

# Is shrub-encroached grassland a stable state in the semi-arid steppe region?

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## Abstract

Understanding community restoration state and the corresponding assembly mechanism is helpful to assess the restoration measures and predict community dynamics. We collected plots by shrub cover (low, medium and high) in three *Caragana microphylla* shrub-encroached grasslands which were fenced since 1979, 1983, and 2003 (fencing duration) in the northern China, to explore the effect of fencing duration and shrub cover on the community restoration by vegetation investigation and phylogenetic approach. There were significant differences in community composition among different fencing duration or shrub cover treatments. Species richness in the site of fencing since 1979 or in the plots of high shrub cover was relatively higher than that in any other sites or plots. By phylogenetic analysis, functional traits were phylogenetically convergent. Based on the standardized effect sizes of mean pairwise distance (SESMPD) ranged from -1.96 to 1.96 in six out of nine plots, which suggested that stochastic processes dominated community assembly. SESMPD were lower than -1.96 in the rest three plots which indicated that competitive exclusion drove community assembly. These results indicated that the increase of fencing duration or shrub cover could enhance competitive exclusion. The present findings highlighted the importance of shrub in influencing the community composition and community assembly, supporting that shrub-encroached grassland is another stable state in the semi-arid northern China. Therefore, it is essential to distinct shrub-encroached grassland from degraded grasslands when formulating relevant conservation and management measures in the semi-arid regions.

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**A short informative containing the major key words:** We investigated the community restoration state and assessed the community assembly mechanisms in shrub-encroached grasslands affected by fencing duration and shrub cover crossed.

**Running title :** Is shrub-encroached grassland a stable state?

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**Key words** : Fencing duration; Shrub cover; Community assembly; Phylogenetic pattern; Shrub-encroached grassland

## 1 INTRODUCTION

Understanding the mechanisms underlying community assembly and predicting community dynamics has long been a central problem in ecology (Kraft et al., 2007; Dong et al., 2019), especially in the context of restoration communities (Jones et al., 2019). Proposed mechanisms range from niche-based assembly rules (Diamond 1975) to neutral assembly (Hubbell, 2001). Early workers relied on taxonomic ranks (e.g., species-to-genus ratios; Elton 1946) to infer ecological processes that structure communities. Recently, the availability of molecular phylogenies has provided a new method for more accurately measuring evolutionary relatedness, and has led to a rapidly growing body of research in this field (Weeb, 2002). A local community can be considered as a subset of a regional pool. Processes contribute to local community assembly (Karft et al., 2007; Liu et al., 2018), including environmental filtering, competitive exclusion and stochastic processes, and outcomes of these processes may be reflected in patterns of relatedness within a community (Cornwell et al., 2006).

Recently, assessing phylogenetic conservatism, i.e., assessing relationships between phylogenetic relatedness and ecological similarities, is usually done before give a conclusion by phylogenetic approach, because more and more ecologists found that ecological traits are not always conserved in evolution but convergent sometimes (Cavender-Bares et al., 2004; Liu et al., 2018). Phylogenetic conservatism indicated that the closely related species tend to be similar of ecological and functional traits (conserved traits). Under this condition, phylogenetic clustering pattern suggests environmental filtering, phylogenetic overdispersal indicates competitive exclusion, and phylogenetic random pattern implies neutral processes dominating the community assembly (Webb et al. 2002; Kembel & Hubbell, 2006; Liu et al., 2018). However, when ecological traits are convergent, phylogenetic clustering suggests competitive exclusion, and phylogenetic overdispersal indicates environmental filtering driving the community assembly processes (Karft et al., 2007; Liu et al., 2018).

Due to global changes, large areas of terrestrial communities are degrading, especially in arid and semi-arid regions (Eldridge et al., 2011; Chen et al., 2014; Dong et al., 2019). For example, in the semi-arid Inner Mongolia Steppe in the northern China, large areas of native climax communities, *Stipa grandis* – *Leymus chinensis* communities, degraded or even thicketed because of drought caused by climate change and overgrazing by cattle or/and sheep, with the loss of plant diversity and the decline of ecosystem functioning (Peng et al., 2013; Dong et al., 2019). In response, a number of restoration practices were carried out to restore the semi-arid grassland vegetation, and fencing is regarded as an effective management practice (Wu et al., 2009). Therefore, many researches have been focused on the restoration processes (or secondary succession) of different communities, including forest (Muscarella et al., 2015; Liu et al., 2018; Pinho et al.,

2018), wetlands (Lozada-Gobilard et al., 2019), grasslands (Conradi et al., 2017; Barber et al., 2019; Dong et al., 2019) and abandoned agricultural fields (Dinnage, 2009).

Secondary succession is community assembly in action with changes in species richness as well as community composition, and therefore, it offers a chance to test the relative importance of different assembly processes along restoration duration and its consequences on the structure and functioning of plant communities (Pinho et al., 2018). As for the secondary succession of non-shrub-encroached degraded grasslands, both arrivals of propagules from outside and germinations from soil seed bank are very important at the the beginning of restoration, thereby, both random processes (arrival) and environmental filtering (conditions for germination) are expected to dominate the community assembly during the early stage of succession (Meiners et al., 2015; Marteinsdóttir et al., 2018). As successions proceed, competitive exclusion increasingly influences community assembly due to the improvement of abiotic conditions (Chen and Tang, 2016) and interspecific competition (Purschke et al., 2013; Dong et al., 2019). Consequently, the community gradually tends to be relatively stable (Zhang et al., 2019). However, there is still a lack of research on the community assembly mechanisms in secondary succession of shrub-encroached grassland considering the significant differences between non-shrub-encroached degraded grassland and shrub-encroached grassland. Although some reseachers pointed out that shrubs can facilitate the establishment of surrounding herbaceous communities (Koyama et al., 2015) by reducing abiotic stress (Maestre et al., 2009; Soliveres and Eldridge, 2014), many studies confirmed that shrub encroachment influenced the cycling of nutrient such as nitrogen, carbon and surface water and energy flow (Peng et al., 2013), causing grassland patches or degradation (Xiong et al., 2004; Zhou et al., 2018; Pierce et al., 2019).

For shrub-encroached grassland, shrub formed different cover at the landscape scale along the degree of shrub encroachment (Eldridge et al., 2011; Peng et al., 2013; Soliveres and Eldridge, 2014). On one hand, the increase of shrub cover across entire landscapes may affect the heterogeneity of soil nutrients and light availability, provide more niche and promote plant differentiation (Breshears, 2006; Soliveres and Eldridge, 2014). On the other hand, microclimatic conditions (Soliveres et al., 2012; Zhou et al., 2018) affected by shrubs could act as environmental filters for plant communities. So far, no research has explored the impact of shrub encroachment on the assembly of plant community in shrub-encroached grasslands although some studies have shown that shrub encorachment has changed the plant composition, community structure and abiotic environment of local habitat (Eldridge et al., 2011; Peng et al., 2013).

In this study, we chose communities treated by different fencing duration and shrub cover in the *Caragana microphylla* shrub-encroached grassland in the northern China, and used phylogenetic distances to assess assembly mechanisms along fencing duration and shrub cover gradient. We aimed to answer the following questions: 1) did the increase in fencing duration or shrub cover promote the restoration of herbaceous communities? 2) were key traits of species in the community phylogenetically conserved? and 3) how did fencing duration and shrub cover affect phylogenetic structure of herbaceous community and which process(es) played important roles in affecting community assembly.

## 2 MATERIALS AND METHODS

### 2.1 Study region and experiment design

The present study was conducted in the restoration area of *C. microphylla* shrub encroachment (43deg33'-43deg38' N, 116deg40'-116deg44'E) in the middle of the Inner Mongolia Steppe, China. The climate in this region is typical semi-arid continental climate with a mean annual temperature of -0.4, ranging from -22.3 (minimum monthly temperature, January) to 18.8 (maximum monthly temperature, July), and mean annual precipitation ~348mm. The typical vegetation type of this region belongs typical steppe, and the perennial rhizome grass *Leymus chinensis* (Trin.) Tzvel. and the perennial bunchgrass *Stipa g randis* P. Smirn. dominate these communities (Bai et al., 2004). The major soil types of this area are calcic chestnuts and calcic chernozems (Schonbach et al., 2011). Due to the irrational utilization, most of this area has degraded with the deceasing plant diversity and vegetation cover. In the seriously degraded area, *C. microphylla* shrub expanded rapidly and showed a gradient of cover along the degradation degree (Peng et al., 2013).

Three sites that had been fenced since 1979, 1983 and 2003 were selected, where *C. microphylla* shrub encroachment had emerged before fencing. These sites are characterized by an average altitude of 1,182 m, a mild slope less than 5%, and shared the similar climatic conditions (because the geographical distance between pairwise sites was less than 5000 m). Within each site, there was a shrub cover gradient along the disturbance intensity.

In August 2018, we selected three plots of 100 m x 100 m, representing a gradient in shrub cover and density from low (<10%) to medium (10%-30%) to high (>30%) within each site. We quantified shrubs cover and density using the average value method in each plot within three 20 x 20 m sub-plots (established randomly). Within each plot, we sampled five 0.5 x 0.5m quadrats placed entirely beneath the canopy of shrubs and the same number of quadrats in open areas. In each quadrat, each vascular plant species and its density (cloned plants were calculated by tiller number) were recorded.

## 2.2 Trait selection

We selected seven plant traits for each species: specific leaf area (SLA), leaf dry matter content (LDMC), leaf area (LA), leaf thickness (LT), leaf carbon content (LCC), leaf nitrogen content (LNC) and vegetation height (VH). These traits are widely recognized as good predictors for reflecting different plant species strategies for acquiring, using and conserving resources, including light, nutrients and water (Maire et al., 2009; Pinho et al., 2018). All of these were measured in field, based on standard methods of Perez-Harguindeguy et al. (2013). More details of the trait information were listed in Supplementary material (Table S1).

## 2.3 Measurement of species richness, species composition and community stability

We calculated species number in a quadrat as species richness. To distinguish differences in species composition among different fencing duration or shrub cover treatments, non-metric multidimensional scaling (NMDS) based on Bray-Curtis distance was run. We used the `envfit` function from `vegan` to test the community composition difference between treatments of different fencing duration or shrub cover, with treatment type as a categorical factor variable. Significance of these differences was tested using Analysis of Similarity (Anosim) by calculating 999 random permutations. These analyses were performed in the ‘vegan’ package in R (R Core Development Team).

M-Gordon stability index, a popular international method, is used for determining community stability (Hou et al., 2019). It uses the number and frequency of all species in a plant community to establish a mathematical model, and calculates the reference point of the community. Then, the stability of the plant community was calculated according to the reciprocal of the distance between the reference point and the stable point. More details for calculation methods of M-Gordon stability index see Supplementary material. The closer the reference point to the stable point, the higher the stability of plant community (Hou et al., 2019).

## 2.4 Measurement of phylogenetic structure and trait phylogenetic conservatism

Before quantifying phylogenetic structure and phylogenetic conservatism, we constructed a phylogenetic tree for our study species. All the 19 species investigated in this study were identified based on Angiosperm Phylogeny Group III system (The Angiosperm Phylogeny Group, 2009) using the Plant List database (<http://www.theplantlist.org/>). And then, the completed phylogenetic tree with branch lengths (Figure. S1) was directly assembled within the online tool Phylomatic (<http://phylodiversity.net/phylomatic/>) based on the ultrametric phylogeny of Zanne et al. (2014). Next, a phylogenetic tree was randomly solved by ‘multi2di’ function, and then we ultrametrized the tree using ‘compute.brln’ function from ‘ape’ package in R (R Core Development Team).

To characterize community phylogenetic diversity and structure, we calculated Faith’s phylogenetic diversity (PD) (Faith 1992), mean nearest taxon distance (MNTD) and mean pairwise distance (MPD) (Webb, 2000). PD is defined as the total branch length among all taxa in a community. MPD generally reflects phylogenetic structure across the entire tree, whereas MNTD is sensitive to patterns among closely related species towards the tips of the phylogeny (Blaimer et al., 2015). In addition, we calculated the standardized effect sizes

(SES, differences of observed values versus null communities) of MPD and MNTD ( $SES_{MPD}$  and  $SES_{MNTD}$ ) by weighting species abundance and their associated quantiles ( $p$  value) to assess statistical significance.

To further strengthen the conclusion, we analyzed the SES with two null models: 1) randomizing the community data matrix ('independent swap') and 2) shuffling taxon labels randomly across the phylogeny ('taxa labels'), this algorithm randomizes the relatedness of species to one another, but it maintains species occurrence frequency and abundance.  $SES_{MPD}$  and  $SES_{MNTD}$  are equivalent to net relatedness index (NRI) and nearest taxon index (NTI) multiplied by  $-1$ . Phylogenetic clustering is indicated by negative SES values and low quantiles ( $p < 0.05$ ), corresponding to  $-1.96$ , and overdispersion by positive SES values and high quantiles ( $p > 0.95$ ), corresponding to  $1.96$ . Non-significant positive or negative values indicate observed values close to the median of the random dispersion.

To quantify trait phylogenetic conservatism, we employed a widely used Blomberg's K statistic with 999 iterations (Blomberg et al., 2003). Blomberg's K statistic is assessed by using the Brownian motion (BM) of trait evolution, and is considered as one of the most powerful approaches (Pavoine and Ricotta, 2013). K-value close to 1, greater than 1 and less than 1 indicate that the trait distribution perfectly matches BM expectation of trait evolution on the phylogeny, is more conserved and is less conserved than BM expectation, respectively (Kraft et al., 2007). K-values between 0.4 and 0.7 are hereby in the following considered to represent intermediate, whereas values below 0.4 express low levels of conservatism. We assessed the statistical significance of K by comparing observed variances of phylogenetic independent contrasts to a null model generated by shuffling tips randomly across the phylogeny. Analyses of phylogenetic structure and trait phylogenetic conservatism were performed in the 'picante' package in R (R Core Development Team).

## 2.5 Statistical analysis

All data used in this study met the Shapiro–Wilk test of normality and the Levene's test of homogeneity of variance.

Two-way ANOVA of general linear models were used to assess the effects of fencing duration, shrub cover and their interaction on species richness, phylogenetic diversity and phylogenetic structure. A one-way ANOVA analysis with Tukey's HSD test was conducted to evaluate the difference among levels of fencing duration or shrub cover when interaction were not significant. Alternatively, a simple-effect analysis was used to evaluate the difference of levels of one factor under a certain condition of another factor when significant interaction were found. Linear model was applied to test the relationship between phylogenetic diversity and species richness.

## 3 RESULTS

### 3.1 Species richness, species composition and community stability

Nineteen species from 17 genera of 11 families were recorded in these plots, and Poaceae, Amaranthaceae and Amaryllidaceae comprise of 57.9% of the species recorded (Table S2). Species richness was significantly higher in the site of fencing since 1979 than that in site of fencing since 1983 or 2003; and species richness was significantly higher in the high shrub cover plots than that in the low shrub cover plots (Figure 1). The results of NMDS plots showed strong segregation in species composition among different fencing duration communities ( $p < 0.05$ ) (Figure 2A; Table S3). In addition, there was a significant segregation ( $p < 0.001$ ) in species composition between plots of high shrub cover and any of the other two shrub cover treatments, but there was no significant segregation between plots of low and medium shrub cover ( $p = 0.407$ ) (Figure. 2B; Table S3). The site of fencing since 2003 had a lower M-Gordon stability index of the plant community, and the plots of high shrub cover had higher M-Gordon stability index (Figure. S2; Table 1).

### 3.2 Community phylogenetic diversity and structure

Fencing duration significantly increased phylogenetic diversity (PD), and PD in the sites of fencing since 1979 was the highest (Figure 3). There was no significant difference in PD among different shrub cover treatments.

There was a significantly positive relationship between phylogenetic diversity and species richness ( $R^2 = 0.824$ ,  $p < 0.001$ ) (Figure. 4).

$SES_{MPD}$  (TL) was significantly affected by fencing duration, shrub cover and their interaction.  $SES_{MPD}$  (TL) was significantly lower in the plots of high shrub cover (around -1.96, phylogenetic clustering) than in the plots of low or medium shrub cover under fencing since 2003 and 1983 (higher than -1.96 and lower than 1.96, phylogenetic random); and significantly lower in the plots of low shrub cover under fencing since 1979 (around -1.96, phylogenetic clustering) than in the plots of low shrub cover under fencing since 1983 or 2003 (around zero, phylogenetic random). There was no significant difference in  $SES_{MPD}$  (IS) among different fencing duration or shrub cover treatments (Figure. 5B).

The values of  $SES_{MNTD}$  (IS or TL) were between -1.5 and 1, which were significantly affected by shrub cover (Table 2), and  $SES_{MNTD}$  in the high shrub cover plots was obviously lower than that in the low or medium shrub cover plots.

### 3.3 Trait phylogenetic conservatism

All K values of investigated traits were less than 1, ranging from 0.15 to 0.53 (Table 1), which indicated that all traits exhibited low to intermediate levels of conservatism. In addition, the  $p$  values of five traits (VH, LNC, LCC, LDMC, LT) were less than 0.05.

## 4 DISCUSSION

Community phylogenetic approach reveals the relationship of evolutionary processes, regional environments, and interactions between species, which is important to understand the mechanisms of species co-occurrence and community assembly (Cavender-Bares et al., 2009). The present findings demonstrated that the restoration processes of shrub-encroached grassland were affected by fencing duration and shrub cover.

The increase of fencing duration increased the species richness and phylogenetic diversity, suggesting that longer fencing duration was conducive to the restoration of community diversity in shrub-encroached grasslands (Yoshihara et al., 2010; Soliveres & Eldridge, 2014; Koyama et al., 2015). Generally, species richness usually increases first and then decreases in secondary succession of grazing exclusion (Chen et al., 2014). In this study, species richness did not decline even in the communities that had been fenced since 1979. The result of NMDS confirmed the differences in species composition between fencing durations. Meanwhile, there were only 19 species in species pool in this study area, far lower than the number 27-58 in the typical grassland and degraded grassland of this region (Bai et al., 2000). These results indicated that the community was still in recovering stage, but the restoration rate was slow. We also found that the communities with higher fencing duration were more stable. However, the restoration processes of shrub-encroached grassland were inconsistent with non-shrub-encroached grassland which could return to the climax state after about 11-year fencing (Li et al., 1993). Previous studies have suggested that *C. microphylla* had higher water capturing capability than grasses, it will lead to irreversible stage once *C. microphylla* dominated the landscape (Peng et al., 2013), mainly due to that there was a threshold point for transitions between states (Xiong et al., 2004; D'Odorico et al., 2012). In addition, the existence of shrub canopy would ameliorate abiotic environment for the growth of neighbor species, enhancing plant richness and influencing community composition (Maestre et al., 2009; Koyama et al., 2015). This also explains why the species richness and community composition of high shrub cover communities were significantly different from that of low and medium shrub cover community.

Phylogenetic diversity reflects evolutionary history of coexisting species and clarifies their capacity to adapt to environmental changes (Alberti et al. 2017). In addition, it can represent independent aspects of community structure (Barber et al., 2019; Jones et al., 2019). Previous studies found that phylogenetic diversity was positively correlated with species richness but differed in their temporal or successional patterns (Purschke et al., 2013). In the present study, the phylogenetic diversity positively correlated with species richness overall. However, phylogenetic diversity didn't increased obviously with species richness in different succession gradient of shrub cover. This result indicated that most plant lineages present in the high shrub cover

communities also occurred in the low and medium shrub cover and that they are generally less speciose in the last two shrub cover communities (Blaimer et al., 2015; Bares et al., 2019). This also reflected that the impact of shrub cover on species diversity was not consistent with fencing duration.

In this study, we found that the majority of traits were phylogenetically convergent, with six out of seven phylogenetic signals were lower than 0.40. Pipenbahr et al. (2013) found traits were convergent for most of traits when they tested phylogenetic signal in the North Adriatic Karst grassland. However, most studies in tropical and temperate forests demonstrated that traits were phylogenetically conservatism (Muscarella et al., 2015; Dobert et al., 2017). This difference may occur, because compared with woody plants, herbaceous plants tend to have shorter life cycles, more rapid reproduction and more adaptable to the environmental changes (Donoghue, 2008). The evolutionary relatedness of ecological traits have an important influence on the interpretation of community assembly (Liu et al., 2018). Cavender-Bares et al. (2004) emphasized the importance of detecting phylogenetic conservatism, and suggested that the results of testing phylogenetic conservatism should be combined with the observed phylogenetic dispersion pattern in order to make a strong inference on the ecological and functional similarity of coexisting species.

The phylogenetic pattern varied widely among different fencing duration and shrub cover communities. The low shrub cover communities under fencing since 1979 and high shrub cover communities under fencing since 1983 and 1979 showed evidence of phylogenetic clustering ( $SES < -1.96$ ), with co-occurring species significantly more closely related than would be expected by chance. In contrast, phylogenetic random dispersion ( $-1.96 < SES < 1.96$ ) dominated in low shrub cover communities under fencing since 2003 and 1983, medium shrub cover under fencing since 2003, 1983 and 1979 and high shrub cover communities under fencing since 2003 (based on MPD). All of communities showed a random assembly regarding MNTD, but it showed a trend of phylogenetic clustering with fencing duration or shrub cover increasing. When ecological traits evolve convergently, the interpretation of the community assembly becomes complicated, and its predictive ability declines (Kraft et al. 2007). Convergent traits can result in clustering patterns even though competitive exclusion is the dominant process of community assembly (Webb et al. 2002). With regard to the present results, clustering in low shrub cover communities under fencing since 1979 and high shrub cover communities under fencing since 1983 and 1979 could be linked to competitive exclusion rather than habitat filtering. The combination of phylogenetic clustering and convergent trait evolution seen in our study has also been found in other studies (Pipenbahr et al., 2013; Purschke et al., 2013; Liu et al., 2018). Research results from non-shrub-encroached grassland indicate that competitive exclusion would play more important role in driving the community assembly with the fencing duration due to the improvement of abiotic conditions (Wellstein et al., 2014; Conradi et al., 2017; Barber et al., 2019). Our results indicated that the communities assembly during the fencing restoration in shrub-encroached grassland was consistent with that of non-shrub-encroached grassland. In addition, we also found shrub cover significantly affected the community assembly process. On one hand, previous studies have shown that higher shrub cover could increase soil fertility (Maestre et al., 2009; Pipenbahr et al., 2013) and ameliorate abiotic environment (Molina-Montenegro et al., 2016; Thompson et al., 2017). The decrease in abiotic stress and the increase in species richness may intensify resource competition among herbaceous species (Chapin et al., 1994; Purschke et al., 2013). On the other hand, there may be direct competition between shrubs and herbaceous species, and higher shrubs have a higher competitive advantage in light and water resources than herbaceous species (Peng et al., 2013; Pierce et al., 2019), accelerating competitive ability for resources. Thus, niche overlap in open space should become significant and competition should consequently intensify.

More recently, the importance of random colonization and stochasticity in early community has become evident (Ulrich et al. 2016; Marteinsdottir et al., 2018; Liu et al., 2018). Our results found a random assembly process ( $-1.96 < SES < 1.96$ ) in six out of nine communities (Figure 5). Generally, random community assembly is considered to be strongest in communities that are dominated by colonization. In arid and semi-arid communities, colonization was often limited by unfavorable local environmental conditions (Conradi et al., 2017; Marteinsdottir et al. 2018), seed availability (Knappova et al., 2017) or lack of facilitating species (Callaway, 1995). At sites like Inner Mongolia steppe with low soil nutrient status, limited, seed rain and low seedling survival (Koyama et al., 2015), a successful colonization requires a sequence of favorable events.

This also proved that these communities have lower stability than those with longer fencing duration and higher shrub cover (Figure S2; Table 1).

## 5 CONCLUSION

The present findings showed that the presence of shrubs inhibited the restoration rate and suggested that shrub-encroached grasslands is another stable state in this region. If we want to restore the diversity and composition of grassland community and maintain its function, it can not be achieved only fencing, especially in the high shrub cover area of *C. microphylla*. It may require some human interference practices that inhibit *C. microphylla* growth (Xiong et al., 2004; Alados et al., 2019) and incorporates biotic perspectives into the framework of restoration (Koyama et al., 2015; Pierce et al., 2019).

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## CONFLICT OF INTEREST

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Table 1 Results of community stability analysis.

Plot	Fitting formula	R <sup>2</sup>	Intersection coordinate	Stability index
2003	y = -0.0108x <sup>2</sup> + 1.9364x + 12.686	0.99	(33.98, 66.02)	5.06
1983	y = -0.0115x <sup>2</sup> + 1.8767x + 23.604	0.94	(30.20, 69.80)	6.93
1979	y = -0.0126x <sup>2</sup> + 2.0686x + 14.552	0.98	(32.07, 67.93)	5.86
Low	y = -0.0129x <sup>2</sup> + 2.0761x + 16.495	0.97	(31.24, 68.76)	6.29
Medium	y = -0.0102x <sup>2</sup> + 1.7441x + 23.978	0.97	(31.36, 68.64)	6.22
High	y = -0.0145x <sup>2</sup> + 2.2532x + 13.357	0.96	(30.88, 69.12)	6.50

Table 2 The results of general linear models for evaluating the effect of fencing duration, shrub cover and their interactions on species richness and phylogenetic structure. IS: null model based on independent swap algorithm; TL: null model based on taxa labels algorithm. SES<sub>MPD</sub>: standard effect size of mean pairwise distances; SES<sub>MNTD</sub>: standard effect size of mean nearest taxon distance.

Variable	Fencing duration (df=2)	Fencing duration (df=2)	Fencing duration (df=2)	Shrub cover (df=2)	Shrub cover (df=2)
	<i>F</i>	<i>P</i>	ω <sup>2</sup> (%)	<i>F</i>	<i>P</i>
SES <sub>MPD</sub> (TL)	12.574	<b>0.000</b>	18.03	11.843	<b>0.000</b>
SES <sub>MPD</sub> (IS)	0.789	0.458	1.77	1.229	0.292
SES <sub>MNTD</sub> (TL)	3.017	0.055	5.87	5.698	<b>0.018</b>
SES <sub>MNTD</sub> (IS)	1.222	0.300	2.61	3.751	<b>0.025</b>

Table 3 Results of analyses of phylogenetic signal, comparing the K-statistic and its associated p-value for seven measured species functional traits.

Trait	Type of variable	Range	<i>K</i>	p
Vegetation height (VH)	Continuous (cm)	13.00-44.48	0.32	<b>0.048</b>
Specific leaf area (SLA)	Continuous (m <sup>2</sup> /kg)	11.58-26.37	0.22	0.225
Leaf nitrogen concentration (LNC)	Continuous (g/kg)	2.32-4.73	0.31	<b>0.046</b>
Leaf carbon concentration (LCC)	Continuous (g/kg)	35.37-47.17	0.53	<b>0.008</b>
Leaf area (LA)	Continuous (cm <sup>2</sup> )	0.56-113.07	0.15	0.746
Leaf dry matter content (LDMC)	Continuous (mg/g)	93.01-404.19	0.33	<b>0.035</b>
Leaf thickness (LT)	Continuous (mm)	0.11-0.93	0.34	<b>0.021</b>

## Figure captions

**Figure 1** Species richness of different fencing duration and shrub cover communities.

**Figure 2** Non-metric multidimensional scaling (NMDS) ordination of community taxonomic composition. A) Fencing duration treatments; B) Shrubs cover treatments. Each point represents the species composition in a given quadrat. Lines represent the confidence ellipse at the 0.95 level.

**Figure 3** Phylogenetic diversity of different fencing duration and shrubs cover communities.

**Figure 4** Relationship between phylogenetic diversity and species richness.

**Figure 5** The standardized effect sizes SES of mean pairwise distance ( $SES_{MPD}$ ) and mean nearest taxon distance ( $SES_{MNTD}$ ). The significant difference from the null model is shown by asterisks. The same English letters indicate non-significant differences ( $p > 0.05$ ) among different fencing duration treatments; and the same Greek letters indicate non-significant ( $p > 0.05$ ) differences among different shrubs cover conditions.





