

Positive Plant Diversity-Soil Stability Relationships are Mediated through Roots in the Songnen Grassland: Chronosequence Evidence

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Abstract

Living plant diversity (excluding the litter issue) may affect below-ground properties and processes, which is critical to obtaining an integrated biodiversity-ecosystem functioning theory. However, related patterns and underlying mechanisms have rarely been examined, especially lacking long-term evidence. We conducted a factorial crossed sample survey to examine the effects of plant diversity on soil stability over succession based on space-for-time substitution in the Songnen Steppes, North-Eastern China. The results indicate that, under natural colonizing conditions, species-poor systems achieved lower soil stability than species-rich systems, regardless of successional stage. However, soil stability was significantly regulated by plant species richness (number), composition (identity), density (abundance), and functional roles. Our results confirm that a long-term positive plant diversity-soil stability relationship exists in steppe succession. In particular, this enhanced effect of biodiversity on soil stability may operate via diversifying plant root traits. Our results may underpin an integrated biodiversity-ecosystem functioning theory, and improve human use and conservation management of natural resources at an integrated ecosystem level.

Keywords: biodiversity-ecosystem functioning, density compensation, diversity-stability relationship, functional role, mechanism, soil detachment rate, succession, root form

Introduction

The past two decades have seen extensive research in ecology on biodiversity and ecosystem functioning (BEF), as ecologists strive to estimate the consequence of widespread biodiversity loss while confronting the growing dominance of ecosystems much-altered by humans (Loreau, 2000; Loreau *et al.*, 2001; Schwartz *et al.*, 2000; Hooper *et al.*, 2005; Maestre *et al.*, 2012; Yang *et al.*, 2012). Specifically, pursuing the relationships between biodiversity and ecosystem stability (BSR) has fascinated ecologists over an even longer history (Tilman, 1999; McCann, 2000; Tilman *et al.*, 2006; Isbell *et al.*, 2009; Haddad *et al.*, 2011). For instance, the earlier campaign arose directly from the ‘diversity-stability hypothesis’ that greater diversity begets stability (MacArthur, 1955; Elton, 1958), but could be traced back to Darwin (Loreau, 2000; Hector and Bagchi, 2007). However, this has never been an easy task – the BSR patterns obtained have been multiple and contentious. Moreover, ‘different theoretical results contradict each other, empirical results are inconsistent, and theoreticians and empiricists often disagree’ (Ives and Carpenter, 2007). To date, the reached consensus has indicated that fully understanding the strength and pattern of BSRs not only requires detailed knowledge of species interactions and the impacting environmental contexts, but

also of the multiplicity or multifacetedness of definitions of both biodiversity and stability (Pimm, 1984; McCann, 2000; Ives and Carpenter, 2007). Moreover, to better understand the BSRs and ecosystem management, investigating the underlying mechanisms may be more valuable (Ives and Carpenter, 2007).

The most relevant and frequently used terms of community/ecosystem stability include variability (e.g. the prevailing coefficient of variance based measures), resistance, invasibility, and resilience, respectively (Ives and Carpenter, 2007). Temporal stability (pertaining to the variability school), which has been defined as a lack of fluctuations over time and is usually calculated from measurements of biomass (Tilman, 1999; Isbell *et al.*, 2009), cover (Sankaran and McNaughton, 1999; Dovciak and Halpern, 2010; Yang *et al.*, 2012), or abundance/density (Valone and Hoffman, 2003), has often been addressed. While not without exceptions (Bezemer and van der Putten, 2007; Sasaki and Lauenroth, 2011), a major positive BSR relationship tends to occur at the community level (Tilman, 1996; Lehman and Tilman, 2000), but intense debates concentrate at the population level (Valone and Hoffman, 2003; Dovciak and Halpern, 2010; Yang *et al.*, 2012). For example, although the population stability of individual species can have negative, neutral or positive relationships with diversity (van Ruijven and Berendse,

2007), negative BSR patterns of populations of individual species seem to predominate in experimental grassland communities (Tilman *et al.*, 2006; van Ruijven and Berendse, 2007), whereas positive or neutral relationships have been observed in natural assemblages (Valone and Hoffman, 2003; Romanuk *et al.*, 2009). Exceptions are more common in other schools of stability, e.g., there is a dispute as to the role of 'insurance hypothesis' in maintaining the stability (resistance) of ecosystem functions and services provision (Pfisterer and Schmid, 2002; Valone and Barber, 2008).

In general, BSRs have been studied in three ways: above-ground versus above-ground, below-ground versus below-ground, and above-ground versus below-ground (Wardle *et al.*, 2004). However, most of the experiments have concerned the effects of plant diversity on above-ground primary production or nutrient retention in grasslands during limited periods of time, often failing to detect significant effects on below-ground processes (Loreau *et al.*, 2001) or long-term ecosystem consequences. Although below-ground BEFs (e.g. Hedlund and Ohrn, 2000; Heemsbergen *et al.*, 2004; Wilson *et al.*, 2009) and mutualistic linkages between above- and below-ground subsystems such as the effect of plant litter diversity on soil properties/decomposition (Wardle *et al.*, 1997; Bardgett and Shine, 1999) or below-ground biodiversity effects on above-ground traits (van der Heijden *et al.*, 2008; Wagg *et al.*, 2011) have been examined substantially, the effects of living plant diversity on below-ground ecosystem properties and processes and underlying mechanisms have not been well understood (Bever, 1994; Loranger-Merciris *et al.*, 2006; Wardle, 2006; Orwin *et al.*, 2010; Sylvain and Wall, 2011). In particular, Jiang *et al.* (2008) suggest that diverse BEF relationships are possible for non-biomass functions due to negative selection effects. Moreover, little is known about how and why BSRs may vary over successional time (Dovciak and Halpern, 2010); the sufficiently long term evidence is lacking. All these invite more empirical evidence to enhance the assumption for resolving the dilemma.

Theory predicts that diversity can regulate community stability through mechanisms such asoveryielding, the covariance effect (complementarity), and the portfolio effect (statistical averaging) (e.g. Doak *et al.*, 1998; Tilman, 1999; Lehman and Tilman, 2000). The 'insurance hypothesis' has also been regarded as a critical mechanism (Yachi and Loreau, 1999). Furthermore, recent empirical studies have also indicated a sampling effect (e.g. Sasaki and Lauenroth, 2011), where stability was regulated by dominant species rather than diversity. Jiang (2007) suggests that, in competitive communities, diversity may not affect ecosystem functioning due to density compensation. However, under current consensus (Ives and Carpenter, 2007), it is most likely that more, not necessarily mutually exclusive, mechanisms are to be unravelled. For example, soil aggregation enhancement through enriched arbus-

cular mycorrhizal fungi (AMF) may be a mechanism for increasing sequestration of carbon (Rillig, 2004; Rillig *et al.*, 2007; Wilson *et al.*, 2009). The interspecific functional dissimilarity may be another mechanism through which below-ground biodiversity drives soil processes (Heemsbergen *et al.*, 2004).

The interplay between organisms and their physicochemical environment plays a key role in both evolution and ecosystem functioning (Loreau, 2010), and plants can affect soil processes either directly or indirectly (Loreau *et al.*, 2001; Sylvain and Wall, 2011). Here, we conducted this study to examine the effects of above-ground living plant diversity upon below-ground ecosystem stability in natural steppe communities in northeastern China. To separate the effects of organisms and their physicochemical environment, we assigned study plots that have relatively homogenous matrix conditions within a localized small area (no more than 100 ha). Here, we focus on one type of stability (i.e., soil aggregate stability) and four components of biodiversity (species richness, identity or composition, density or abundance, and functional traits). We assessed soil stability based on soil detachment rates (SDR; See De Baets *et al.*, 2007), i.e., soil resistance to water dispersion as a function of perturbation. Higher SDR values indicate lower stability, and vice versa. The steppe represents the typical local vegetation along the eastern part of the Eurasian Steppes, the world's largest grassland biome, which has undergone intensifying perturbations and is shrinking (Wang *et al.*, 2009). However, under changing diversity conditions during succession, the pattern and mechanism of a living plant diversity-soil stability relationship has yet to be investigated. Hence we attempt to address the following three questions: (1) what is the relationship between living plant diversity and soil stability in native steppe communities over successional cycles? (2) what plant community traits determine soil stability in steppe ecosystems? (3) what are the mechanisms that maintain plant diversity-soil stability relationships?

Materials and methods

Study site

The fieldwork was conducted on July 9, August 14, and September 11, 2010 in the northern steppe (44°35'N, 123°30'E), Northeast Normal University Changling Grassland Ecology Research Station, Jilin Province, China. The study site is a 100 ha fenced area within a Natural Reserve for grassland conservation established during the 1960s. The area has a monsoon climate with a long-term mean annual precipitation of about 470 mm (85% of which occurs in June – September). Mean annual temperature is about 4.6 °C and average monthly temperatures range from -17.6 °C in January to 21.7 °C in July. The soil is meadow soil, although zonally it is Chernozem. The vegetation is temperate steppe dominated by *Stipa baicalensis*, a species becoming increasingly rare owing to historical

steppe-cropland conversion. Nevertheless, the widespread recalcitrant saline-alkali soils plus complex micro-topology create an abundance of non-zonal meadow steppes – *Leymus chinensis*, a rhizome grass species dominating meadows (Wang *et al.*, 2009), emerging as the edaphic climax indication in the steppes.

About 357 grass species have been recorded that form the native *L. chinensis*-dominated associations at the site, although no fewer than 750 grass species have been reported in the steppes (Li, 2011). In the past, due to irrational land uses such as overgrazing and steppe-cropland conversion, steppe degradation was common. Three major stages of succession, either progressive or converse, can be readily identified and verified as follows: the *L. chinensis* monospecific community in late succession, mature and stable; mixed communities dominated by *L. chinensis* and others in transitional succession, with less stability; and communities dominated mainly by annual herbs like *Suaeda glauca* or *Chloris virgata* in the early colonizing stage, often heavily degraded and unstable. Since 2001, the initiation and ongoing implementation of the Grain for Green Project (GGP) has generated a continuing recovery effect in the steppes (Cao, 2011). Many steppes, by excluding grazing and cultivation, are thus in the process of progressive succession during natural restoration. The succession series often run in the following sequence: bare land → *S. glauca* or *C. virgata* dominated community → *L. chinensis* + *Artemisia anethifolia* or *S. corniculata* or *C. virgata* or *Puccinellia tenuiflora* or *Hordeum brevisubulatum* or *Convolvulus ammannii* or *Allium polyrrhizum* dominated communities → *L. chinensis* + *Carex duriuscula* or *A. neriniflorum* or *Cleistogenes squarrosa* dominated communities → *L. chinensis* or *L. chinensis* + weeds or *Lathyrus quinquevenerius* or *Thalictrum simplex* or *Galium verum* or *Eleocharis intersita* or *Potentilla flagellaris* dominated communities (Li and Guo, 2011).

Experimental design

Our design was based on the diversity-time hypothesis that plant species diversity is always a transitional property changing with community successions (Ricklefs, 2007). In view of criticisms regarding the use of experimental communities (Jiang *et al.*, 2009) including the uncertainty about the actual length of typical succession cycles in long-term experiments, we chose space-for-time substitution to build steppe successional chronosequences based on indicator species and community composition expertise in local steppe succession (Pickett, 1989; Li and Guo, 2011). The field design thus emerged was a factorially crossed sample survey upon the defined chronosequences. Biodiversity here was broadly defined, including terms that are numerical (both richness and density), compositional (identity), and functional. Because of the regularity in definition and nature of measuring functional diversity (Mouillot *et al.*, 2005), we measured the functional roles of the component plant species by examining their root

life forms (architecture). Three root architectures were recognized, i.e., rhizomatous root, tap root, and fibrous root, which usually played respective roles in affecting soil properties and processes (Li, 2011). Therefore, changing plant diversity during succession meant a concomitant varying placement of proportionate root traits within a soil volume.

Four treatments were thus recognized regarding succession: mature stage (climax), transitional stage (mixtures), early colonizing stage (pioneer), and a referenced bare-land stage. The community density/abundance factor was discerned by dividing overall coverage and stem density into two levels: higher density (coverage > 0.7) and lower density (coverage < 0.4) (Tab. 1). As a result, seven 2 m × 2 m plots were carefully selected and placed, each representing a combination of key stage and community density in the succession (including a reference plot). Three replicates for each treatment were also made.

Vegetation sampling

Plant cover was measured using a 1 m × 1 m frame with equally distributed 2 cm × 2 cm grids. During the measurement, the frame was placed above the canopy in each quadrat. The percent cover of the community was visually point estimated in all grids and summed across grids for each plot. Within each plot, species richness was recorded as the number of plant species, and the species composition, including both species identity and abundance (i.e., the number of plant stems of each species) were also recorded). Compositions were thus characterized with regard to the dominant species (one or more) with higher abundance. This facilitated calculation of the functional weight of some species groups by linking their above-ground abundance and below-ground placement of root forms (Tab. 1).

Soil sampling and stability test

After the vegetation survey, the above-ground biomass was cleared by hand clipping such that intact soil sampling could be performed using rectangular polymer soil sample boxes (10 cm wide, 20 cm deep and 30 cm long; De Baets *et al.*, 2011). The dispersal test apparatus consisted of a plastic basin (46 cm in diameter, 30 cm deep) and a metal sieve of appropriate diameter with 0.25 mm holes. Therefore, water-stable soil aggregates (WSA) were separated as microaggregates (< 0.25 mm in diameter) and macroaggregates (> 0.25 mm in diameter). Before the test, the sieve was placed in the basin, 10 cm above the basin bottom. The root permeated soil sample was put on the sieve in the basin, and tapwater was gently added to the basin without disturbing the soil sample until it was submerged. This spontaneous dispersal process lasted 4 hours. The metal sieve together with the remaining soil on it was then carefully removed from the basin; and the detached soil particles were separated from the undetached aggregates (defined as > 5 mm fractions) on the sieve and both

Tab. 1. The characteristics in above- and below-ground communities at the study site

Plot	Succession stage	Plant coverage	Plant diversity in soil sample	Species composition	Percentage of species present (%)	Stem density (stems ha ⁻¹)	Root life-form
Climax							
1	Higher stem density	0.8	1	<i>Leymus chinensis</i>	100	2,783,300	Rhizamatous root
2	Lower stem density	0.2	1	<i>L. chinensis</i>	100	695,830	Rhizamatous root
Transition							
3	Higher stem density	0.9	6	<i>Phragmites communis</i>	30	1,000,000	Rhizamatous root
				<i>Kalimeris integrifolia</i>	50	1,670,000	Rhizamatous root
				<i>L. chinensis</i>	10	333,300	Rhizamatous root
				<i>Artemisia mongolica</i>	5	166,700	Rhizamatous root
				<i>Puccinellia tenuiflora</i>	4	133,332	Fibrous root
				<i>Cleistogenes squarrosa</i>	1	33,330	Fibrous root
4	Lower stem density	0.2	4	<i>P. communis</i>	25	173,750	Rhizamatous root
				<i>K. integrifolia</i>	65	451,750	Rhizamatous root
				<i>L. chinensis</i>	5	34,750	Rhizamatous root
				<i>A. mongolica</i>	5	34,750	Rhizamatous root
Pioneer							
5	Higher stem density	0.9	1	<i>Chloris virgata</i>	100	3,131,200	Fibrous root
6	Lower stem density	0.2	1	<i>C. virgata</i>	100	695,800	Fibrous root
7	Bare land	0	0	<i>Bare soil</i>	0	0	No

were weighted after air drying for 10 days. Detached soil passing through the sieve was also measured to obtain the microaggregate SDR. SDR was thus calculated as:

SDR = Detached soil aggregates weight / Tested soil sample weight × 100%.

In this research, we measured three SDRs, i.e., above-sieve or water-stable macroaggregates SDR (ADR), below-sieve or microaggregates SDR (BDR), and the summed SDR (TDR). The soil stability was thus defined to be 1/SDR, which emerged as a value without dimension and encompassed three stability measures, i.e., AS=ADR⁻¹ (macroaggregate stability), BS=BDR⁻¹ (microaggregate stability), and TS=TDR⁻¹ (overall stability).

Root analyses

After the soil dispersal test, for each soil sample, the roots were separated and treated and root variables such as root density (RD) and root length density (RLD) were measured following De Baets *et al.* (2011). Species identities and related root life forms were also considered, such that root measures of respective species, the dominant root-form, and their total were all readily available.

Statistical analyses

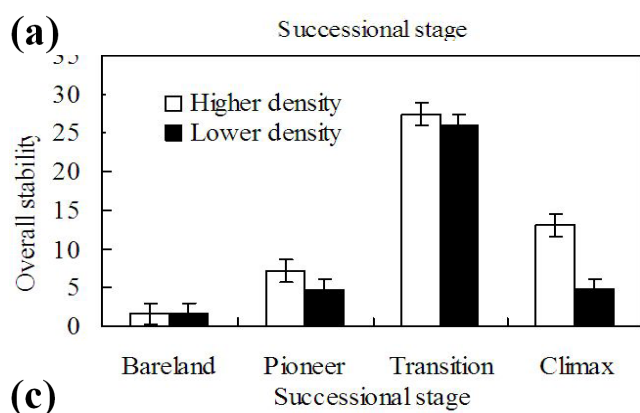
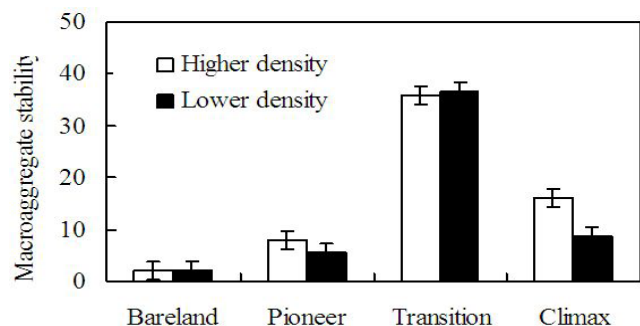
A data normality test was made and if weak, for the sake of maintaining the originality of data, data transformation was avoided by the use of a nonparametric method. Therefore, a multivariate General Linear Model (GLM) analysis was first applied to examine treatment ef-

fects of successional stage and community plant density and their interactions on soil stability in a random-plot design. Since successional stage in effect includes 'hidden treatments' such as species richness and composition, we conducted a multivariate GLM analysis to further test the specific effects of community plant species richness, composition, density, and their interactions on soil stability. In addition, plant composition variation is usually associated with a proportionate change in diversifying root life forms within a soil volume. We thus conducted another GLM analysis to test the effects of species richness, density, root-forms, and their interactions on soil stability. A one-way ANOVA was also carried out to examine effects of species richness on soil stability. Student-Newman-Keuls (SNK-q) tests were used to discern the multiple means' grouping performance. To draw the BSR, we performed a regression analysis to fit the variations in diversity during succession and accompanying soil stability. To explore the underlying mechanisms for the observed BSR, a regression analysis was used to distil possible links between key root variables and concomitant soil stability. All the analyses were performed using SPSS 13.0 for Windows (SPSS Inc., 1989-2004, USA).

Results

Species richness variation and compositions during succession

Our vegetation survey indicated that species richness increased drastically throughout succession, from 1 (3 in the plot) at the pioneer stage to about 5 (16 in the plot) at the transitional stage, and then decreased to 1 (3 in the plot) again at the climax stage (Fig. 1d; Tab. 1).



The referenced bare-land stage had the lowest soil stability, while the transitional stage had the highest, and both early and late successional stages exhibited intermediate levels of stability (Fig. 1a-c). In contrast, high plant density was related to greater soil stability (Fig. 1; Fig. 2).

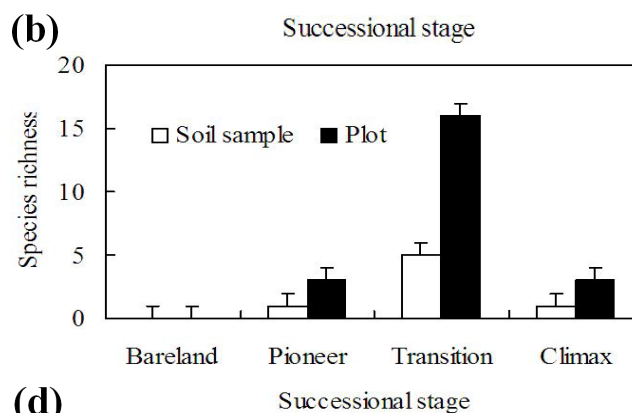
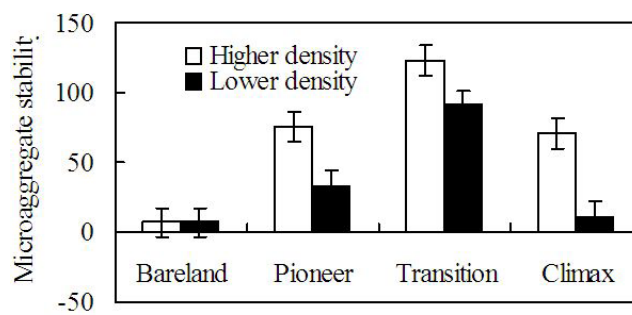


Fig. 1. Effects of successional stage and plant density on soil stability (\pm SE). (a): macroaggregate stability (AS); (b): microaggregate stability (BS); (c): overall soil stability (TS); (d): species richness variations along successions

Also, plant community compositions evolved from early colonizing annual herbs (e.g. *Chloris virgata*) to complicated mixtures (annuals and perennials) and then to the climax-indicator dominated perennials (Tab. 1).

Effects of successional stage, species richness, composition, and plant density on soil stability

Multivariate GLM revealed that both successional stage and plant density had significant effects on all soil stability measures, i.e., microaggregate stability, macroaggregate stability, and overall soil stability, but no significant interactive effects ($\alpha = 0.05$; Tab. 2, Fig. 1a-c).

Tab. 2. Effects of successional stage (ST), plant density (D), and their interactions on soil stability, using multivariate analysis of General Linear Model (GLM). DF is degrees of freedom

	AS		BS		TS		
	df	F	P	F	P	F	P
ST	2	165.77	<.001	21.19	<.001	124.36	<.001
D	1	4.49	0.052	25.89	<.001	12.27	0.004
STxD	2	2.91	0.088	0.77	0.480	3.37	0.064

However, plant density had a stronger effect on BS ($p < 0.01$) and TS ($p < 0.01$) than on AS ($p = 0.052$).

Further test results substantiated that the ‘hidden treatments’ of successional stage, e.g., species richness and composition, together with plant density, also had significant effects on soil stability, but still no interactive effects ($\alpha = 0.05$; Tab. 3, Fig. 2a-f).

Tab. 3. Interactive effects of species richness (SP), composition (C) or root-forms (R) and plant density (D) on soil stability using multivariate GLM

	AS		BS		TS		
	df	F	P	F	P	F	P
SP	1	190.72	<.001	40.15	<.001	173.65	<.001
D	1	3.34	0.089	24.04	<.001	10.48	0.006
C (R)	1	10.85	0.005	1.63	0.222	4.50	0.052
SPxD	1	2.236	0.154	0.927	0.350	1.942	0.183
SPxC (R)	0						
DxC (R)	1	2.06	0.173	0.61	0.449	4.00	0.065
SPxDxC (R)							

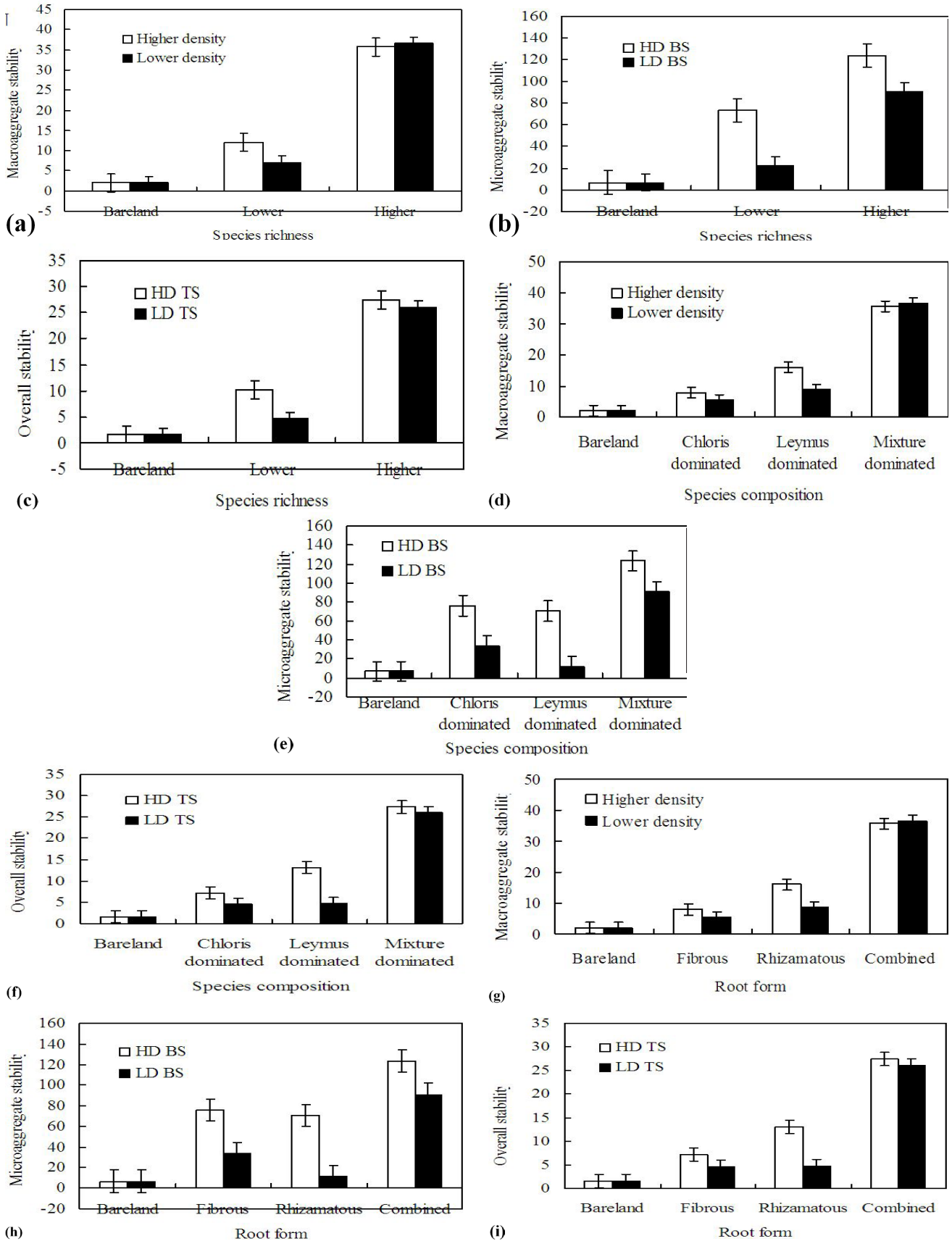


Fig. 2. Effects of species richness, composition and dominant root-form on soil stability (\pm SE). See Fig. 1 for treatment abbreviations

Species richness positively affected all three types of soil stability measures significantly ($p < 0.01$) – species-rich systems had higher soil stability than species-poor systems, whereas species composition had much stronger effects on AS ($p < 0.01$) than on TS ($p = 0.052$) but not on BS ($p = 0.222$; Tab. 3). Nevertheless, microaggregates appeared to be more stable than macroaggregates under all treatments, with generally higher BS values than AS or TS (Fig. 1; Fig. 2).

Diversity-stability relationship and possible mechanisms

Similar to the results for each successional stage, throughout the entire successional cycle, all three measures of soil stability also showed positive relationships with plant species richness (Fig. 3a-c). Root-soil stability analysis indicated that, with the changing direction of plant diversity during succession, plant root-form scenarios affected soil stability, consistent with the effect of species compositions (Tab. 3; Fig. 2g-i). In general, bare-land (no roots) had the lowest soil stability, while communities with combinations of fibrous-root- and rhizomatous-root species generated the highest soil stability, with intermediate performances seen for species with either solely fibrous roots or rhizomatous roots (Fig. 2g-i).

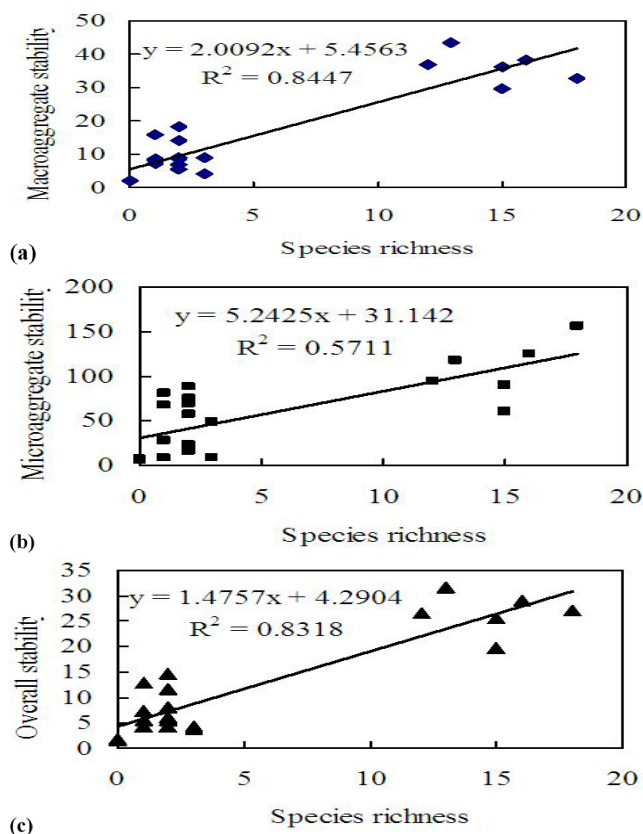


Fig. 3. The relationship between species richness and soil stability for macroaggregate stability (AS; a); microaggregate stability (BS; b); and overall soil stability (TS; c)

A further plant root-soil stability analysis corroborated that soil stability was positively correlated with both root density (RD) and root length density (RLD) (Fig. 4a-f). However, RD (biomass) showed a stronger correlation with soil stability than RLD (Fig. 4).

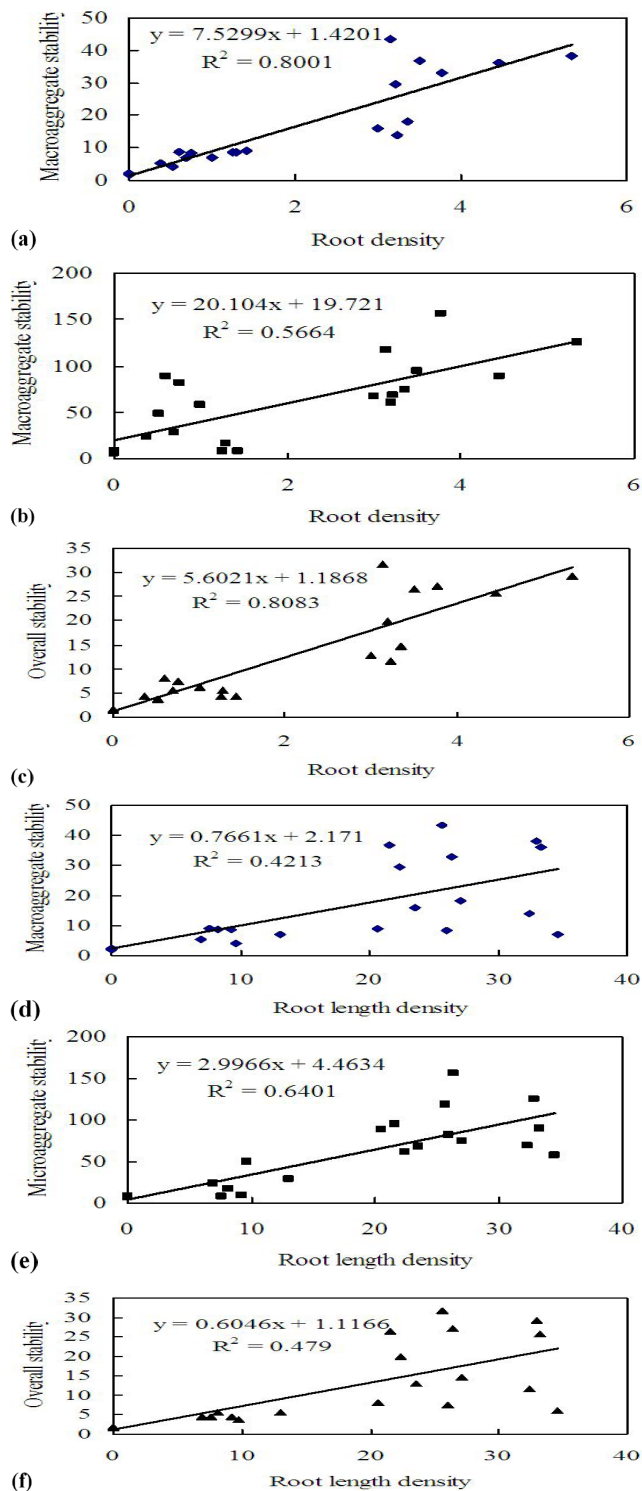


Fig. 4. The relationship between plant root and soil stability for macroaggregate stability (AS; a,d); microaggregate stability (BS; b, e); and overall soil stability (TS; c, f)

Discussion

Species richness and composition during succession

Our results exhibit a general trend of changing plant species richness and associated community compositions in parallel with steppe succession, consistent with the diversity-time hypothesis (Ricklefs, 2007). This trend is also in line with other mainstream ecological theories, e.g., the succession theory (Peet and Christensen, 1980; Huston and Smith, 1987), the competition theory (Rajaniemi *et al.*, 2003), and the climax hypothesis (Phillips, 1934). During the early colonizing phase of succession, growing conditions are usually harsh and plant growth is slow, which often results in long-term declines in plant species diversity (Cammeraat *et al.*, 2005) and dominance of short-span early successional species, e.g., annual herbs. Moreover, these species may be better colonists but poorer competitors in the communities (Tilman *et al.*, 2007). In contrast, during transitional stages from early to late succession, species richness tends to increase toward an acme, with intensifying resource use efficiency and species interactions during community assembly, exhibiting a tendency toward saturation of the communities. While in late succession, intense competitions and other evolutionary factors lead to a major exclusion effect and thus decline in species richness, finally generating the climax communities (Ricklefs, 2007). Meanwhile, in the course of succession, early successional species are replaced by more persistent species, e.g., perennial herbs or woody species, causing manifest variations in community species composition. Moreover, annual herbs with fibrous roots in the early stage were gradually replaced by perennial grasses with rhizomatous roots dominating the late successional stage, with a transitional period dominated by a mix of annuals and perennials with their own specific respective root-forms. Based on these findings, our results thus suggest that space-for-time substitution rather than permanent-plot experiments may indeed be a robust tool to facilitate long-term ecological studies, such as exploring long-term BSRs, in agreement with Foster and Tilman (2000).

What are the determinants of soil stability under changing plant diversity conditions during succession?

Soil stability, as measured by soil aggregate stability (resistance to disturbance) or fluctuating rates of other soil properties or processes (variability; e.g., Wardle *et al.*, 2000; Wang *et al.*, 2012), is an elementary component of ecosystem stability. It has been suggested that the mechanisms by which plants stabilize soil aggregates may be manifold (Pohl *et al.*, 2009). First, the above-ground accumulated plant litter can increase carbon input (soil organic matter) in the soils through decomposition (Wardle, 2006; Sylvain and Wall, 2011), causing an enhanced soil aggregation process (Franzluebbers *et al.*, 2000; Cammeraat *et al.*, 2005). Second, the below-ground root systems may form anchors that can stabilize the loose soil (Gyssels and Po-

esen, 2003; Pohl *et al.*, 2009). Third, dead roots can also accumulate, thus increasing carbon input into the soil, as is often seen in higher productivity environments (Sylvain and Wall, 2011). Fourth, other above- and below-ground factors favouring rhizospheric activities will also favour soil aggregation by increasing glomalin, e.g., increased biomass production, higher AMF abundance (Wilson *et al.*, 2009), or other enriched soil biodiversity conditions. In particular, all these may be found over successional phases (Dovciak and Halpern, 2010), an idea also borne out in our investigation. For example, vegetation successional stage has been reported to have progressively increasing effects on the population stability of forest herbs (Dovciak and Halpern, 2010). However, no vegetation successional effect has been observed on slope stability in abandoned lands (Cammeraat *et al.*, 2005). Yet in our case, although successional stage had significant effects on soil stability, these effects did not exert themselves progressively over time (Fig. 1).

It has been further assumed that many factors attributable to successional stage or life history may play respective roles in regulating ecosystem stability (Dovciak and Halpern, 2010). First, higher plant diversity may indeed enhance soil stability. For example, Pohl *et al.* (2009) found that, in disturbed alpine ecosystems, plant diversity can enhance soil stability. Wang *et al.* (2012) also report that, in secondary succession in a semi-humid evergreen broadleaf forest, plant species richness can enhance soil conservation, although the effect may be relatively weak compared to the contributions of plant cover and density. In particular, plant functional richness may also exert effects on some below-ground properties in natural communities but not on the resistance to perturbation of any of the ecosystem properties considered in artificial communities (Wardle *et al.*, 2000; see also Gastine *et al.*, 2003 study in temperate grassland communities). In addition, litter diversity may not always support the view that enhanced species richness improves ecosystem functions (Wardle, 1997). Despite these debates, it is believed that ecosystem stability can be maintained if diversity has facilitative effects, or if stability is a precursor rather than a response to diversity (Dovciak and Halpern, 2010; see also Bai *et al.*, 2004; Wang *et al.*, 2012).

Second, however, the composition of the above-ground plant species (or the identity of a functional group) may affect the responses of below-ground properties to disturbance and thus affect ecosystem stability, albeit the relative effects of plant richness, composition and others are still disputed on occasion (Hooper and Vitousek, 1997; Tilman *et al.*, 1997; Wardle *et al.*, 2000; Wang *et al.*, 2012).

Third, plant density may also affect ecosystem functioning (Jiang, 2007). However, density dependence may not be always prevalent, particularly in a highly heterogeneous ecosystem (Luo *et al.*, 2012). Consistent at least in part with previous findings, our results stipulate that plant

species richness, composition (also functional), and plant density simultaneously affect soil aggregate stability in the Songnen steppe.

Apart from the above, disturbance may also influence ecosystem stability either directly or indirectly through the richness, composition, or functions of the governing species. For instance, on some occasions mowing may increase population and community stability, and nutrient addition may have the opposite effect, but they do not alter the BSR patterns (Yang *et al.*, 2012). However, grazing of pastures may have little detrimental effect on soil aggregate stability in the Southern Piedmont USA (Franzuebbers *et al.*, 2000). By contrast, compared to other factors such as skiing in general, heavy disturbances like machine-grading on ski slopes can have strong effects on soil stability and species richness as well as on the BSRs in disturbed alpine ecosystems (Pohl *et al.*, 2012). Therefore, vegetation factors, including species richness, cover, and root character, are more important for soil stability at machine-graded sites than at sites with little disturbance. In our study, disturbance has not been considered as a separate factor affecting soil stability, since one of our goals is to investigate the native Eurasian steppes subjected to no major anthropogenic perturbations over entire successional cycles. To investigate the likely effects of a specific disturbance on soil stability, new studies with specific field or experimental designs are needed. However, despite the difficulty of pinpointing all the factors affecting soil stability, and their likely intrinsic linkages among each other, our results suggest that the determinants of soil stability in relation to plant diversity are of multivariate sources operating over entire successional cycles.

Positive diversity-stability relationships and underlying mechanisms

Interestingly, the positive species richness-soil stability relationships observed in this study are in agreement with major theories that biodiversity begets stability (e.g. Doak *et al.*, 1998; Tilman *et al.*, 1998; Tilman, 1999; Lehman and Tilman, 2000) and many empirical studies including above-ground (e.g., Tilman and Downing, 1994; Tilman *et al.*, 2006; Dovciak and Halpern, 2010; Allan *et al.*, 2011), below-ground (e.g., Heemsbergen *et al.*, 2004; Wilson *et al.*, 2009; Wagg *et al.*, 2011), and above- versus below-ground (e.g., Porazinska *et al.*, 2003; Zak *et al.*, 2003; Orwin *et al.*, 2010; Eisenhauer *et al.*, 2011) experiments. In particular, it is also consistent with the recent efforts on biodiversity-ecosystem multifunctionality relationships (Hector and Bagchi, 2007; Maestre *et al.*, 2012). However, although the positive plant species richness - soil stability relationship is strong over the course of succession in our study, species richness is not the only major driver of soil stability and there is a definite cause-and-effect relationship. In our study, plant density, together with species richness and composition, all significantly affected soil stability, which is consistent with Wang *et al.* (2012). But

the relative importance of species richness was greater than that of density and composition in affecting soil stability in our case. Therefore, compared to other efforts in solely above-ground or solely below-ground systems, our efforts linking above- and below-ground elements may reveal particular mechanisms governing plant diversity and soil stability relations. In this light, the positive plant species richness-soil stability relationships obtained throughout entire succession cycles in our study may only be a by-product of multivariate co-working mechanisms of major plant community traits or others.

Unlike some theoretical interpretations trying to elucidate mechanisms for maintaining ecosystem stability (e.g. Tilman *et al.*, 1998; Yang *et al.*, 2012), we seek interpretations based on likely mechanistic pathways leading to the diversity-dependent stability of the steppe soils. Fortunately we found that, moving along the direction of succession, there is always some regular rhythmic fluctuation of soil stability in response to variations in species richness, community composition and plant density across successional stages (Figs. 1, 2). This is because plant community succession often involves simultaneous changes not only in species richness (number), but also in species compositions (identity) and community density (abundance). Therefore, it would be very odd to relate species richness only to stability, when other diversity-related variables may also affect stability at the same time. In our research, with the variations in species richness with succession, species-poor communities often have simple compositions and fewer root types with lower root biomass, while species-rich communities have more complex compositions with mixed root types and higher root biomass (Tab. 1; Figs. 2, 3, 4). To our knowledge, the enhanced root biomass and mixing of diverse root types in the communities can result in a positive relationship between soil stability and plant species richness (Pohl *et al.*, 2009; Sylvain and Wall, 2011). In contrast, density compensation may be equally important for maintaining soil stability (Jiang, 2007). However, in some situations, density may actually override the effect of species richness-induced elements, posing a conversion of roles between species richness and density in governing richness-stability relationships.

Conclusions

We conclude as follows: (1) Under natural colonizing conditions in the Songnen Steppes, species-poor systems achieved lower soil stability than species-rich systems, regardless of successional stage; (2) Soil stability was dramatically regulated by plant species richness (number), composition (identity), density (abundance), and functional roles; (3) A long-term positive plant diversity-soil stability relationship exists in steppe succession. Particularly, this enhanced effect of biodiversity on soil stability operates via diversifying plant root traits.

In summary, the compelling and realistic threat of global biodiversity loss has forced us to cope with biodiversity conservation issues seriously. However, these concerns may need an integrated approach and a unifying BEF theory that can guide conservation activities. In this regard, our findings on plant diversity and soil stability relationships over entire successions may be a good addition to currently major BEF theories and empiricisms. Indeed, incorporating both above- and below-ground patterns and processes may help form a unifying BEF theory that incorporates major subsystems of ecosystems and reconciles all kinds of relations amongst them.

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