

Plant functional diversity mediates the effects of vegetation and soil properties on community-level plant nitrogen use in the restoration of semiarid sandy grassland



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ABSTRACT

Species-rich plant communities use nitrogen (N) more efficiently in grassland ecosystems; however, the role of plant functional diversity in affecting community level plant N-use has received little attention. We examined plant N content, stock and N-use efficiency at community-level along a restoration gradient of sandy grassland (mobile dune, semi-fixed dune, fixed dune and grassland) in Horqin Sand Land, northern China. We used the functional trait-based approach to examine how plant functional diversity, reflected by the most abundant species' traits (community-weighted mean, CWM) and the dispersion of functional trait values (FDis), affected N-use efficiency in sandy grassland restoration. We further used the structure equation model (SEM) to evaluate the direct or indirect effects of plant species richness, biomass, functional diversity and soil properties on community-level plant N-use efficiency. We found that plant biomass and its N stock increased following sandy grassland restoration, and there were lower plant N content and higher N-use efficiency in semi-fixed dune, fixed dune and grassland as compared with mobile dune. N-use efficiency was positively associated with plant species richness, biomass, CWM plant height, CWM leaf C:N, FDis and soil gradient, but SEM results showed that species richness, CWM leaf C:N, plant biomass and FDis controlled by soil properties were the main factors exerting direct effects. CWM plant height also had a positive effect on N-use efficiency through its indirect effect on plant biomass. Soil gradient increased N-use efficiency through an indirect effect on vegetation rather than a direct effect. Final SEM models based on different plant functional diversity explained over 74% of variances in N-use efficiency. Effects of plant functional diversity on N-use efficiency supported both the mass ratio hypothesis and the complementarity hypothesis. Our results clearly highlight the important role of plant functional diversity in mediating the effects of vegetation and soil properties on community level plant N-use in sandy grassland ecosystems.

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1. Introduction

Nitrogen (N) is a primary limiting resource in determining net productivity in temperate grassland ecosystems (LeBauer and Treseder, 2008). Nitrogen-use efficiency (NUE) can be defined as the amount of biomass produced per unit of N taken up by plants from soil (Fornara and Tilman, 2009; Selman et al., 2013). NUE has

been widely used to analyze the response of plant growth to different N availabilities (Fan et al., 2013; Funk, 2013). Changes in NUE also play an important role in N cycling and retention in terrestrial ecosystems (Knops et al., 2002; Selman et al., 2013). Plants with high NUE were presumed to be advantageous in the environment of low N availability (Boerner, 1984; Aerts and de Caluwe, 1994), and high community-level NUE may mitigate the effect of N deposition on ecosystem (Selman et al., 2013). Numerous studies have also reported the complex effects of species identity (Epstein et al., 1998; Vázquez de Aldana and Berendse, 1997), species diversity (Hooper and Vitousek, 1998; Niklaus et al., 2006) and soil properties (Aerts and de Caluwe, 1994; Funk and Vitousek, 2007; Funk, 2013; Selman et al., 2013) on leaf or community-level N uptake and

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storage in natural ecosystems. The trait-based approach can clearly reveal the effects of plant functional diversity on plant community structure and function (Butterfield and Suding, 2013; Lavorel, 2013; Le Bagousse-Pinguet et al., 2014). However, our knowledge on responses of NUE to those biotic and abiotic factors is still limited. To reveal the driver of NUE is very important for the understanding of N storage, retention and cycling (Selmants et al., 2013; Wilkinson et al., 2015) and how plant adapts to environment with different N availability in natural ecosystems (Vázquez de Aldana and Berendse, 1997).

Plant species differing in their ability to capture, store and release N represent a major driver of N cycling in grassland ecosystems (Knops et al., 2002). Plant communities with higher species richness can use N more completely and efficiently due to species' complementarity in resource use (Tilman, 1997; Hooper and Vitousek, 1998; Mueller et al., 2013; Selmants et al., 2013). Fast-growing plants exhibit high specific leaf area and leaf N content (Kazakou et al., 2007). High plant NUE often resulted from both an increase in biomass and a decrease in leaf N content (An et al., 2005; Aerts and Chapin, 2000; McCulley et al., 2009). High leaf carbon-nitrogen ratio (C:N) is associated with high plant NUE (Aerts and Chapin, 2000; McCulley et al., 2009). Increased soil N availability often leads to a decline in plant NUE because of an enhancement of plant N concentrations (Boerner, 1984; Aerts and de Caluwe, 1994). Nutrient-rich habitats have fast-growing species with high rates of nutrient turnover and nutrient-poor habitats favor slow-growing species with low nutrient-loss rates (Funk and Vitousek, 2007; Fan et al., 2013; Funk, 2013). So, studying effects of plant diversity, plant C:N and soil fertility on community-level plant NUE to improve our understanding of how biotic and abiotic factors influence plant N use, which will in turn be helpful for the management of natural ecosystems.

A number of studies have shown that ecosystem function may be driven by plant functional diversity as reflected by the abundant functional trait values (community-weighted mean, CWM) and the variety of functional trait values (FD) (Butterfield and Suding, 2013; Schumacher and Roscher, 2009). The CWM effects may primarily be attributed to the biomass ratio hypothesis (De Deyn et al., 2008; Lavorel, 2013), while FD effects support the niche complementarity hypothesis (Gamfeldt et al., 2008; Lavorel and Garnier, 2002; Tilman, 1997). According to the mass ratio hypothesis (Grime, 1998), the role of species in the ecosystem is proportional to their biomass; therefore, the most abundant species have the strongest effects on ecosystem processes and properties (Conti and Díaz, 2013; Lavorel, 2013; Butterfield and Suding, 2013). Plant height, leaf dry matter content (LDMC), specific leaf area (SLA) and leaf C:N, as four important traits related to plant size, structural properties and tissue quality, greatly affect the plant growth rate, species richness and biomass (Dechaine et al., 2014; Spasojevic et al., 2014b). Functional dispersion (FDis) represents the mean space of each species, weighted by relative abundances, to the centroid of all species in the community (Spasojevic et al., 2014a), which may be regarded as a surrogate measure of functional richness and functional divergence. In particular, higher FDis may reflect the coexistence of different functional strategies and an increase in niche-complementarity among species (Schleicher et al., 2011; Spasojevic et al., 2014a), thus resulting in higher plant N use in the community. Therefore, examine roles of CWM traits and FDis in affecting ecosystem-level N use can provide profound insights into the mechanism of how plant functional diversity affects ecosystem processes and properties.

Horqin sandy grassland is located in the semi-arid area of south-east Inner Mongolia and is one of the most severely desertified regions of China (Zhao et al., 2005). However, thanks to annual precipitation of 350–500 mm, mobile dunes can be gradually stabilized via vegetative succession after excluding grazing (Liu et al.,

2009; Zhang et al., 2005; Zuo et al., 2009). Previous studies have documented that species richness, biomass, soil organic C, total N, electrical conductivity, very fine sand and silt and clay increased along a vegetation succession from sand pioneer plants in mobile dunes to low shrub communities in semi-fixed dunes, then toward annual herbaceous communities in fixed dunes (Liu et al., 2009; Zhang et al., 2005; Zuo et al., 2009, 2012b). Soil properties are the primary driver of plant distribution and community composition following sandy grassland restoration (Zuo et al., 2009). However, little is known about how community-level plant NUE responds to vegetation and soil properties along a restoration gradient of sandy grassland.

In this paper, we used the structural equation model (SEM) to assess the direct and indirect effects of plant diversity, biomass, functional diversity and soil properties on NUE in sandy grassland restoration. We also attempted to identify how CWM traits and FDis affect community-level plant NUE in sandy grassland ecosystems. Specifically, we tested the three hypotheses: (1) community-level plant NUE was positively associated with plant species richness in sandy grassland restoration; (2) high community-level plant NUE was associated with high leaf C:N of the most abundant species, because of a decrease of leaf N content following an increase of biomass in sandy grassland restoration; and (3) increasing functional dispersion (FDis) increased plant NUE due to the effect of niche-complementarity among species.

2. Methods

2.1. Site description

This study area is located in the southwest part of Horqin Sandy Land (42°55' N, 120°42' E; 360 m elevation), Inner Mongolia, Northern China. This area has a typical continental semi-arid climate with a warm summer and a very cold winter. The mean annual temperature is around 6.4 °C and mean annual precipitation is 360 mm, with 75% of the total in the growing season of June to September. Soils that consist of coarse and fine sand with loose structure are vulnerable to wind erosion. The pattern of natural vegetation is characterized by a mosaic of sand dunes and grasslands, including mobile dunes, semi-fixed dunes, fixed dunes and grasslands (Liu et al., 2009; Zuo et al., 2012a). Mobile dunes are dominated by sand pioneer plants, *Agriophyllum squarrosum* (an annual forb). Semi-fixed dunes are dominated by the low shrub, *Artemisia halodendron*. An annual forb, *Artemisia scoparia*, is the dominant plant in fixed dunes. Grasslands are dominated by an annual forb, *A. scoparia* and perennial grasses, *Phragmites communis* and *Pennisetum centasiaticum*.

Within the study area, 24 plots (20 m × 20 m) up to 8 km apart were selected to span a typical restoration gradient of sandy grassland, including mobile dune with less than 10% vegetation cover (MD), semi-fixed dune with 10–60% vegetation cover (SFD), fixed dune with more than 60% vegetation cover (FD) and grassland with more than 60% vegetation cover (G) (Liu et al., 2009; Zuo et al., 2012b). Each habitat type had six replicate plots. Semi-fixed dunes and fixed dunes were naturally restored from mobile dunes by excluding grazing, from approximately 1995 and 1980, respectively. Before grazing exclusion, the landscape of these dune sites was characterized by areas with mobile dunes. Grassland sites also excluded livestock grazing by fencing to restore vegetation from 1996, thus grassland represents a relatively good vegetation type in this region.

2.2. Sampling and measurement

In mid-Aug 2013, five 1 m × 1 m quadrats were set up at the center and the four corners in each plot to carry out vegetation survey

and soil sampling. We estimated vegetation cover and recorded the number of plant species within each quadrat. Then we harvested the above-ground biomass of each species and collected litter in each quadrat. Above-ground plant biomass and litter were dried at 60 °C for 48 h and weighed in the lab. The biomass of each species and litter in each quadrat were ground by a mill, and analyzed for carbon (C) and nitrogen (N) contents by an elemental analyzer (vario Macro cube, Elementar, Germany). Plant biomass N stock (g N m^{-2}) was calculated from both N content (%) in plants and their biomass. We calculated community-level plant N-use efficiency (NUE) as above-ground biomass produced per unit mass of N (Fornara and Tilman, 2009; Selmants et al., 2013).

We collected three random soil cores at depth of 0–10 cm within the quadrat using a 3 cm-diameter soil auger and pooled these to form one composite sample for laboratory analysis. Concurrently, one soil core was taken to measure soil water content within the quadrat using the same auger. Other soil samples were collected for soil bulk density using a soil auger equipped with a stainless-steel cylinder (5 cm in both diameter and height). Soil textures from international and USDA classification systems were determined by the wet sieving method (Li et al., 2012). Soil pH and electrical conductivity (EC) were determined in a 1:1 soil-water slurry and in a 1:5 soil-water aqueous extract (Multiline F/SET-3, Germany), respectively. Soil total C and N were determined by an elemental analyzer (vario Macro cube, Elementar, Germany). Vegetation characteristics and soil properties at four different habitats are summarized in Table S1.

Environmental soil variables from 24 plots (see Table S2 in Supporting Information) were analyzed using a principal component analysis (PCA) along the restoration gradient of sandy grassland (Le Bagousse-Pinguet et al., 2014; Zuo et al., 2012a). The results of PCA indicated that the first axis could explain over 86% of the total variability. Hereafter, we refer to plot scores along the first PCA axis (soil PCA) as 'soil gradient', which was positively correlated with total soil carbon, nitrogen, C/N, pH, EC, very fine sand, silt and clay and soil water content and negatively with soil bulk density and coarse sand. Soil PCA was used in all subsequent statistical analyses as a single variable representing soil conditions.

2.3. Plant functional traits and diversity

Four key traits related to resource use and plant growth were chosen to characterize the vegetative and regenerative phases. For the 34 most abundant species (representing over 90% of plant biomass from 45 species), 5–10 individuals of each species were collected in each quadrat. 10–20 leaves per species in each quadrat were taken for the determination of leaf traits (Cornelissen et al., 2003). For each individual sample, specific leaf area (SLA), leaf dry matter content (LDMC), leaf C:N and plant height were measured by using the standard methodologies (Conti and Díaz, 2013; Cornelissen et al., 2003; Spasojevic et al., 2014b). Single-trait (community-weighted mean, CWM) and multi-trait indices (function dispersion, FDis) were used to calculate functional diversity using the FDiversity package (Butterfield and Suding, 2013; Casanoves et al., 2011; Spasojevic et al., 2014b). CWM showing the trait values of the most abundant species was simply calculated as the abundance-weighted mean trait value for a community (Butterfield and Suding, 2013; Violle et al., 2007): $\text{CWM}(\text{trait}_x) = \sum p_i x_i$, where CWM (trait_x) is the CWM for a X trait, p_i is the relative biomass of the i -th species in the community and x_i is the trait value of i -th species. FDis showing the degree of trait dissimilarity among species was measured as the multiple traits dispersion within the functional volume of each plant community and calculated from the standardized four traits (Schleicher et al., 2011). The detailed formula of FDis was described by Casanoves et al. (2011).

2.4. Statistical analysis

We first used 24 plots to test correlations among soil PCA, vegetation characteristics and functional diversity in sandy grassland restoration. Then we used linear regression analyses to obtain the most important predictors of community-level plant NUE. Finally, to investigate the direct and indirect effects of the combination of factors on NUE, we used the structural equation model (SEM), a technique well-suited to evaluating relationships among networks of variables (Spasojevic et al., 2014b; White et al., 2014; Zuo et al., 2012a). A complex initial model was created to show all significant variables from plausible interaction pathways. We removed non-significant variables to find the model with the lowest AIC (Lamb, 2008; Spasojevic et al., 2014b; Zuo et al., 2012a). We assessed the model fit with a Chi-square test ($P > 0.05$), root mean square error of approximation (RMSEA < 0.05) and goodness-of-fit index (GFI > 0.95) (Lamb, 2008; White et al., 2014).

All data were expressed as mean \pm 1 SE ($n = 6$) and the analysis of variance (ANOVA) was across the plot level. All difference statistical tests were carried out using SPSS (version 16.0). All functional diversity indices were calculated using the statistical package FDiversity v. 2011 (Casanoves et al., 2011). The structural equation modeling was conducted using AMOS 20.0 software.

3. Results

We found that habitat changes following sandy grassland restoration had a significant effect on plant biomass, N content and storage in plant biomass and plant NUE at community-level (Fig. 1, $P < 0.001$). Plant biomass and its N storage increased along the restoration gradient of sandy grassland. Plant N content in mobile dune was higher than that in semi-fixed dune, fixed dune and grassland, whereas NUE showed a reverse trend (Fig. 1). Plant N content and NUE did not differ among semi-fixed dune, fixed dune and grassland ($P > 0.05$).

Habitat changes following sandy grassland restoration had significant effects on four community-weighted mean traits and functional dispersion (Table 1, $P < 0.05$). Plant height increased along the restoration gradient of sandy grassland. SLA and LDMC in semi-fixed dune were lower than mobile dune, fixed dune and grassland. Leaf C:N in mobile dune was higher than semi-fixed dune, fixed dune and grassland. FDis in grassland was higher than mobile dune and fixed dune, but no difference between semi-fixed dune and fixed dune. So, plant functional traits and diversity had strong responses to habitat changes following sandy grassland restoration.

Correlation analyses indicated that there were important relationships among vegetation characteristics, functional diversity and soil properties (Table 2). Soil gradient was significantly and positively associated with vegetation characteristics and functional diversity components (Table 2, $P < 0.05$). There were significantly positive correlations among plant species richness, biomass, biomass N storage, plant height, leaf C:N and FDis ($P < 0.05$). We also found that FDis represented different dimensions of functional compositions (Table 2). Soil gradient, functional diversity components and vegetation characteristics significantly explained community-level plant N use (Fig. 2). NUE was significantly and positively correlated to soil gradient, species richness, plant biomass, plant height, leaf C:N and FDis ($P < 0.05$).

Based on above analyses, we created the initial structural equation model (SEM) to describe their influencing relationships among soil gradient, vegetation characteristics, functional diversity components and NUE (Fig. 3a). Final SEM models based on different functional diversity components provided the best fit and explained over 74% of variances of NUE, including plant height

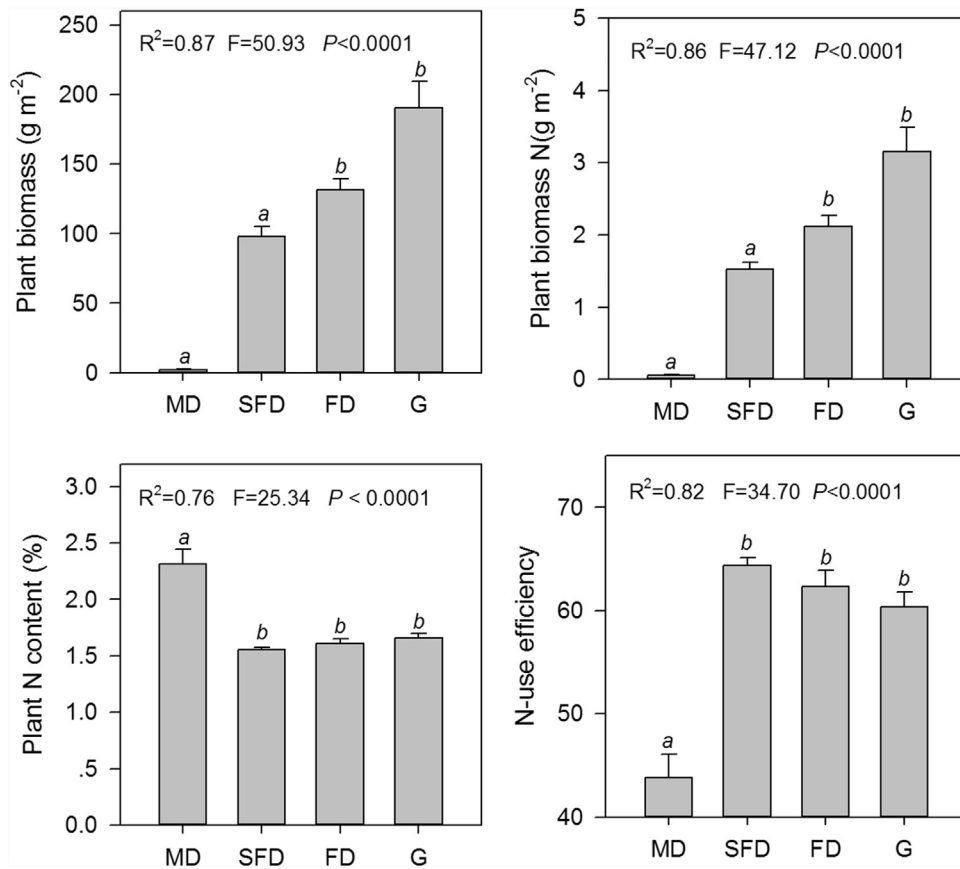


Fig. 1. Comparisons of plant biomass, nitrogen content and storage in plant biomass and community-level nitrogen-use efficiency at four habitats of sandy grassland. Values represent means \pm standard errors ($n=6$). Different letters in mean values indicate statistical difference among different habitats at $P<0.05$.

Table 1

Functional diversity components at four habitats of sandy grassland (mean \pm SE, $n=6$).

	MD	SFD	FD	G	F	P
<i>Community weighted means (CWM)</i>						
Plant height (cm)	9.23 \pm 0.99 ^a	24.95 \pm 2.63 ^b	43.12 \pm 2.11 ^c	64.80 \pm 5.81 ^d	49.66	<0.001
SLA (m ² kg ⁻¹)	16.83 \pm 1.58 ^a	12.93 \pm 0.85 ^b	19.12 \pm 0.56 ^a	19.48 \pm 0.77 ^a	8.82	<0.01
LDMC (g kg ⁻¹)	261.12 \pm 9.76 ^a	207.68 \pm 9.43 ^b	270.42 \pm 11.33 ^a	279.98 \pm 10.22 ^a	10.03	<0.001
Leaf C:N	14.72 \pm 0.65 ^a	20.11 \pm 0.96 ^b	19.79 \pm 0.44 ^b	20.23 \pm 0.87 ^b	12.38	<0.001
<i>Multi-traits functional diversity index</i>						
Functional dispersion	0.02 \pm 0.01 ^a	0.08 \pm 0.02 ^{bc}	0.05 \pm 0.01 ^{ac}	0.10 \pm 0.01 ^b	8.56	<0.01

MD, mobile dune; SFD, semi-fixed dune; FD, fixed dune; G, grassland; SLA, specific leaf area; LDMC, leaf dry matter content, C:N, carbon-to-nitrogen ratio. Different letters in from mean values indicate statistical difference among different habitats at $P<0.05$.

(Fig. 3b, $R^2=0.77$, $\chi^2=0.87$, $P=0.65$; RMSEA=0.00; GFI=0.99), leaf C:N (Fig. 3c, $R^2=0.81$, $\chi^2=0.05$, $P=0.82$; RMSEA=0.00; GFI=1.00) and function dispersion (Fig. 3d, $R^2=0.75$, $\chi^2=2.18$, $P=0.54$; RMSEA=0.00; GFI=0.96). Three final models were found to

indicate the positively direct effects of species richness and plant biomass on NUE ($P<0.01$), while soil gradient increased NUE via its indirect effects on community structures (Fig. 3b–d, Table 3). Increasing plant height, leaf C:N and function dispersion increased

Table 2

Correlation coefficients among soil PCA, vegetation characteristics and function diversity in sandy grassland restoration.

	Soil PCA	Species richness	Plant biomass	Biomass N storage	SLA	LDMC	Height	Leaf C:N
Species richness	0.68**							
Biomass	0.85**	0.69**						
Biomass N storage	0.87**	0.67**	0.99**					
SLA	0.52**	0.37	0.25	0.27				
LDMC	0.50*	0.15	0.21	0.24	0.37			
Height	0.93**	0.65**	0.96**	0.97**	0.40	0.36		
Leaf C:N	0.51*	0.65**	0.67**	0.64**	-0.01	-0.03	0.57**	
Function dispersion	0.53**	0.42*	0.48*	0.49*	0.06	-0.04	0.44*	0.72**

SLA, specific leaf area; LDMC, leaf dry matter content, C:N, carbon-to-nitrogen ratio.

* $P<0.05$.

** $P<0.01$.

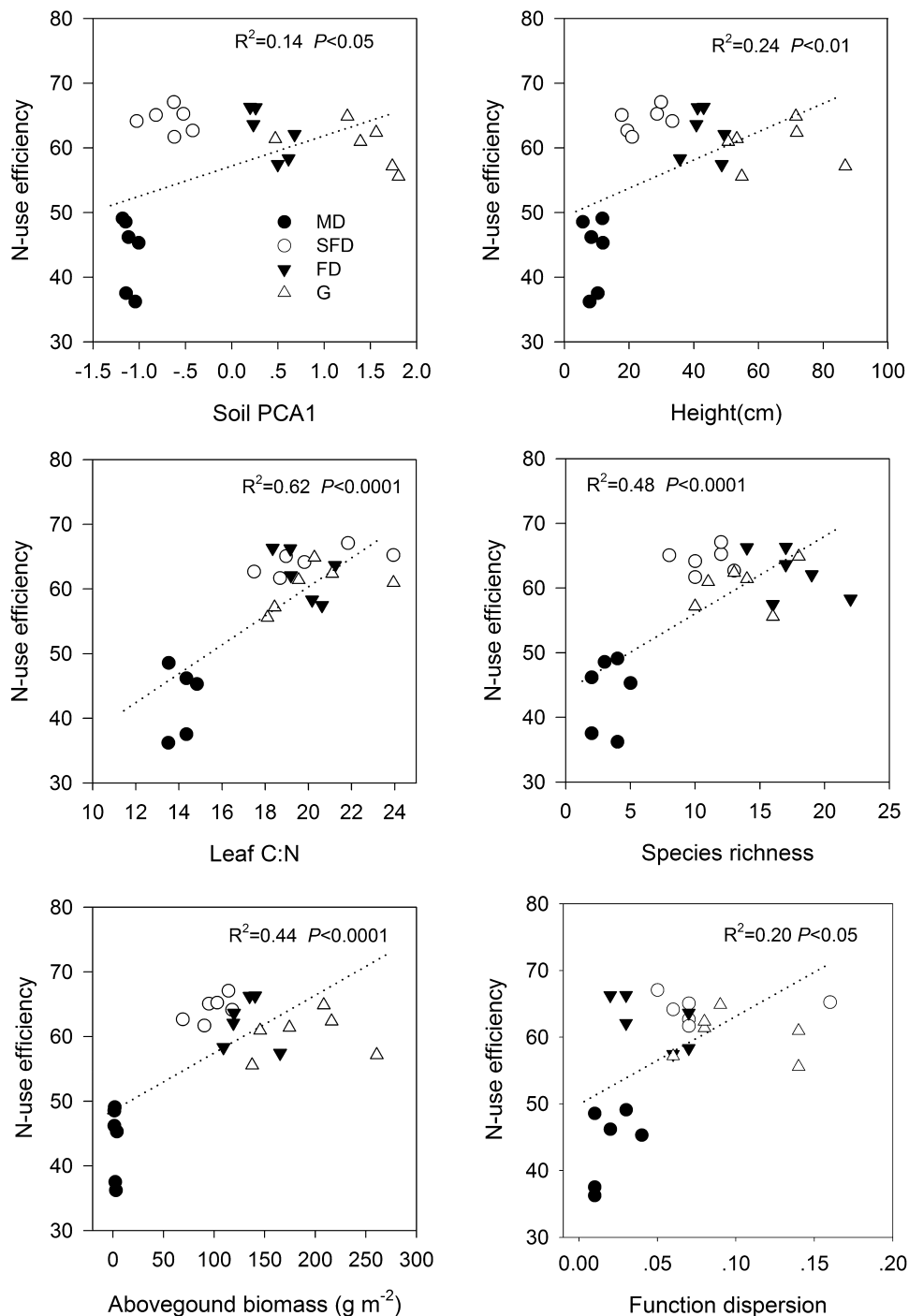


Fig. 2. Simple linear regression analyses of soil PCA, plant height, leaf C:N, species richness, plant biomass and function dispersion with community-level nitrogen-use efficiency in sandy grassland restoration. MD, mobile dune; SFD, semi-fixed dune; FD, fixed dune; G, grassland.

NUE through different pathways, e.g. plant height through its direct effect on plant biomass, leaf C:N through both its direct and indirect effect on species richness and plant biomass, and function dispersion through its direct effect (Table 3). In addition, the direct effects of plant height and leaf C:N on plant biomass indicated that CWM traits controlled by soil properties could explain changes of plant biomass in sandy grassland restoration.

4. Discussion

This study demonstrates a first attempt to assess the role of plant functional diversity controlled by soil properties in

affecting community-level plant NUE in sandy grassland restoration. Our study is consistent with other findings that plant N concentration has important implications on its NUE, with increasing NUE at lower plant N concentrations (Aerts and Chapin, 2000; McCulley et al., 2009). Our results supported three hypotheses that NUE was affected by species richness, leaf C:N of the most abundant species and function dispersion (Fig. 3, Table 3). Effects of species richness, CWM leaf C:N and function dispersion on NUE were direct rather than indirect, while CWM plant height and soil properties acted indirectly (Table 3). These results have suggested that both the mass ratio hypothesis and the complementarity hypothesis can better explain the NUE in sandy grassland restoration. Results from

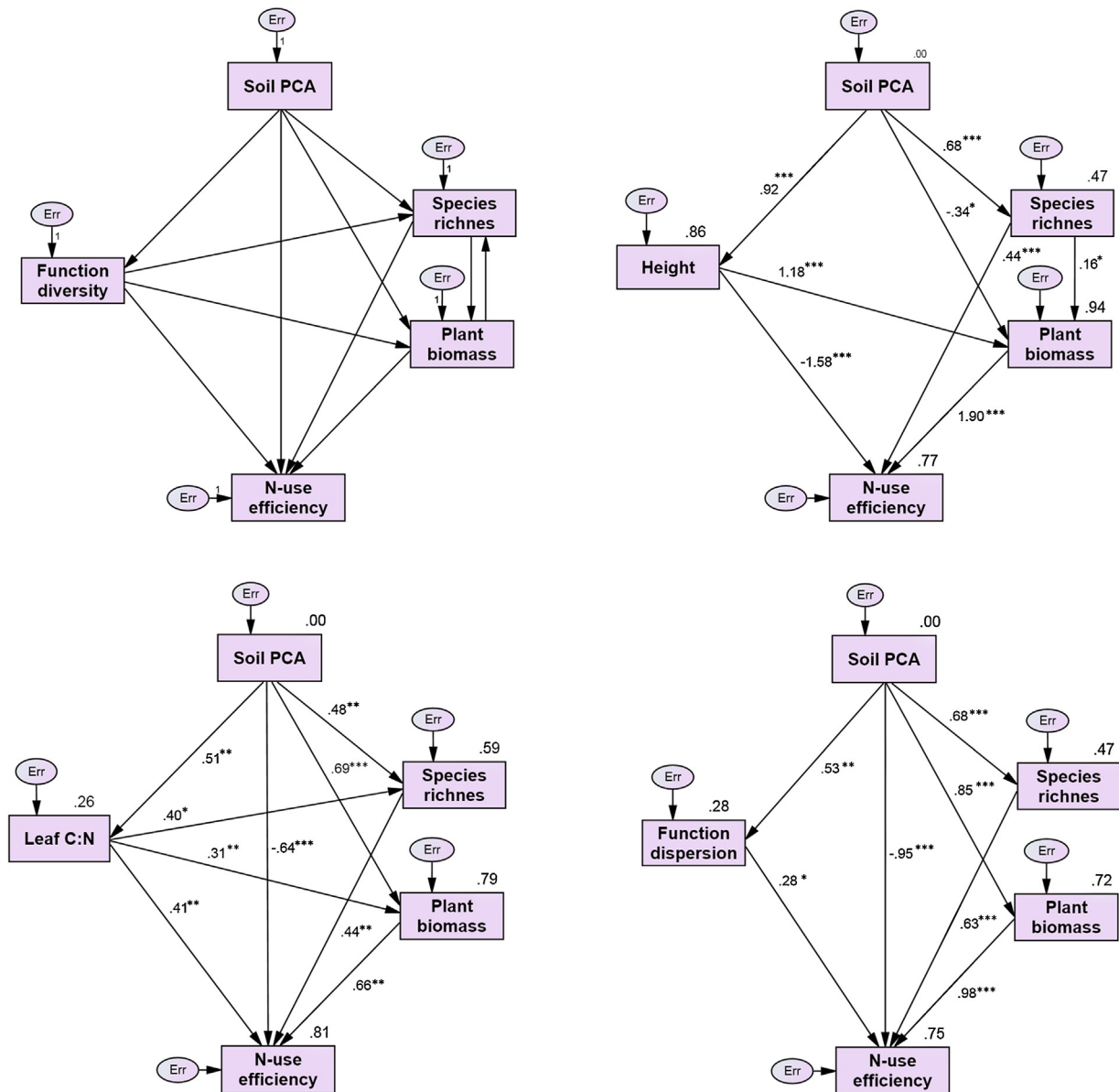


Fig. 3. Structural equation model showing all interaction pathways of functional diversity, vegetation characteristics and soil properties. (a) Initial conceptual model: single headed arrows indicate paths and double-headed arrows show the correlations included in the initial model. The exogenous unobserved variables (Err) account for the unexplained error. The fitted multi-group structural equation model for (b) height, (c) leaf C:N and (d) function dispersion. Standardized regression weights (along path) and total variance explained as a result of all predictors pointing to that variable (top right corner of rectangle). *, ** and *** indicate statistically significant paths at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.

simple linear regression analyses were in agreement with SEM, but SEM analysis results indicated that SEM could provide new insights into the underlying ecological relationships (Lamb, 2008; White et al., 2014).

We found that NUE did increase with increased species richness in sandy grassland restoration. This is consistent with other studies from grassland ecosystem, supporting species-rich plant communities use N more completely and efficiently (Hooper and Vitousek, 1998; Mueller et al., 2013; Selmants et al., 2013). This can be explained by that increased species richness can increase above-ground plant biomass due to the species' complementarity, which can further result in high NUE (Cardinale et al., 2011). In this study, it was found that increased plant biomass was associated with an increase in species richness ($r = 0.69$ in Table 2, Fig. 3b), supporting the positive relationship of plant diversity and ecosystem functioning in diversity-manipulation experiments (Hector et al., 1999;

Tilman et al., 1997) and natural grassland ecosystems (Han et al., 2007). Increased biomass in a species-rich community was not only associated with increased N uptake but also with decreased N concentrations in plant biomass (Fornara and Tilman, 2009; van Ruijven and Berendse, 2005), which supports a relatively higher plant N use in species-rich plant communities.

Community-level plant height and leaf C:N determined by soil properties had significant effects on NUE in sandy grassland restoration. This is in agreement with the finding that ecosystem properties are mainly driven by the CWM traits in communities (Butterfield and Suding, 2013; Conti and Díaz, 2013; Petchey and Gaston, 2006), supporting the mass ratio hypothesis (Grime, 1998). Our results also support the finding that high-standing plants may have high plant biomass (Conti and Díaz, 2013; Lavorel and Grigulis, 2012), thus contributing indirectly to high NUE. In addition, increasing competition for light in species-rich communities

Table 3Direct, indirect and total effects on community-level nitrogen-use efficiency based on standardized values of statistically significant SEM paths ($P < 0.05$).

Functional diversity metric	Predictor	Pathway to N-use efficiency effect	Effect
Height	Soil PCA	Direct	NS
		Indirect	0.46
		Total	0.46
	Species richness	Direct	0.44
		Indirect	0.29
		Total	0.73
	Height	Direct	−1.58
		Indirect	2.22
		Total	0.64
	Plant biomass	Direct	1.90
		Indirect	NS
		Total	1.90
Leaf C:N	Soil PCA	Direct	−0.64
		Indirect	1.07
		Total	0.43
	Species richness	Direct	0.44
		Indirect	NS
		Total	0.44
	Leaf C:N	Direct	0.41
		Indirect	0.38
		Total	0.79
	Plant biomass	Direct	0.66
		Indirect	NS
		Total	0.66
Functional dispersion	Soil PCA	Direct	−0.95
		Indirect	1.41
		Total	0.84
	Species richness	Direct	0.63
		Indirect	NS
		Total	0.63
	Function dispersion	Direct	0.28
		Indirect	NS
		Total	0.28
	Plant biomass	Direct	0.98
		Indirect	NS
		Total	0.98

are more productive, resulting in the investment allocation of C and N into plant tissues and maximizes plant height (Fornara and Tilman, 2009; van Ruijven and Berendse, 2005). The positive association of NUE with community-level leaf C:N suggests that low N-use efficiency at leaf level also supports high N-use efficiency at community level, due to the varying plant N turnover strategies of plants in rich species communities (Aerts and Chapin, 2000; McCulley et al., 2009).

Function dispersion (FDis) directly affected NUE in sandy grassland restoration, which supplied the obvious evidence for the niche-complementarity model (Tilman, 1997). This complementarity effect implies that NUE depends on the functional characteristics of the constituent species in sandy grasslands. These results are also consistent with other findings that plant communities with a wide dispersion of species trait space can use N more efficiently, because different species can use N in a complementary way and facilitate each other (Hector et al., 1999). In this case, with the increase of species richness following sandy vegetation succession occurring from the sand pioneer to low shrub communities, then toward the annual herb dominated communities (Zuo et al., 2009), niche partitioning among plant species and their positive species interactions allow for a more complete use of soil resources (Tilman, 1997). These results further indicate that changes in both functional diversity and species richness will greatly affect community-level plant N use in sandy grassland ecosystems.

Although soil properties had an indirectly negative effect on NUE in sandy grassland restoration, the total effect was positive through its indirect effect (Fig. 3, Table 3). In agreement with an association between plant biomass and soil gradient, NUE increased along soil gradient, supporting the finding that changes of soil properties also strongly affected NUE (Vázquez de Aldana and Berendse,

1997). In our study, species growing in nutrient-poor soil environments tended to maintain a high plant N concentration, thereby implying a low NUE. This study supplies evidence to weaken the soil fertility-trait relationship hypothesis that increased soil fertility should result in an increase in plant tissue N contents because plant species use N less efficiently (Hobbie, 2015). Structural equation models have suggested that the strong effects of plant height, leaf C:N, plant richness and biomass on NUE are directly affected by changes in soil properties. So, our studies stress the critical role of local variation in soil properties for determining plant trait selection, species compositions and plant biomass, which significantly drives NUE changes following sandy grassland restoration.

5. Conclusions

This study is one of the first to demonstrate that plant species richness, biomass, functional traits and diversity, as well as their responses to soil properties, need to be considered when community-level plant N-use is investigated in sandy grassland ecosystems. Our study clearly illustrates the direct and positive effects of plant richness, biomass, CWM leaf C:N, and functional dispersion determined by soil properties on N-use efficiency in sandy grassland restoration. The effects of plant functional diversity on N-use efficiency support both the mass ratio hypothesis and the niche-complementarity hypothesis. Niche complementarity among species plays an important role in community-level plant N use in sandy grassland ecosystems. Plant functional diversity may enhance the explanation of associations of N-use efficiency with plant community structure and soil properties in sandy grassland restoration. One important implication of this study is that conservation of higher plant diversity may maintain higher

N-use efficiency in sandy grassland ecosystems. An effort should be made to increase species richness and functional dispersion by selecting some higher standing plants with higher leaf C:N in order to improve community-level plant N use in sandy grassland restoration. In addition, community-level plant N use efficiency is relatively easy to measure, and thus becomes an effective tool in understanding plant N use and its underlying mechanisms in grassland ecosystems. However, our conclusions need to be taken with caution when comparing ecosystems that differ in resource base as a consequence of different species compositions, water availability and soil nutrient levels.

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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.ecolind.2016.01.012>.

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