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LETTER

Decoupled responses of above- and below-ground beta-diversity to nitrogen enrichment in a typical steppe

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Abstract

Increased atmospheric nitrogen (N) deposition affects biodiversity in terrestrial ecosystems. However, we do not know whether the effects of N on above-ground plant β-diversity are coupled with changes occurring in the soil seed bank. We conducted a long-term N-addition experiment in a typical steppe and found that above-ground β-diversity increased and then decreased with increasing N addition, whereas below-ground β-diversity decreased linearly. This suggests decoupled dynamics of plant communities and their soil seed bank under N enrichment. Species substitution determined above- and below-ground β-diversity change via an increasing role of deterministic processes with N addition. These effects were mostly driven by differential responses of the above-ground vegetation and the soil seed bank β-diversities to N-induced changes in environmental heterogeneity, increased soil inorganic N concentrations and soil acidification. Our findings highlight the importance of considering above- and below-ground processes simultaneously for effectively conserving grassland ecosystems under N enrichment.

KEYWORDS
beta diversity components, community assembly, environmental heterogeneity, grassland ecosystems, nitrogen deposition, soil seed bank

INTRODUCTION

Increased N inputs from the atmosphere into terrestrial ecosystems usually enhance biomass production (Bobbink et al., 2010) and reduce plant diversity (Zhang et al., 2021). Previous studies have shown that N enrichment decreases species richness and abundance (e.g. α-diversity) in above-ground vegetation (Midolo et al., 2019) and the soil seed bank (Basto et al., 2015). However, studies on the effects of N enrichment on the β-diversity of above-ground vegetation, a measure of spatial turnover of species composition, have not yet reached a consensus, with some studies reporting positive (Chase, 2010), negative (Liu et al., 2021) and neutral effects (Yang et al., 2019). The response patterns of soil seed bank β-diversity to N enrichment are even less clear. Different plant species in a community have different responses to changes in biotic and abiotic factors caused by N enrichment (Lebrija-Trejos et al., 2010; Midolo et al., 2019). Therefore, N enrichment may alter the β-diversity of both above-ground vegetation and the soil seed bank in grassland ecosystems. This in turn affects the diversity from local (α-diversity) to regional (γ-diversity) levels (Hautier et al., 2020; Wang & Loreau, 2016). Understanding changes in β-diversity of above-ground vegetation and the soil seed bank is crucial for detecting changes in regional biodiversity and the underlying mechanisms of community assembly under N enrichment.

Changes in β-diversity can be caused by two different processes (Baselga, 2017): (1) species substitution, whereby individuals of some species in one site are substituted with the same number of individuals of different species from another site, and (2) abundance difference, whereby some individuals are absent from one site compared to others (Baselga, 2017). Seed input predominantly depends on the reproduction of local above-ground vegetation (Meyer-Grunefeldt et al., 2015); the regeneration of above-ground vegetation, especially of those species that rely on seed germination for population recruitment, mainly depends on seeds from the soil seed bank (An et al., 2020). Therefore, species substitution and abundance difference in the above-ground vegetation and the soil seed bank influence each other, leading to the coupling of β-diversity between above-ground vegetation and the soil seed bank. However, under accelerating global changes, β-diversity of above-ground vegetation and soil seed bank may become decoupled. It has been suggested that global change (e.g. N deposition) may frequently result in a disruption of the connections among species, as well as among species and their environment before the most obvious impacts can be detected (Ochoa-Hueso, 2016). This may partly be attributed to different sensitivities within the community or ecosystem properties to the new environmental conditions (Ochoa-Hueso et al., 2021). For example, in the case of the above-ground vegetation and soil seed bank, N deposition may result in a decoupling of these above- and below-ground components due to a lagged response of the soil seed bank (Zhang et al., 2019). Moreover, adding N to soils may stimulate the predation/decomposition of seeds with high C:N ratio by N-limited soil organisms, thus again causing a mismatch between the above- and below-ground compartments (Ochoa-Hueso & Manrique, 2010). In this regard, the soil seed bank may play a limited role in the restoration of degraded grasslands due to a depleted abundance of seeds (Leishman et al., 2000). Therefore, studying the β-diversity of both the above-ground vegetation and soil seed bank can help to identify linkages between the above- and below-ground processes that modulate plant community assemblies and provide effective guidance for biodiversity conservation under changing environments.

Deterministic and stochastic processes may affect species substitution and abundance difference responses to N enrichment. Deterministic processes involve environmental filtering and various biological interactions that result in communities where certain characteristics dominate (Chase & Myers, 2011), whereas stochastic processes are associated with dispersal and ecological drift (Bell, 2001). Nitrogen enrichment typically intensifies the competition between plants for resources such as light and nutrients (Hautier et al., 2009; Rajaniemi et al., 2003) and changes the physical and chemical properties of the soil, including increasing concentrations of NH_4^+-N and cations and reducing pH. Therefore, deterministic community assembly processes may lead to the survival of only a few species that are nitrophilous or acidophilous, consequently decreasing the chance for species substitution. In such circumstances, the β-diversity of above-ground vegetation would be reduced (Chalcraft et al., 2008; Conradi et al., 2017). However, seeds arriving and accumulating at a site are usually less sensitive to small-scale changes in soil conditions than are growing plants and seldom experience interspecific competition (DeMalach et al., 2017; Walck et al., 2011). Therefore, species substitution and abundance differences in the soil seed bank may not be reduced at low N-addition rates, leading to different β-diversity responses to N addition in above-ground vegetation and the soil seed bank. Nitrogen enrichment can also increase plant community productivity, leading to greater stochasticity in community assembly (Chase, 2010). Alternative states can occur in highly productive communities, leading to greater variation in species substitution or abundance difference, consequently increasing the β-diversity of above-ground vegetation (Steiner & Leibold, 2004). Increased plant cover and biomass due to N enrichment also constitute a mechanical barrier to seed input to the soil seed bank and suppress seed germination (Gross, 1998), which may result in a decoupling between the above-ground vegetation and soil seed bank β-diversity. However, to date, few studies have considered the relative importance of different above- and below-ground mechanisms in determining changes in the β-diversity of above-ground vegetation and the soil seed bank under N enrichment.

Here, we use a 12-year multi-level N-addition experiment in the temperate steppe of northern China to explore the responses of the β-diversity of the above-ground vegetation and soil seed bank to N enrichment. We asked the two following questions: First, how does the β-diversity of the above-ground vegetation and soil seed bank respond to N enrichment? We hypothesized that the β-diversity of the above-ground vegetation and soil seed bank would decrease at different rates, with the above-ground portion responding much stronger than the below-ground counterpart. Second, what are the main response mechanisms of the β-diversity of the above-ground vegetation and soil seed bank to N enrichment? We hypothesized that reduced species substitution would mainly determine the reduction in β-diversity with increasing N addition. We also predicted that deterministic processes might mainly govern the reduction in β-diversity in above-ground vegetation and the soil seed bank.

MATERIALS AND METHODS

Study site and experimental design

We conducted a field experiment in a steppe grassland (43°32′45″ N, 116°40′30″ E) in Xilinhot, Inner Mongolia Autonomous Region, China. This area received approximately 349.6 mm of annual precipitation (1980–2014), with 72% occurring during the growing season (May–August). The average annual temperature was 0.9°C. The lowest temperature occurred during January (−21.1°C), and the highest occurred during July (19.8°C). According to the US soil taxonomy classification, the soil type was a Calcic–Orthic Aridisol. Two perennial grasses, namely *Leymus chinensis* and *Stipa grandis*, account for more than 60% of the above-ground plant biomass during the peak growing season.

The N-addition experiment was initiated in September 2008 at a relatively flat and geologically uniform site. The study site had been fenced in 1999 to prevent grazing by large animals and received no fertilization prior to the experiment. The experiment used a random-block design with 10 blocks of 45 × 70 m each and a 2 m buffer between each block. Each block was divided into 8 × 8 m experimental plots. Nitrogen was added evenly to each plot in the form of NH_4NO_3 with nine different loads (0, 1, 2, 3, 5, 10, 15, 20 and 50 $\mathrm{g\,N\,m^{-2}\,year^{-1}}$) and two frequencies, that is, twice or 12 times per year, with 10 replicates for each treatment. The experiment started on 1 September 2008 and N was added on the first day of each month thereafter under the high-frequency treatment of 12 times per year. For the low-frequency treatments twice per year, NH_4NO_3 was added to the plots on 1 November 2008, and subsequently on 1 June and 1 November annually. By 2020, all the plots had received 12 consecutive years of treatment. Detailed information on this experimental design can be found in Zhang et al. (2014). This experiment aimed to examine how the amount and frequency of N addition affected species turnover during the growing season (Zhao et al., 2022). In this study, we only used high-frequency additions that mimicked chronic N deposition in the real world. This allowed us to explore how increased N deposition would change the spatial diversity of the above-ground vegetation and the soil seed bank.

Above-ground vegetation and soil seed bank sampling

In April 2019, we established a fixed quadrat of 0.5 × 2 m in each plot to conduct monthly surveys of the richness and abundance of the above-ground vegetation. These surveys were conducted in the middle of each month from May to September. This was repeated in newly established subplots in 2020. For the above-ground vegetation data, we only used the survey results from August of each year because they represented the period with the greatest number of species and peak biomass.

The soil seed bank was sampled in April 2019 and 2020. Soil samples were collected using a soil corer (diameter 5 cm) to a depth of 10 cm. Five soil cores were sampled from each plot and evenly mixed to form one composite sample. Ninety soil samples were collected in total, placed in cloth bags, air-dried for 15 days and sieved to 2 mm. The seed germination method was used to identify the species and seed density of the soil seed bank. Fine sand was spread in a 40-cm-long, 24-cm-wide and 8-cm-deep plastic tray and covered with nylon fabric with a mesh diameter of 0.2 mm. Soil samples were evenly spread onto the mesh. We also prepared 10 trays only containing fine sand as a control to detect the presence of any germinating seeds that were not from the research site. The trays were watered daily, and seedlings were removed after identification. Seedlings that could not be identified were left in place for subsequent identification at later stages. When no new seeds had germinated for 2 weeks, the soil in each plastic tray was air-dried for 5–6 days, and then evenly mixed. Germination was then allowed to continue until no new seeds germinated for 5 days. Germination experiments were performed in a greenhouse at the Institute of Botany, Chinese Academy of Sciences, Beijing, and the duration of the experiment was 8 months.

Sampling and measurement of soil variables

At the end of August 2019 and 2020, three soil cores 0.5 m apart with a depth of 10 cm were collected using a soil auger with a 3 cm diameter near each plant sampling quadrat. Each set of three soil samples was thoroughly mixed to form a composite sample. Soils were then sieved through a 2 mm mesh for soil NH_4^+-N, NO_3^--N and soil moisture content (%) analyses. Sub-samples were air-dried for soil pH, soil total carbon (C) concentration, soil total N concentration and soil effective cation exchange capacity (ECEC; the sum of charge equivalents of exchangeable cations, such as Ca^{2+}, Mg^{2+}, K^+ and Na^+) analyses. Soil inorganic N was extracted using 50 mL of

2.0 M KCl solution. The concentrations of soil NH_4^+-N and NO_3^--N were measured using a flow injection auto-analyser (FLAstar 5000 Analyser; Foss Tecator, Hillerød, Denmark). The soil pH was measured in water suspension (soil:water = 1:2.5) using a pH meter (FE20-FiveEasy, Mettler Toledo, Columbus, Ohio, USA). The concentrations of total C and N were measured using an elemental analyser (Elementar Analysensysteme GmbH, Hanau, Germany). The ECEC was analysed by extraction with 1 M NH_4OAc, and exchangeable Ca, Mg, K and Na were measured using an atomic absorption spectrophotometer (AA-6800, Shimadzu, Japan).

Statistical analysis

Beta diversity was calculated based on the abundance-weighted Bray–Curtis dissimilarity index. This is a commonly used method for calculating β-diversity that is robust to sampling errors (Schroeder & Jenkins, 2018). β-diversity may also depend on community size (Chase & Myers, 2011; Kraft et al., 2011). Therefore, we performed a null model analysis to disentangle the variations in β-diversity from variations in α-diversity. We calculated the standard effective size of β-diversity (β-deviation) by comparing the observed β-diversity values with those generated by a null model. We generated 999 null local communities for each amount of added N and randomly placed individuals into each local community with probabilities proportional to the regional relative cover/abundance of each species, while retaining the local α-diversity (Ning et al., 2019). The standardized effect size (β-deviation) was calculated as the difference between the observed and mean β-diversity of the null communities, divided by the standard deviation of the β-diversity of the null communities (Ning et al., 2019). β-deviation values approaching 0 indicate that β-diversity does not deviate from stochastic expectations, whereas negative or positive β-deviation values indicate lower or higher β-diversity than expected by chance. We tested the significance of N addition effects on the β-diversity and β-deviation using permutational analyses of variance. To explore the various ecological processes associated with β-diversity patterns based on species abundance, β-diversity was partitioned into balanced variation in abundance, whereby the individuals of some species in one site are substituted with the same number of individuals of different species from another site, and abundance gradients, whereby some individuals are absent from one site compared to others (Baselga, 2017). We used (1) species substitution as a proxy of balanced variation in abundance and (2) abundance difference as a proxy for the abundance gradient. These components were calculated based on the Bray–Curtis distance matrix (abundance-based assemblage dissimilarity) and were analogous to the division of β-diversity into species replacement and richness differences based on Sørensen dissimilarities (presence-/absence-based assemblage dissimilarity) (Baselga, 2017).

We also quantified the magnitude of stochastic processes using the normalized stochasticity ratio (NST) (Ning et al., 2019). This method assumes that the communities consist of a combination of two types of species based on their type of assembly. One type of species is under completely deterministic assembly, and the other type is under completely stochastic assembly (Ning et al., 2019). The NST measures the relative position of the observed value between extremes of pure deterministic and pure stochastic assemblies. Therefore, it reflects the contribution of stochastic assembly relative to deterministic assembly and is based on the magnitude rather than the difference between the observed and null expectations. Therefore, NST can be considered a quantitative measure of stochasticity. NST was determined using 50% as the boundary point between the deterministic (0%–50%) and stochastic (50%–100%) assemblies (Ning et al., 2019).

Based on six soil physicochemical indicators, namely soil moisture content, soil pH, soil total C concentration, soil total N concentration, soil inorganic N concentration and soil ECEC, we quantified the environmental heterogeneity of 10 duplicate plots in the same treatment via total pairwise Euclidean distances. The *Z*-score was standardized for the six indicators before calculating environmental heterogeneity. Pearson's correlation coefficients between predictor variables, namely environmental heterogeneity and physicochemical indicators, and β-deviation of the above-ground vegetation and soil seed bank were calculated using Pearson's chi-squared test. Multicollinearity of the predictor variables was evaluated by calculating the variance inflation factors prior to the analysis. This led to the exclusion of the soil total N concentration and soil ECEC. Soil moisture content, soil inorganic N concentration, environmental heterogeneity, soil pH, and soil total C concentration were used as predictor variables because of their low collinearity. The relative contribution of each predictor variable to the β-deviation was determined using the hierarchical partitioning method (Lai et al., 2022). The relative contribution of above-ground vegetation β-deviation to soil seed bank β-deviation and vice versa were also considered. To compare the relative contributions of different functional groups to the species substitution and abundance difference of the community, a 'new' community was formed by removing either grasses or forbs. Following this, the contribution of grasses and forbs to the overall species substitution and abundance difference of the community was measured indirectly by comparing the degree of correlation coefficient between them in the new and original communities respectively (Lennon et al., 2004). In addition, we calculated the species similarity in the above-ground vegetation and soil seed bank using Jaccard's similarity index (Real & Vargas, 1996):

$$SJ = S/(S1 + S2 - S),$$

where S1 and S2 are species richness of above-ground vegetation and soil seed bank, respectively, and S is the number of species shared by above-ground vegetation and soil seed bank.

All the statistical analyses were performed using R version 3.4.0 (R Core Team, 2018). β-diversity was calculated by applying the 'vegdist' function in the package 'vegan' (Oksanen et al., 2012). The environmental Euclidean distance was calculated by applying the 'dist' function in the package 'stats'. The null communities were generated by applying the 'commsim' function in 'vegan' (Oksanen et al., 2012). Permutational analyses of variance were performed using the 'adonis2' function in 'vegan' (Oksanen et al., 2012). The species substitution and difference in abundance were calculated using the functions 'beta.pair.abund' and 'beta.multi.abund' in the package 'betapart' (Baselga et al., 2013). NST and relative importance values were calculated using the 'NST' and 'relaimpo' packages respectively (Ning et al., 2019). Hierarchical partitioning was performed using the 'rdacca.hp' package (Lai et al., 2022).

RESULTS

Responses of β-diversity and β-deviation to N enrichment

The average above-ground vegetation β-diversity increased linearly with N additions up to 10 g N m^{-2} year^{-1} and then slightly decreased ($n=18$, $p<0.05$; Figure 1a; Table S1). In contrast, the soil seed bank β-diversity decreased linearly in response to N ($n=18$, $p=0.06$; Figure 1b; Table S1). The β-deviation also exhibited a similar pattern to the β-diversity, particularly for the soil seed bank (Figure 1d). However, the magnitude of change in β-deviation of the above-ground vegetation was greater than that of the β-diversity, with a sharp decrease at N additions above 5 g N m^{-2} year^{-1} (Figure 1c). We also found that the species similarity between the above-ground vegetation and the soil seed bank decreased with increasing N addition (Figure S1). *L. chinensis and Agropyron cristatum* occurred less frequently in the soil seed bank and more frequently in the above-ground vegetation under N enrichment (Figure S2a,b). However, *Chenopodium aristatum* and *C. glaucum*, which occurred more frequently in the soil seed bank, were less common in the above-ground vegetation (Figure S2c,d).

Contribution of the species substitution and abundance difference to β-diversity, and the β-deviation as affected by deterministic and stochastic processes

Species substitution in the above-ground vegetation first increased and then decreased ($n=18$, $p<0.01$), whereas abundance difference continuously increased with the increasing amounts of N addition ($n=18$, $p=0.05$; Figure 2a). Species substitution in the above-ground vegetation was also negatively correlated

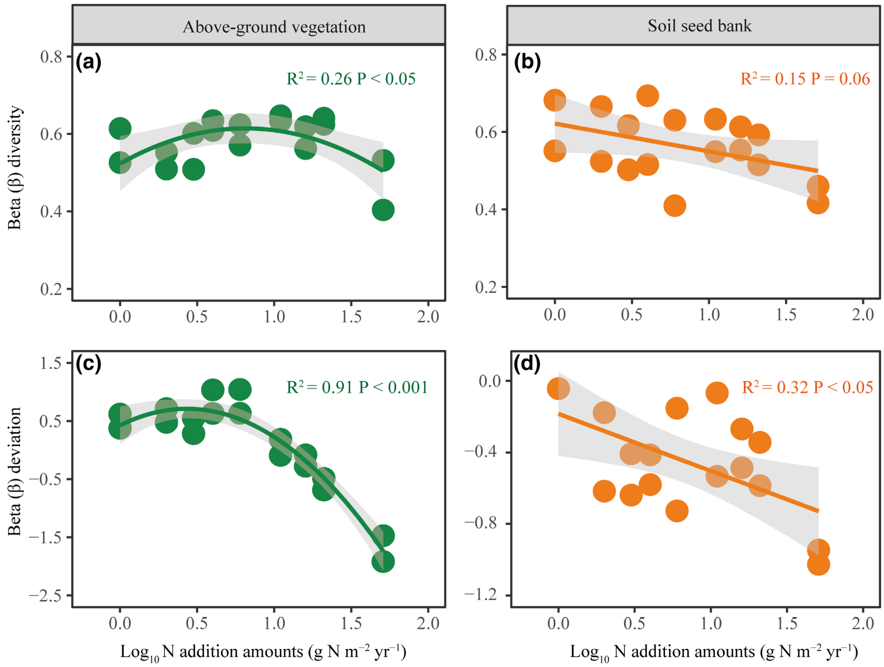

FIGURE 1 β-diversity (a, b) and β-deviation (c, d) of the above-ground vegetation and soil seed bank in the two studied years (2019 and 2020), among replicate plots within each N addition treatment. Negative β-deviation values indicate lower β-diversity than expected by chance, whereas positive β-deviation values indicate the opposite. The green and orange lines represent the above-ground vegetation and soil seed bank respectively. The shaded area is the 95% confidence interval of the regression lines.

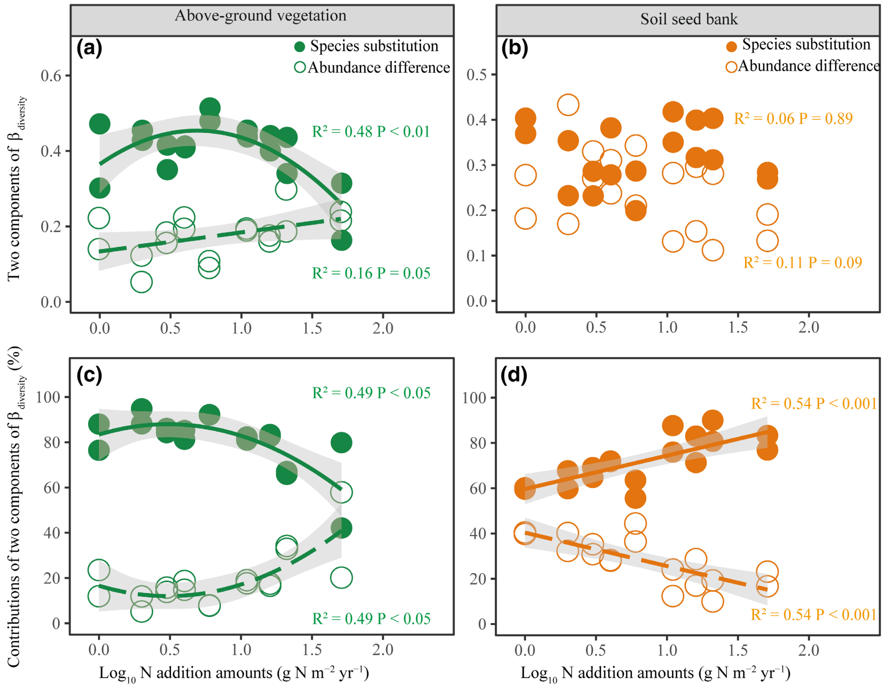

FIGURE 2 Relationship between the N addition amounts and species substitution and abundance difference of the above-ground vegetation (a) and soil seed bank (b) in the two studied years (2019 and 2020). The relative contributions of the species substitution and abundance difference to the β-diversity change of the above-ground vegetation (c) and soil seed bank (d) at each N addition amount in the two studied years (2019 and 2020). The green and orange lines represent the above-ground vegetation and soil seed bank respectively. The shaded area is the 95% confidence interval of the regression lines.

with soil inorganic N ($n=18$, $p<0.01$; Figure S3b) and positively correlated with soil pH and environmental heterogeneity ($n=18$, $p<0.1$; Figure S3c,d). Further analysis showed that the decrease in species substitution of above-ground vegetation was predominantly determined by forbs under N enrichment, whereas the increase in abundance difference was mainly determined by the dominant grass species (Table S2). We found that the proportion of forbs decreased and the proportion of *L. chinensis* abundance increased in the above-ground vegetation under N enrichment (Figure S4). Overall, neither species substitution nor abundance difference in the soil seed bank showed any significant relationships with increasing N ($n=18$, $p>0.05$; Figure 2b), but species substitution in the soil seed bank decreased under high N addition amounts ($n=18$, $p<0.05$; Figure S5).

The contribution of the species substitution to changes in above-ground β-diversity decreased from 82.25% to 60.98%, whereas the contribution of the abundance difference increased from 17.75% to 39.02% with increasing N amounts (Figure 2c). For the soil seed bank, the contribution of the species substitution to the changes in β-diversity increased from 59.88% to 80.11%, whereas the contribution of the abundance difference decreased from 40.12% to 19.89% with increasing N addition amounts (Figure 2d). The roles of stochastic processes in regulating the above-ground vegetation and soil seed bank under N enrichment were similar. The normalized stochasticity ratio decreased from 61.19% to 24.85% for above-ground vegetation but decreased gradually from 62.51% to 39.47% for the soil seed bank with increasing N (Figure 3).

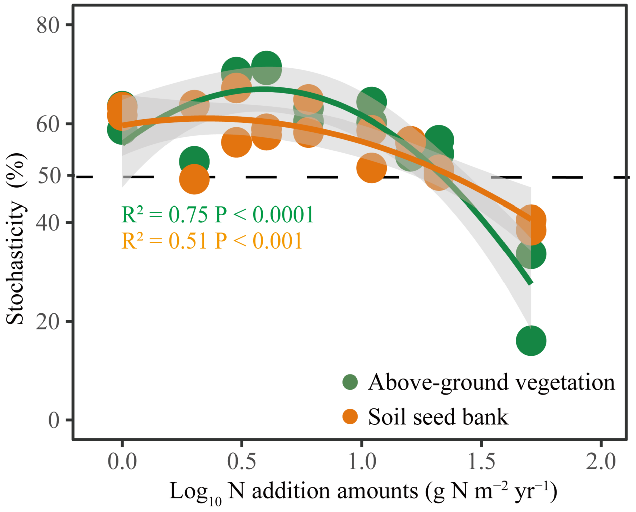

FIGURE 3 Magnitude of the stochasticity in the two studied years (2019 and 2020), quantified as the normalized stochasticity ratio, in regulating the above-ground vegetation and soil seed bank under each added N treatment. The values were calculated using Bray–Curtis dissimilarity. The green and orange lines represent the above-ground vegetation and soil seed bank respectively. The shaded area is the 95% confidence interval of the regression lines.

Relative importance of the environmental heterogeneity and soil properties for β-deviation

Nitrogen enrichment significantly increased soil inorganic N concentrations and reduced soil pH ($n = 18$, $p < 0.05$; Figure S6b,c). Environmental heterogeneity, based on six soil variables, increased in response to N loads up to 10 g N m^{-2} year^{-1} and then decreased with increasing N addition amounts ($n = 18$, $p < 0.001$; Figure 4a). The β-deviation of the above-ground vegetation and environmental heterogeneity were significantly positively correlated ($n = 18$, $p < 0.05$; Table S3). The soil seed bank β-deviation was not affected by environmental heterogeneity ($n = 18$, $p > 0.05$; Table S3). In turn, it was significantly positively correlated with the soil pH, soil total C concentration and β-deviation of the above-ground vegetation, but significantly negatively correlated with the soil inorganic N concentration ($n = 18$, $p < 0.01$; Table S3). Hierarchical partitioning analysis showed that soil inorganic N concentration, soil pH, environmental heterogeneity, soil total C concentration, β-deviation of the soil seed bank and soil moisture content accounted for 77.5% of the variation in β-deviation of the above-ground vegetation. Among these variables, soil inorganic N concentration, soil pH and environmental heterogeneity were the three most important factors regulating above-ground β-deviation (Figure 4b). Nevertheless, the same model explained only 20.5% of the variance in the soil seed bank β-deviation. Soil inorganic N concentration, β-deviation of above-ground vegetation and soil total C concentration were identified as the three most important factors responsible for the β-deviation variance in the soil seed bank (Figure 4c).

DISCUSSION

We found that the β-diversity of the above-ground vegetation and soil seed bank responded differently to N enrichment. The β-diversity and β-deviation of above-ground vegetation increased with N addition amounts of <10 and 5 g N m^{-2} year^{-1} respectively. This suggests that the grassland ecosystem we studied is limited by

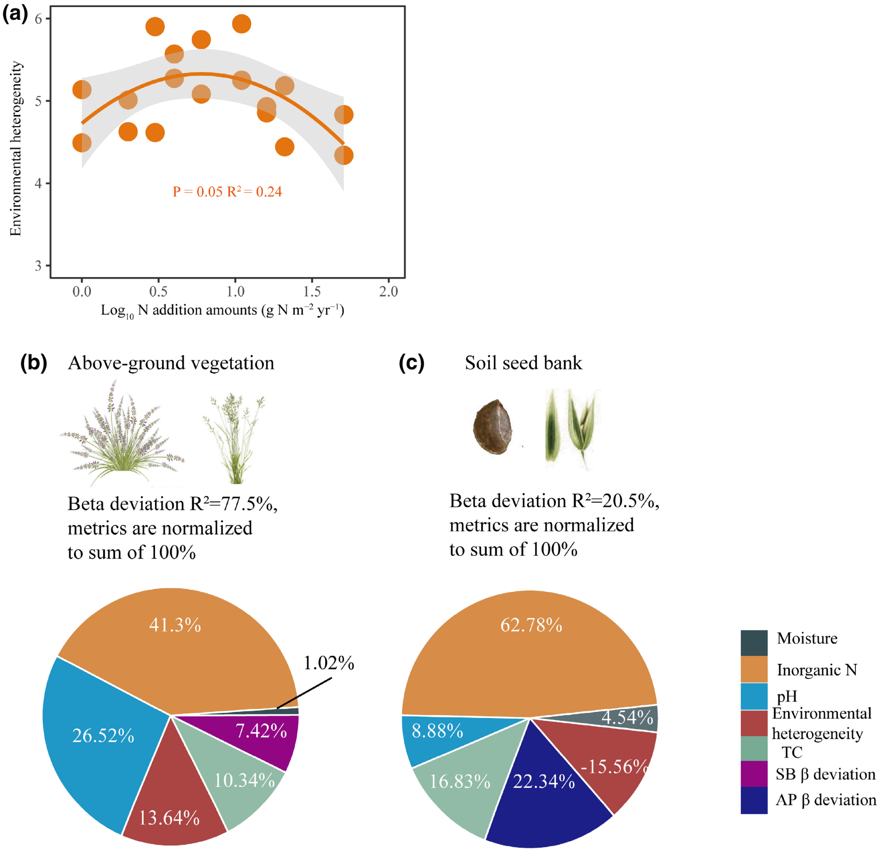

FIGURE 4 Relationship between soil environmental heterogeneity and N addition amounts in the two studied years (2019 and 2020) (a) and hierarchical partitioning analyses between explanatory variables and β-deviation of the above-ground vegetation (b) and soil seed bank (c). Environmental heterogeneity was calculated as the pairwise Euclidean distance among six standardized soil physicochemical variables (soil moisture content, soil pH, soil total C concentration, soil total N concentration, soil inorganic N concentration and soil effective cation exchange capacity) among 10 replicate plots under same treatment. Moisture indicates soil moisture content; Inorganic N is soil inorganic N concentration; pH is soil pH; Environmental heterogeneity is soil environmental heterogeneity; TC is soil total C concentration; SB β-deviation is β-deviation of soil seed bank; AP β-deviation is β-deviation of above-ground vegetation.

N, possibly due to historical livestock grazing and hay harvesting (Bai et al., 2004). However, beyond these thresholds, these two metrics decreased. In contrast, the β-deviation and β-diversity of the soil seed bank decreased with increasing N addition. Moreover, under N enrichment, species substitution played a more important role than did abundance difference in governing changes in β-diversity in the above-ground vegetation and soil seed bank. This suggests that the identity of species, instead of their abundance, mainly determines β-diversity under N enrichment.

Previous studies have shown that N enrichment has inconsistent effects on β-diversity in above-ground vegetation (Houseman et al., 2008; Koerner et al., 2016). For example, Chalcraft et al. (2008) found that in North American grasslands, N enrichment increased β-diversity of above-ground vegetation in low-productivity areas while it decreased β-diversity in high-productivity areas. They attributed this productivity-dependent response to N to the fact that, in low-productivity areas, N enrichment increased the richness differences, while in high-productivity areas, N enrichment decreased the richness differences. Moreover, evidence from the literature indicates that in low-productivity areas, high animal foraging efficiency may contribute to increase in environmental heterogeneity, leading to more species colonization and, consequently, higher β-diversity. In contrast, in the high-productivity areas, the effect of N enrichment on biomass outpaces herbivory, leading to species competition and homogenization and, consequently, to lower β-diversity (Borer et al., 2020; Chase, 1999). The grassland at our study site had low productivity owing to limited precipitation and N availability, as well as grasshopper and rodent herbivory. Therefore, our experiment partially coincides with the results of Chalcraft et al. (2008) for above-ground vegetation in low-productivity areas. However, under high N addition, the decreased β-diversity of above-ground vegetation is inconsistent with the above findings. This result underscores the importance of considering the amount of added N in shaping the relationship between N enrichment and the β-diversity and β-deviation of above-ground vegetation. Therefore, long-term, multiple-level N enrichment experiments in grassland ecosystems should be conducted to obtain general patterns of β-diversity under changes in N availability.

We found that species substitution was the main driver of β-diversity changes in both the above-ground vegetation and soil seed bank. Increased β-diversity in the above-ground vegetation and decreased β-diversity in the soil seed bank under low N treatments may be influenced by stochastic processes. For increased β-diversity in the above-ground vegetation under low N addition amounts, one possible reason is historical contingency, by which species colonize different areas of the same habitat in different orders. Subsequently, when nutrient conditions become favourable, priority effects strengthen, generating multiple stable equilibria in different locations, and thus high species substitution and β-diversity (Chase, 2003, 2010; Ejrnaes et al., 2006). Seed dispersal limitation often causes β-diversity to decrease between nearby communities and increase between distant communities (Chase, 2010; Loreau, 2000; Mouquet & Loreau, 2003). In our study, sampling plots were located relatively close to each other. Therefore, stochastic seed dispersal may lead to decreasing β-diversity of the soil seed bank (Forbes & Chase, 2002; Mouquet & Loreau, 2002, 2003).

The β-diversity of the above-ground vegetation and soil seed bank declined under high N treatments because of a homogenized environment and directional environmental filtering. We found that environmental heterogeneity decreased with increasing N addition, which promotes the chance of colonization of the same species or species with similar characteristics (Xu et al., 2017). Therefore, species composition became more similar and β-diversity consequently decreased. High N addition amounts also increased the soil inorganic N concentration and decreased soil pH. For the above-ground vegetation, a tall and nitrophilous grass, *L. chinensis* was dominant, but some forbs that were intolerant to high soil inorganic N concentration or low soil pH were excluded under the high N treatments (Van Den Berg et al., 2005). Therefore, the reduction in forbs may decrease the chance of species substitution among different plots under high N addition, which leads to a decrease in above-ground vegetation β-diversity. Increased soil inorganic N may stimulate seed germination, or increase deterioration by fungi, both of which would reduce the species richness and abundance of the soil seed bank, leading to decreased species substitution or abundance difference (Ochoa-Hueso & Manrique, 2010; Zhang et al., 2019). Moreover, directional environmental filtering may also retain species with long seed longevity (Pakeman et al., 2012), or retain the seeds of some species that rapidly switch to dormancy under stressful conditions such as soil acidification (Davis et al., 2005; Erenler et al., 2010). These deterministic processes can lead to soil seed bank homogenization, which reduces its β-diversity (Royo & Ristau, 2013). Overall, the deterministic processes can be considered to be the main driving force regulating changes in β-diversity under high N treatments.

The differential responses of the β-diversity of above-ground vegetation and the soil seed bank to N enrichment revealed a decoupling between above-ground vegetation and the soil seed bank. Greater environmental heterogeneity under low N addition possibly caused differences in seedling survival and increased species substitution via different environmental filters or by changing competitive relationships between plant species. However, species substitution in the soil seed bank is insensitive to increased environmental heterogeneity, and seeds do not experience interspecific competition as growing plants do. Therefore, seeds in the soil can survive and germinate, but some plants may fail to establish, resulting in the decoupling of the β-diversity of above-ground vegetation and the soil seed bank under low N addition. Under high N addition, the response of the β-diversity of above-ground vegetation and the soil seed bank to N enrichment was similar, but the species composition became decreasingly similar between the above-ground vegetation and soil seed bank when N addition increased. This may be due to the fact that some species such as the dominant grass *L. chinensis* and *A. cristatum* tend to have a high degree of clonal expansion under increased soil inorganic N (Prati & Schmid, 2000), or that N-induced soil acidification inhibits the seed germination of other species such as *C. aristatum* and *C. glaucum* (Davis et al., 2005). We acknowledge that the decoupling of the above-ground vegetation and soil seed bank may also occur in undisturbed natural ecosystems. However, human disturbances of natural ecosystems may exacerbate the decoupled linkages between above- and below-ground processes. Nonetheless, our study provides empirical evidence that two closely linked life-history stages in plant communities may exhibit different patterns and underlying mechanisms in response to anthropogenic environmental change (Figure 5), such as N enrichment, which had not previously been explored.

By exploring the β-diversity of above-ground vegetation and the soil seed bank in response to N enrichment in temperate grassland, we found that differences in community assembly mechanisms led to different response patterns in the β-diversity of above-ground vegetation and soil seed bank under N enrichment. These results also provide important information for the adoption of management strategies that can effectively conserve the biodiversity and integrity of degraded ecosystems in the face of increasing N deposition. Priority should be given to protecting the diversity of above-ground vegetation. This helps to maintain the plant community stability and soil seed bank diversity. Improving soil conditions may be a more important strategy than adding seeds for restoring grasslands degraded by N enrichment. Our findings also shed new light on the different responses of above-ground vegetation and the soil seed bank to environmental changes, suggesting that above- and below-ground processes should be considered simultaneously to predict changes in grassland ecosystem multifunctionality and to adopt effective adaptive management strategies under global change and human disturbance.

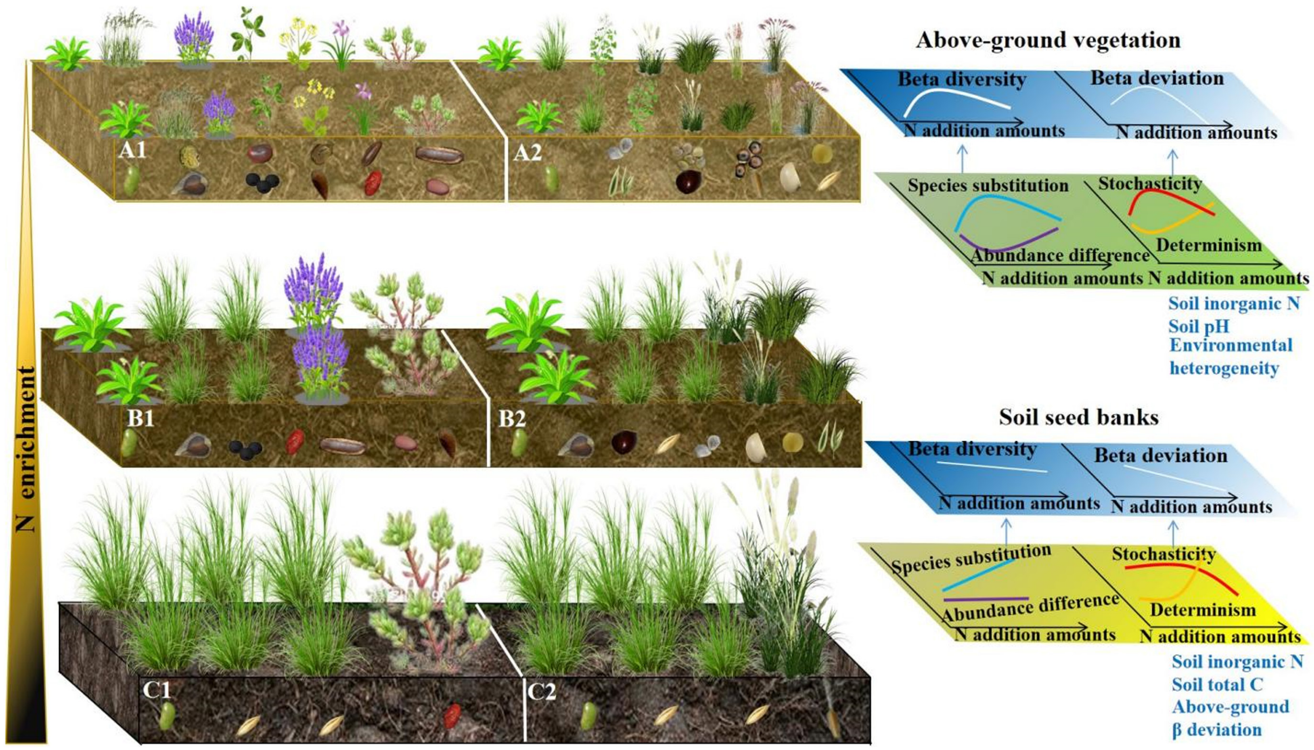

FIGURE 5 A sketched diagram summarizing the key findings of our study. It illustrates the differences in the relative importance of the explanatory variables (soil physical and chemical properties, environmental heterogeneity and β-deviation of the above-ground vegetation and soil seed bank), species substitution (represented by the blue lines), abundance difference (represented by the purple lines), deterministic processes (represented by the orange lines) and stochastic processes (represented by the red lines) in regulating β-diversity and β-deviation of the above-ground vegetation and soil seed bank under increasing N amounts. Increased N addition amounts changed patterns of community assembly of the above-ground vegetation and the soil seed bank (left panel). The changes in β-diversity and β-deviation with increasing N addition amount are also shown in relation to the main drivers and processes (right panel).

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AUTHOR CONTRIBUTIONS

Ming Zhao and Xingguo Han planned and designed the study. Ming Zhao, Junjie Yang and Yunhai Zhang performed the experiments, conducted field and lab work and managed the research site. Ming Zhao and Hongxiang Zhang performed the modelling and analysed the output data. Ming Zhao, Hongxiang Zhang and Xingguo Han wrote the first draft of the manuscript, and Ming Zhao, Michel Loreau, Hongxiang Zhang, Heyong Liu, Yong Jiang, Raúl Ochoa-Hueso and Xingguo Han contributed substantially to the revisions.

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CONFLICT OF INTEREST STATEMENT

The authors have no competing interests.

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DATA AVAILABILITY STATEMENT

The data and codes underlying the study are available at the Dryad repository (https://doi.org/10.5061/dryad.m0cfxpp8z).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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