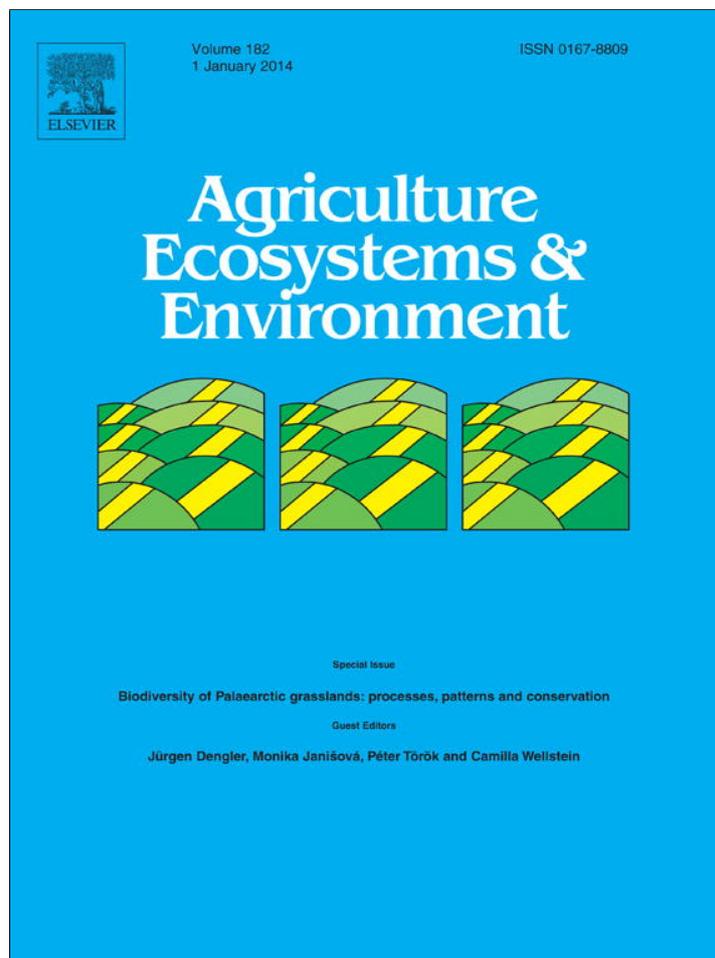


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# Fertilization decreases species diversity but increases functional diversity: A three-year experiment in a Tibetan alpine meadow



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## ABSTRACT

The link between biodiversity and grassland productivity remains a central puzzle for both theoretical and applied ecologists. Little is known about the response of functional diversity (FD, i.e. the extent of trait differences between organisms in a given community) to increased grassland productivity following fertilization. Species diversity is often used as a surrogate for FD but evidence shows that FD is often independent from species diversity.

We conducted a three-year (2004–2006) experiment in a Tibetan alpine grassland to examine the effect of fertilization on FD and SD, and its relationship with productivity. We measured specific leaf area, plant height and seed size for 32 species in control, low- and high-fertilization treatments over three years, and quantified three components of FD: functional richness (FRic), functional evenness (FEve) and functional divergence using Rao's index (FDrao).

While SD decreased rapidly, as expected, both FRic and FDrao increased significantly with fertilization. Neither species evenness nor FEve was affected by fertilization. Aboveground biomass was not significantly correlated with SD, but it was significantly positively correlated with both FRic and FDrao, with the strongest correlations found in 2006 for FRic and in 2005 for FDrao.

The results showed that FD and SD have opposite responses to short-term fertilization. The increased FD suggests enhanced niche differentiation between species remaining after fertilization, which caused species loss. This study highlights the importance of examining FD in assessing fertilization-induced biodiversity loss and its impacts on ecosystem functioning in grasslands.

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## 1. Introduction

It is generally accepted that fertilization causes plant species diversity loss in communities and increases productivity rapidly in semi-natural grasslands (Suding et al., 2005; Clark et al., 2007; Chalcraft et al., 2008). Although various hypotheses have been proposed to explain the underlying mechanisms of biodiversity loss (Grime, 1973; Newman, 1973; Huston, 1979; Stevens and Carson, 1999; Rajaniemi et al., 2003; Hautier et al., 2009) and increased productivity (Gough et al., 2000; Hooper et al., 2005; LeBauer and Treseder, 2008; Craine and Jackson, 2010), attempts

to link biodiversity to productivity following fertilization are rare (but see Mason et al., 2011; Pakeman, 2011). The consequences of fertilization-induced species loss for ecosystem functioning, therefore, remain unclear.

Functional trait diversity (FD, i.e. the extent of trait differences between species in a given community), rather than species diversity (SD), is directly linked with ecosystem functioning (e.g. productivity). Previous studies have used SD as a surrogate for FD and suggested that FD will decrease with species loss following fertilization (Tilman and Downing, 1994; Hooper et al., 2005; Moonen and Barberi, 2008). A growing body of evidence has shown that FD is not congruent with SD following land use change (Diaz et al., 2007; Flynn et al., 2009; Doležal et al., 2011; Pakeman, 2011). Over the last decade, there has been an increasing interest in estimating FD with various functional trait diversity. By incorporating information on functional trait values and distributions, FD not only takes

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into account the functional differences between species, but also incorporates functional trait variation between populations and across individuals within some populations (Mason et al., 2005; Lepš et al., 2006; Lepš et al., 2011). As such, examining FD in contrasting habitats has been proposed as a promising way to understand trait-based community assembly (Mouchet et al., 2010; Spasojević and Suding, 2012; Mason et al., 2013) as well as identify the consequences of species loss due to land use change for ecosystem functioning (Cadotte et al., 2011; Wellstein et al., 2011).

Previous studies in Tibetan grasslands showed that fertilization increased aboveground biomass, and decreased SD due to increased competition for both light and nutrients (Luo et al., 2006; Niu et al., 2008, 2009; Li et al., 2011) or water limitation (Bagchi and Ritchie, 2010). We hypothesized that changes in FD following fertilization would result from two antagonistic mechanisms. On the one hand, FD will decrease because of competitive exclusion and species loss (Leibold, 1997; Grime, 2006; Mayfield and Levine, 2010). On the other hand, trait divergence, the tendency of species to be functionally different from each other to limit competition between them, will increase because of stronger competition and niche differentiation between persisting species (MacArthur and Levins, 1967; Navas and Violle, 2009; Mouchet et al., 2010; Mason et al., 2011). Ultimately, the FD response to fertilization will depend on the balance between these two mechanisms. Theoretically, a maximal FD would be found at intermediate levels of competition (Navas and Violle, 2009). Accordingly, we assumed that FD will increase in short-term fertilization due to increased niche differentiation despite species loss. We also hypothesized that FD could have a stronger positive correlation with community productivity than SD because it is directly linked to trait-based effects on ecosystem functions (Grime, 1998; Lavorel and Garnier, 2002; Gross et al., 2007). To test these hypotheses, we quantified three components of FD (functional richness, Rao's index of functional divergence, and functional evenness) in response to fertilization. We measured specific leaf area, mature plant height, and seed size, which indicate three key dimensions of life history and ecological strategy (Westoby, 1998; Westoby et al., 2002). These traits were measured on constituent species from both control and fertilized communities.

## 2. Material and methods

### 2.1. Study site

The experiment was conducted in the MaQu branch of the Alpine Meadow and Wetland Ecosystems Research Station of Lanzhou University (N 33°59', E 102°00', and altitude 3500 m). The site is located in MaQu County, in the eastern part of the Tibetan Plateau, Gansu province, China. The mean annual temperature is 1.2 °C, ranging from –10 °C in January to 11.7 °C in July. The mean annual precipitation from 1975 to 2010 was 620 mm, with rain occurring mainly during the short, cool summer. The annual duration of cloud-free solar radiation is about 2580 h. For further details about the field site see Niu et al. (2008, 2009).

### 2.2. Experimental design

A flat 13 ha alpine grassland was enclosed within 58 ha of fenced grassland in October 1999. During the years of the experiment, grazing was allowed in the enclosure only during the non-productive winter months. In late May 2004, thirty 5 m × 8 m plots, each separated by 2 m, were laid out in a 5 m × 8 m area of relatively homogeneous grassland at the enclosed site (SFig. 1a in supplementary material). We randomly allocated control, low- and high-fertilizer addition treatments (30 and 60 g fertilizer,

respectively, per square meter for the latter two) to these plots. Hence, each treatment was replicated ten times (arrangement of randomized plots as in SFig. 1b). 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) was hand-broadcast once annually at the end of May during drizzly days to avoid the need for watering. Each plot was divided into two parts: a 5 m × 5 m subplot for measurements of plant traits and a 5 m × 3 m subplot for community monitoring and biomass harvest (SFig. 1c). In a previous study, we reported that photosynthetically active radiation 10 cm above the soil surface decreased 50–70%, while soil available nitrogen increased 36–66%, in fertilized plots relative to control (Luo et al., 2006; Niu et al., 2009).

### 2.3. Community and trait measurements

In the middle of September 2004, 2005 and 2006, a 0.5 m × 0.5 m quadrat was harvested within the 5 m × 3 m subplot of each plot. Harvested quadrats were located in different places each year. The number of individuals was counted for each species before clipping. For clonal plants, the term “individual” refers to ramets (Luo et al., 2006; Niu et al., 2008, 2009). These are functionally equivalent to tillers in grasses and rosettes or rooting branches in forbs. Aboveground green parts (stems and leaves) were sorted by species and brought to the laboratory. Community aboveground biomass was calculated by summing all dried biomass of harvested individuals within a quadrat.

Based on previous studies, we measured functional traits on the 32 most common species in control, low, and high fertilized plots. These species accounted for 85–95% of the aboveground biomass and 80–90% of the vegetation cover of the total plant community (Luo et al., 2006; Niu et al., 2008, 2009). The Leaf-Height-Seed scheme proposed that three key functional traits—specific leaf area (SLA), mature plant height and seed size—could capture the main axes of variation in ecological strategies between species (Westoby, 1998; Westoby et al., 2002). Accordingly, we chose SLA, mature height and seed size to estimate key dimensions of ecological strategies. In August 2005 and 2006, we randomly sampled 2–3 individuals and mature leaves (one leaf per individual) at fruiting time for each of the 32 species in each 5 m × 5 m subplot to measure mature height and SLA, respectively (Cornelissen et al., 2003). The leaf was scanned to measure leaf area in the field before being dried at 80 °C for 48 h and weighed for biomass to the nearest 10<sup>–4</sup> g using a balance (made by Shanghai Yingpai Scales Co., Ltd). We also collected approximately 500 mature seeds from 20 to 30 individuals of each of the 32 species in the fenced control plots over the three years. Seeds have been found to have a relatively constant size within a species across environments in our long-term investigation. Collected seeds were dried at room temperature (approximately 15 °C). Three replicates of 100 dried seeds were weighed for each species.

### 2.4. Data analysis

We calculated the relative abundance of each species in each quadrat as a ratio of individuals of a given species to the total number of individuals of all species in the quadrat. In each quadrat, species richness, the Shannon index of species diversity, and species evenness were calculated based on the number of species and the number of individuals within each species. We calculated the means of SLA and mature height with 25–30 replicates for each species in control, low- and high-fertilized plots in 2005 and 2006. Since seed sizes were relatively constant, we calculated the means of seed size with 3 replicates in control plots for each species and used these means for fertilized treatments in each year. The means of measured traits in each treatment over three years are listed in the supplementary materials (STable 1). In 2004, we did not

**Table 1**  
Species diversity (SD) and functional diversity (FD) measures.

Index	Formula	Terms meaning	Reference
Species richness	$S = N$	$N$ : number of species	Casanoves et al. (2011)
Shannon index of SD	$H = - \sum_{i=0}^s p_i \ln(p_i)$	$i, j$ : species $i, j = 1, \dots, S$ $p_i$ : relative abundance of the $i$ th species	Casanoves et al. (2011)
Species evenness	$E = H/\ln(S)$		Casanoves et al. (2011)
Functional richness	Quickhull algorithm		Mouchet et al. (2010)
Rao index of FD	$Rao = \sum_{i=1}^{s-1} \sum_{j=i+1}^{s-1} d_{ij} p_i p_j$	$x_{it}$ : $t$ th trait value of $i$ th species $T$ : number of traits $d_{ij}$ : Euclidian dissimilarity between the traits of each pair of species $i$ and $j$ : $d_{ij} = \sum_{t=1}^T (x_{tj} - x_{ti})^2$	Mouchet et al. (2010)
Functional evenness	$FEve = \frac{\sum_{i=1}^{s-1} \min\left(\frac{PEW_i}{s-1}, \frac{1}{s-1}\right) - \frac{1}{s-1}}{1 - \frac{1}{s-1}}$	Partial weighted evenness: $PEW_i = \frac{EW_{bl}}{\sum_{b=1}^{s-1} EW_{bl}}$ Weighted evenness: $EW_{bl} = \frac{d_{ij}}{p_i + p_j}$ $bl$ : branch length	Mouchet et al. (2010)

measure SLA and mature height but instead used the mean trait values measured in 2005 and 2006 due to relatively small changes in trait values between years (STable 1 and Niu et al., 2008, 2009). Moreover, our study focuses on comparing FD between treatments based on interspecific trait variability, rather than intraspecific trait variability or comparing FD between years. It is assumed that species hierarchy was mostly well conserved for SLA and mature height across years and environments (Garnier et al., 2001; Kazakou et al., 2013).

Although various indices have been proposed to disentangle distinct components of FD, Mouchet et al. (2010) recently addressed the fact that most of them are redundant and they recommended using three independent components of FD—functional richness (FRic), functional evenness (FEve) and functional divergence (FDiv). Additionally, Rao's index of quadratic entropy (FDrao) has been widely used to indicate functional divergence of traits as it includes variance and functional dispersion and is strongly correlated with FDiv (Leps et al., 2006; De Bello et al., 2011). Accordingly, we chose FRic, FDrao and FEve to examine how different components of FD responded to fertilization. The calculation of diversity indices is summarized in Table 1. We used the fDiversity software program to calculate these indices using an Euclidean distance and an average linkage (Casanoves et al., 2011) after the traits had been standardized to ensure equal contribution of each trait.

We used linear mixed-effects models to examine the longitudinal (or year-to-year) changes in SD, FD and aboveground biomass for each treatment. The fixed-effects terms were treatment and year. The random-effects term was replicated plots for repeated measures. For all models tested, the observation unit was the plot-based estimate of SD, FD and aboveground biomass and the three levels of variation were (i) variation between treatments, (ii) variation between plots within a treatment and (iii) variation between years within a treatment and within a plot.

Linear mixed-effects models have flexibility in modeling covariance structures for repeated measures data (Zuur et al., 2009). To account for the within-plot time-dependent correlations, we tested three contrasting covariance structures: (i) a simple case in which there is no correlation between the repeated measures on the same plot, (ii) a constant correlation between repeated measures, irrespective of the considered years and (iii) a first-order

autoregressive covariance structure that assumes a constant correlation between year  $n - 1$  and year  $n$ . Model selection was based on the comparison of information criteria. Two criteria were used: the Akaike Information Criteria (AIC), and Schwarz's Bayesian Information Criteria (BIC). Given a set of competing models, we selected the one with the minimum AIC and BIC. For the mixed models, we used the lme functions of the lme4 package (Bates et al., 2011) developed for the statistical software R (R Development Core Team, 2012).

### 3. Results

#### 3.1. Effects of fertilization on aboveground biomass, SD and FD

Aboveground biomass increased by 32–36% and 53–54% in low- and high-fertilized plots relative to control, respectively, in 2005 and 2006 ( $F = 12.4$ – $18.9$ ,  $p < 0.001$ ). Species richness decreased by 16–24% in fertilized plots relative to control in 2005 and 2006 (Fig. 1a, Table 2). Shannon diversity decreased by 5–8% in fertilized plots relative to control in 2004 and 2005 (Fig. 1c, Table 2). Functional richness increased by 11–28% and 13–54% in low- and high-fertilized plots relative to control, respectively, in 2004, 2005 and 2006 (Fig. 1b, Table 2). Rao's index of FD increased by 8–27% in fertilized plots relative to control in 2004, 2005 and 2006 (Fig. 1d, Table 2). Neither species evenness nor functional evenness was affected by fertilization (Table 2). Rao's index of FD and species evenness significantly decreased from the first to the third year, irrespective of the level of fertilization, but neither functional richness nor Shannon diversity nor functional evenness significantly differed among the years (Table 2).

#### 3.2. Relationship between biodiversity and community biomass

Neither species richness (Fig. 2a) nor the Shannon index of SD (Fig. 2c) was positively correlated with aboveground biomass. Species richness was even negatively correlated with aboveground biomass in 2005. Contrastingly, both functional richness and Rao's index of FD were significantly positively correlated with aboveground biomass and the correlations were stronger in 2006 for functional richness (Fig. 2b) and in 2005 for Rao's index of FD (Fig. 2d).

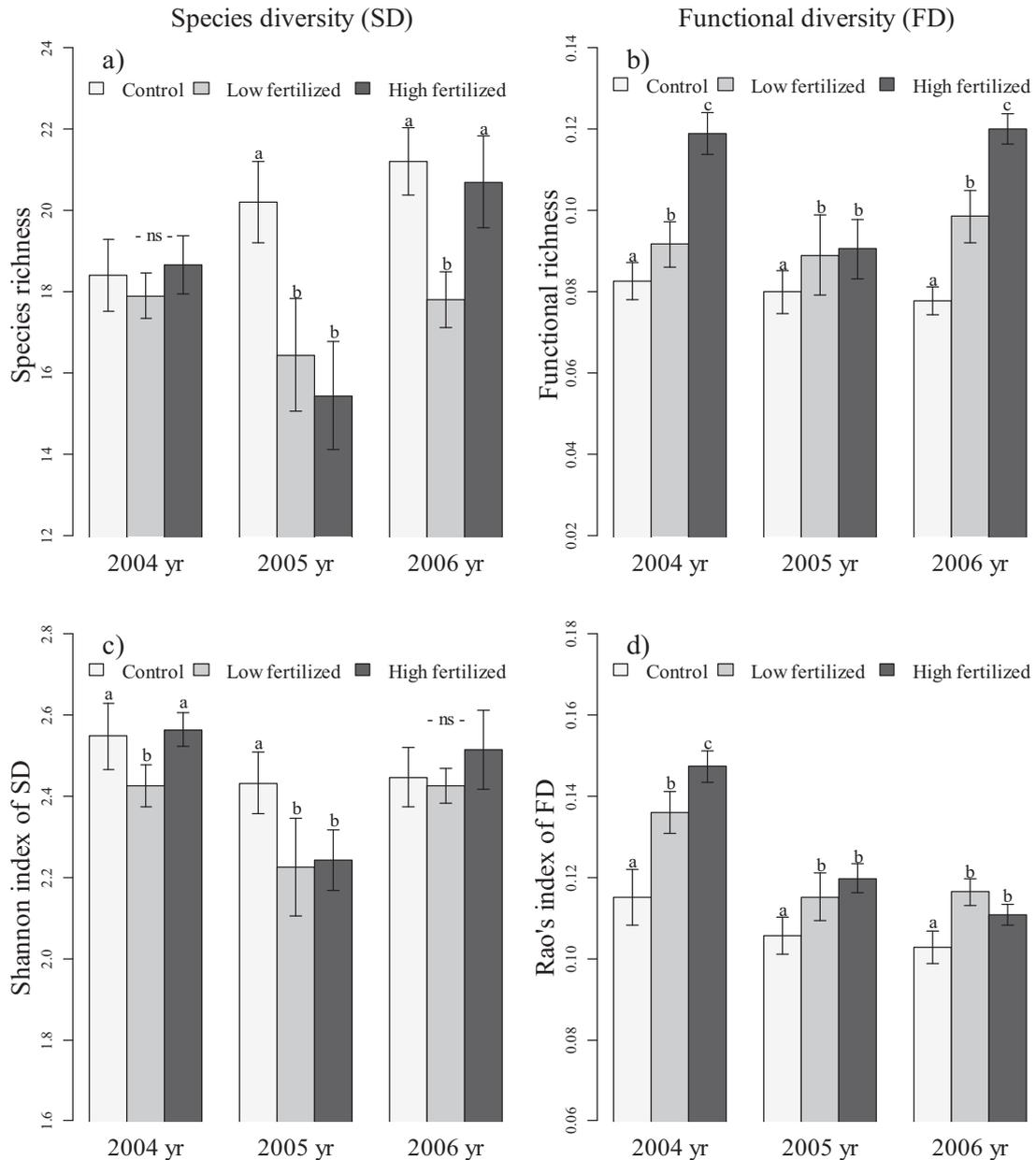


Fig. 1. The response of species diversity (a and c) and functional diversity (b and d) to low and high fertilization in 2004–2006 year.

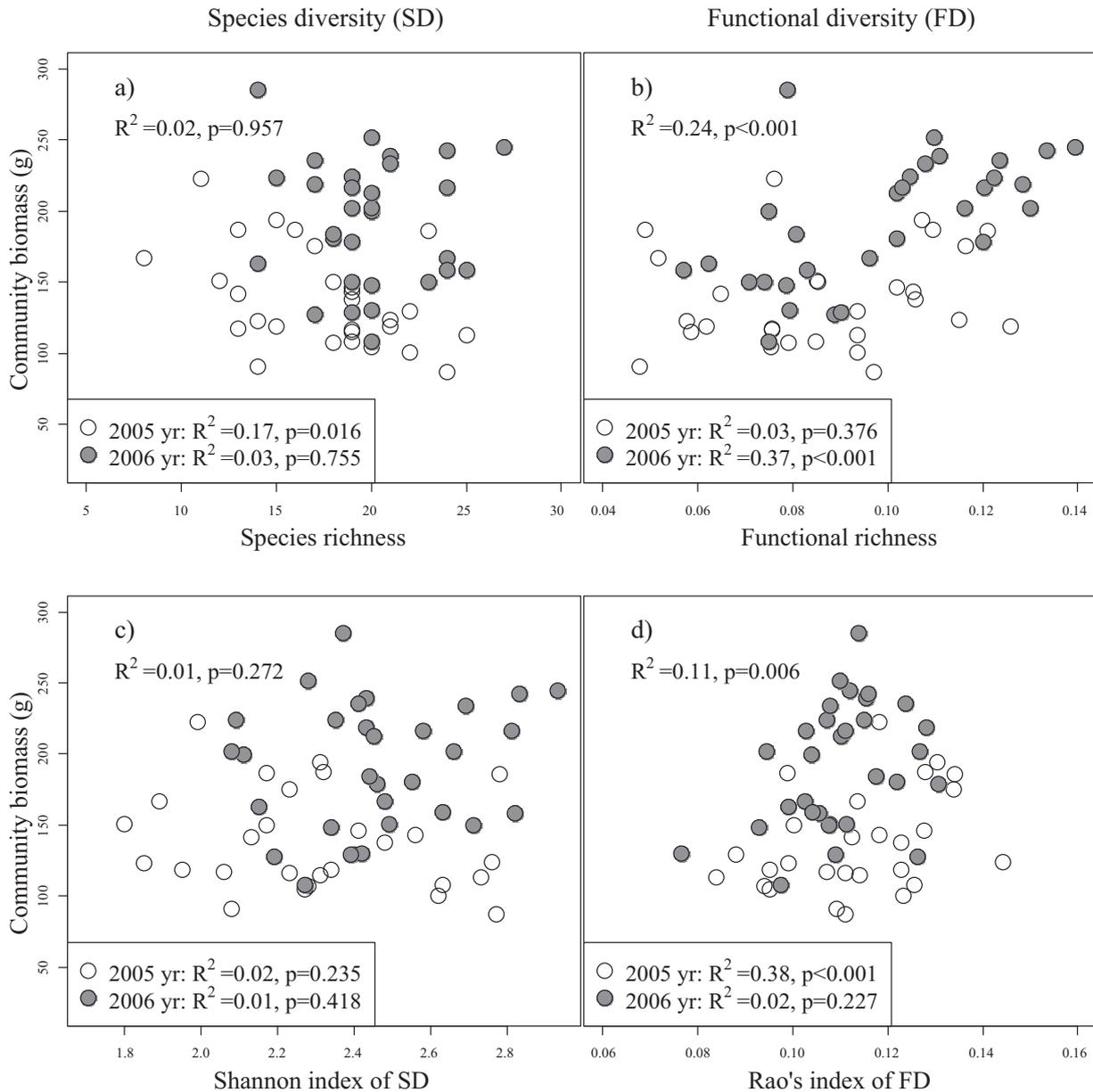
## 4. Discussion

### 4.1. FD increased following short-term fertilization

Previous studies have often used SD as a proxy for FD in examining the functional consequences of species loss following land use and climatic changes. Here we showed that FD and SD in Tibetan grasslands have opposite responses to short-term fertilization. The increase in FD indicated an increase in trait volume filled by the community in terms of mature height, seed size and specific leaf area. This suggests that the increased trait divergence between the persisting species outweighed the decreased trait diversity due to species loss. The increase in trait divergence mainly resulted from asymmetric changes in trait values among species in response to fertilization; e.g. the increase in mature height in dominant grasses was considerably larger than that of rare forbs. These asymmetric changes in trait values often caused increased niche differentiation and limited competition between persisting species in fertilized

plots. Additionally, the decrease of niche overlap may also have resulted from few species persisting in fertilized communities. The decrease of trait and species diversity was either a result of the loss of species with small individuals (Luo et al., 2006) or from the loss of inferior species under enhanced competition for light or soil resources (Niu et al., 2008; Li et al., 2011).

FRic was primarily influenced by changes in species trait values, e.g. significantly asymmetric changes in species height and SLA in response to fertilization, rather than by trait abundance (individual number of species) changes. As a result, FRic increased greatly in high-fertilized plots in the first and third years (Fig. 1b), during which few species disappeared (Fig. 1a) but asymmetric change in traits increased (STable 1). FDrao was highly sensitive to asymmetric changes in the numbers of individuals among species, which resulted in FDrao significantly increasing during the first year as many small individuals were lost (individuals-weighted Shannon index decreased, Fig. 2c), due to self-thinning following fertilization (Stevens and Carson, 1999; Luo et al., 2006). Consequently, in the



**Fig. 2.** The relationships between community biomass and species diversity (SD, a and c) or functional diversity (FD, b and d) overall (regression coefficient at the top) and in 2005 and 2006 year (regression coefficient in legend box).

third year, when many individuals were lost, the decrease in trait diversity was larger than the increase in trait divergence, and  $FD_{\text{Rao}}$  in high-fertilized plots fell. We expect that the communities would have hosted more functionally similar species (decreased FD) with few grass species dominating in fertilized plots if we had continued to apply fertilizer in the grassland after the third year, as predicted in previous studies (Grime, 2006). This indicates that increased FD following short-term fertilization may be a transient phenomenon. In early years, niche differentiation is a dominant assembly process in fertilized plots, while in the later years competitive exclusion may cause persisting species to become more functionally similar. In this light, our results support the hypothesis of Navas and Violle (2009) that FD will increase with competition and maximal FD will be found at intermediate levels of competition. Indeed, some recent studies also report increased trait diversity with niche differentiation or productivity (Doležal et al., 2011; Mason et al., 2011; Reich et al., 2012). In summary, by taking into account the considerably asymmetric change in species traits, we highlight the

importance of species adaptation and niche differentiation in biodiversity assembly in short-term fertilized grasslands. Although the results contradict previous predictions that fertilization should increase similarity in species composition between communities (Grime, 2006; Mayfield and Levine, 2010), we also expect that FD should decrease in fertilized grasslands in the longer term. To clarify the impact of fertilization on trait-based FD, long-term experiments are needed.

#### 4.2. FD positively correlated with aboveground biomass following fertilization

In natural and experimental communities, previous studies have documented that plant biodiversity is positively correlated with productivity due to functional complementarity and selection effects (Huston, 1979; Bai et al., 2004; Balvanera et al., 2006; Cardinale et al., 2007). In fertilized communities, researchers have often failed to explain the relationship between declining species

**Table 2**

Summary of linear mixed effect model for effects on fertilizations and years on species diversity and functional diversity.

Response/AIC/BIC	Fixed effects				
	Value	Std. error	DF	t-Value	p-Value
<i>Species richness/453.61/468.33</i>					
Intercept	19.95	0.50	57	39.70	<0.01
Low fertilized	-2.61	0.72	26	-3.63	<0.01
High fertilized	-1.83	0.73	26	-2.51	0.02
<i>Shannon index of SD/8.09/22.82</i>					
Intercept	2.47	0.03	57	76.01	<0.01
Low fertilized	-0.11	0.05	26	-2.42	0.02
High fertilized	-0.04	0.05	26	-0.81	0.42
<i>Species evenness/-244.58/-229.85</i>					
Intercept	0.85	0.01	56	67.81	<0.01
Years	-0.02	0.01	56	-2.76	0.01
Low fertilized	<0.01	0.02	26	0.00	1.00
High fertilized	0.02	0.02	26	1.03	0.31
<i>Functional richness/-425.68/-413.41</i>					
Intercept	0.08	<0.01	57	22.41	<0.01
Low fertilized	0.01	<0.01	26	2.58	0.02
High fertilized	0.03	0.01	26	5.86	<0.01
<i>Rao index of FD/-469.27/-454.54</i>					
Intercept	0.12	<0.01	56	35.04	<0.01
Years	-0.01	<0.01	54	-5.62	<0.01
Low fertilized	0.02	<0.01	26	3.80	<0.01
High fertilized	0.02	<0.01	26	4.51	<0.01
<i>Functional evenness/-212.54/-197.82</i>					
Intercept	0.66	0.02	56	44.7	<0.01
Years	-0.01	0.01	56	-0.88	0.38
Low fertilized	-0.03	0.02	26	-1.65	0.11
High fertilized	-0.01	0.02	26	-0.44	0.67

AIC, Akaike Information Criteria; BIC, the Schwarz's Bayesian Information Criteria; DF, degrees of freedom.

diversity and increased biomass (Hooper et al., 2005; Jiang et al., 2009). One of the key reasons is the discordance between SD and ecosystem functioning. Consistent with previous studies, our results clearly showed that SD does not positively correlate with aboveground biomass (Fig. 2a and c).

In our results, the correlation between FD and biomass production was either due to a significant FRic-biomass relationship in 2006 (Fig. 2b) or a significant FDrao-biomass relationship in 2005 (Fig. 2d). The discrepancy between FRic and FDrao may result from the different ways in which they handle trait abundance. A strong positive correlation between FRic and aboveground biomass suggests that species complementarity lead to increased productivity in years following fertilization. Species complementarity may have resulted from coexistence between abundant conservative species (e.g. dominant grasses with low SLA and relatively slow growth rates) and exploitative species (e.g. forbs with high SLA and fast growth rates). The increase in FRic advantaged persisting species more adequately use of light (Stevens and Carson, 1999) or even added soil nutrient (Rajaniemi et al., 2003) or water (Bagchi and Ritchie, 2010), which benefited for accumulation of community biomass following fertilization. Since FDrao is more affected by traits of dominant species, a significantly positive relationship between FDrao and aboveground biomass suggests that the mass (selection) effect caused community productivity to increase after fertilization (Grime, 1998; Lavorel and Garnier, 2002; Diaz et al., 2007). Following fertilization, many individual of forbs disappeared due to the increased competition, but grasses became larger, allocated more biomass to vegetative growth and were strong competitors for soil and light resources in fertilized plots (Luo et al., 2006; Niu et al., 2008). Our finding is consistent with previous long-term studies in experimental communities, which found that the selection effect was the main driver for increased productivity in

the early years, but the effects of complementarity increased over time (Fargione et al., 2007; Reich et al., 2012). This is also consistent with the observation that fertilized communities with high canopies and high leaf trait diversity often tend to have higher biomass in the later years.

In conclusion, our work highlights the importance of examining FD in assessing fertilization-induced biodiversity loss and its impacts on productivity in grasslands. Further long-term investigation is needed to evaluate the impact of nutrient enrichment on trait-based FD and its consequences for ecosystem functioning.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.agee.2013.07.015>.

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