individual to community (Niklas 1990, 1994).

As emphasized in plant life-history theories, shifts in biomass allocation can be instrumental in allowing individuals

**Table 2.** Estimates of the allometric coefficients *a* and *b* for interspecific relations of RB and VB. Results are from regression type II analyses (SMA) of  $\log_{10}$ -transformed data. In all cases,  $P < 0.001$ , CI, confidence interval

Life-form	Treatment	<i>n</i>	<i>a</i> (mean and 95% CI)	<i>b</i> (mean and 95% CI)	<i>r</i> <sup>2</sup>
Forbs	Control	71	1.15 (1.00–1.33)	0.094 (0.036–0.243)	0.645
	Grazed	64	1.18 (1.03–1.35)	0.115 (0.049–0.268)	0.712
	Fertilized	51	1.16 (0.96–1.39)	0.062 (0.017–0.218)	0.598
Graminoids	Control	40	0.79 (0.62–1.00)	0.069 (0.242–1.978)	0.457
	Grazed	38	1.04 (0.81–1.34)	0.236 (0.067–0.824)	0.436
	Fertilized	37	0.99 (0.78–1.24)	0.201 (0.05–0.815)	0.547

**Fig. 4.** A summary of the number of forbs (black) and graminoids (white) which significantly responded to grazing (a and c) and fertilization (b and d). Allometric exponents (a and b) and intercepts (c and d) were estimated using regression type II analyses. Tests for an effect of treatment on allometric intercepts were performed when the two allometric models had a common exponent.**Fig. 5.** Interspecific relationships between RB and VB for forbs (a) and graminoids (b). The Standardized Major Axis and the allometric coefficients for each treatment are given in Table 2. The symbols represent the mean values for each species in each of the three treatments.

to succeed in new environments (MacArthur & Wilson 1967; Bloom *et al.* 1985; Bazzaz 1996). For example, increased allocation to stem and leaves at the expense of RB is a typical response to intense light competition (Obeso 2002; Niu *et al.* 2008). Such patterns have been described along successional gradients (Newell & Tramer 1978; Gleeson & Tilman 1990). The results we obtained in the fertilized plots agreed with these predictions as most species exhibited a decrease in the biomass invested in reproductive parts per unit of vegetation biomass (Figs 3b,d and 4d). It is thus concluded that maintaining competitive ability in fertilized communities is achieved partly through size-independent changes in RA.

Many authors have reported a reduced RA in heavily grazed plants (Crawley 1985; Belsky 1986; Whitham *et al.* 1991). It has been shown that grazed ecotypes exhibit smaller RA than conspecific non-grazed ecotypes in several grass species (Jaramillo & Detling 1988; Smith 1998). Our results were not consistent with these patterns, since for a given plant size the RB of most species is higher in grazed plots compared to control plots (Figs 3a,c and 4c). This pattern is noticeable within and across species (Figs 3 and 5). Most species respond to grazing by decreasing their reproductive threshold, that is, by an earlier flowering and fruiting. This response was confirmed by our phenological observations (unpublished). Increased light availability in the grazed plots might promote the developmental shift of meristems to inflorescences and flowers. Apart from severe abiotic constraints, alpine plants from the Tibetan Plateau have experienced a long history of grazing. As predicted in r–K theory, this could explain that perennial plants may have evolved to semelparity, with increased seed production and enhanced seed dispersal, that is, a regenerative trait syndrome that might improve the colonization of new patches of disturbed habitat in heavily grazed conditions (Schaffer 1974; Bazzaz *et al.* 1987; Ranta *et al.* 2000).

#### SIZE-INDEPENDENT CHANGES OF RB

Our results confirmed that RB is strongly dependent on individual size. RA was found to be positively or negatively related to individual size. This was in agreement with previous studies of size-dependent RA (reviewed in Reekie & Bazzaz 2005). Theory predicts that plants could respond to changing environments not only by a size-dependent effect, but also by a size-independent effect (called ‘true plasticity’ in other studies) (Weiner 2004). Here, we found that many species responses included significant changes in allometric coefficients (either slope or intercept), and thus did not solely reflect a size-dependent effect. This means that plants of the same size do differ in their RB because of land use. Our results are consistent with other empirical evidence showing that variation in RB is explainable by the effects of environment on plant biomass partitioning (Ackerly & Jasienski 1990; Sugiyama & Bazzaz 1998; Müller *et al.* 2000). For example, Ericsson (1995) found that *Betula pendula* seedlings can change the shoot : root allocation along a nutrient gradient. A plastic trade-off between vegetative and RB controlled by light and nutrients was also found in a *Plantago* species (Reekie & Sakar 2005). On the

other hand, there have been contrasting views suggesting that these trade-offs between reproductive and VB are relatively stable across different environments (Stearns 1989; Wilson & Thompson 1989; Vincent, Van & Goh 1996). Indeed, from a quantitative perspective, VB may account for many of the variations observed in RB when compared to the effects of environment (Müller *et al.* 2000; Weiner 2004). Yet the ability to modify biomass partitioning among environments may be of evolutionary significance. For example, this variability may serve to buffer populations from environmental changes, thus reducing the risk of extinction (Ghalambor *et al.* 2007).

#### COMPARISON OF FORBS AND GRAMINOIDS

Many studies have shown that forbs and graminoids do not respond similarly to grazing and fertilization (reviewed in Vesik & Westoby 2001; Suding *et al.* 2005). These studies focused on abundance and trait values of different functional groups, but did not examine differences in allometric patterns. Here, we first showed that the changes in VB in response to land use are much more pronounced for graminoids than forbs. Plant morphological constraints may explain these differences. In grazed plots, graminoids may be more affected because of their taller stature or better edibility. In fertilized plots, graminoids benefited more from the supply of new resources, as also reported in many other studies. However, these stronger responses in term of VB did not translate into stronger shifts of RA. Overall, forbs and graminoids showed the same allometric relationships, whatever the land use. In terms of differences, there was only a slight tendency towards a more pronounced reduction of VB–RB allometric exponent for graminoids in grazed plots (Fig. 2c). This might be explained by the greater ability of graminoids to produce new leaves when heavily grazed. This regrowth would be at the expense of reproductive tissues, but, on the other hand, would ensure efficient clonal propagation (Diaz *et al.* 2007). This contrasting response to grazing between forbs and graminoids may also explain why the allometric intercept of the VB–RB relationship between species is significantly higher in grazed plots, as compared to control plots, for forbs, but not for graminoids (Fig. 5).

#### Conclusion

To our knowledge, this is the first comparative analysis addressing changes in RA for most of the component species of grassland. Our results provided evidence that allometric relationships between vegetative and RB vary significantly with land use. Most of the size-independent variations were driven by changes in the allometric intercept of the VB–RB relationship, though the allometric exponent of many species also shifted in response to grazing or fertilization. Large scale comparative studies of plant growth allometry should better take into account the impact of environmental factors on these allometric coefficients. We advocate that a size-independent effect on RA should be considered in an evolutionary perspective. Further studies are needed to understand how this integrated

level of variability increases plant individual fitness and affects population dynamics in the context of land use changes.

## Acknowledgements

We are grateful to S. Lavorel, F. de Bello and all the staff of the TDE research team for their valuable discussion. We thank C. W. Fox, S. D. Wilson and two anonymous reviewers for their helpful comments on previous versions of this manuscript. Invaluable laboratory help was provided by Y. J. Luo, S. T. Zhao, Z. G. Zhao, X. H. Zhou, P. Jia and all the staff at MaQu Rangeland Workgroup. The study was supported by the Key Project of the National Natural Science Foundation of China (Granted No. 90 202 009).

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Received 16 July 2008; accepted 2 October 2008  
 Handling Editor: Scott Wilson

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Table S1.** List of investigated species.

**Table S2.** Effects of grazing and fertilization on vegetative biomass and on allometric parameters of the VB–RB relationship.

**Fig. S1.** Comparative performance of the two-parameter models.

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