


Quantifying the indirect effects of nitrogen deposition on grassland litter chemical traits

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Abstract Litter chemical traits are one of the dominant controls on litter decomposition. Increasing atmospheric nitrogen (N) deposition is expected to alter litter chemical traits at the community level in both direct (altering intraspecific chemistry) and indirect ways (changing species abundance and composition). Compared to intraspecific changes, the role of changes in species composition in driving the responses of litter chemical traits to N enrichment has been seldom quantitatively addressed. We quantified

the relative contribution of intraspecific changes versus changes in community composition on litter traits and how this would be influenced by the magnitude of N deposition by taking advantage of a long-term field N addition experiment in a semi-arid grassland with a wide range of N addition rates. Nitrogen deposition altered plant species abundance by facilitating the dominance of one species with a nutrient acquisitive strategy, producing higher quality litter and being more responsive to N addition at the intraspecific level. Overall, changes in species composition, intraspecific changes and their interaction all

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led to higher litter quality (higher N and lower lignin, cellulose and hemicellulose concentrations) under N deposition treatments. The relative contribution of species composition on the responses of litter chemical traits to N deposition also increased with N addition rate, ranging from 5 to 40% for litter N, and from 2 to ~ 30% for the three structural carbon components. Our results demonstrate the positive impacts of increasing N deposition on litter quality through changing intraspecific C and N chemistry and species turnover, which has potential consequences for litter decomposition and nutrient cycling in ecosystems. Further, we highlight the important contribution of shifts in species abundance to the plant-mediated biogeochemical responses to N deposition.

Keywords Nitrogen deposition · Litter chemistry · Species turnover · Intraspecific variation · Lignin · Litter decomposition · Community composition · Nutrient cycling · Semi-arid grassland

Introduction

Atmospheric N deposition induced from the development of industry and population expansion has multiple effects on ecosystem dynamics, including plant species and functional composition, plant physiology, organic matter turnover and soil biogeochemical cycles (Knorr et al. 2005; Clark and Tilman 2008). Increasing attention has been paid to the changes of functional traits of plant litters in response to N enrichment because of their critical impacts on litter decomposition processes and feedback on plant composition (e.g., Lü et al. 2012). The impacts of N deposition on litter decomposition are both direct (influence of soil N availability on the decomposition process) and indirect (changes in litter quality via change in plant composition and plastic changes) (Aerts et al. 2003; Berg and Matzner 1997). Generally, given the close relationship between litter quality and soil organic matter dynamics (Hobbie 2015), N deposition is thought to accelerate soil organic matter decay in ecosystems dominated by high quality litter but favor soil organic matter buildup in ecosystems dominated by low quality litter (Waldrop et al. 2004; Bardgett 2017).

Plant species composition has been shown to be highly sensitive to N enrichment in diverse herbaceous communities (Bobbink 1991; Clark and Tilman 2008). For example, in the present context of semi-arid grasslands of northern China, the grass *Leymus chinensis* typically increases its dominant position and other species lose their predominance in communities with increased N addition (Bai et al. 2010; Zhang et al. 2014a, b). The ‘Mass Ratio Hypothesis’ predicts that the impacts of N enrichment on the functional traits at the community level would be highly dependent on the species which benefit most from N-enriched conditions (Grime 1998; Garnier et al. 2004). Given that the litter quality generally varies greatly among different species (Hättenschwiler and Jørgensen 2010), even in a single ecosystem (Freschet et al. 2012), the change in plant community composition due to N deposition can lead to substantial changes of litter traits at the community level (Cornelissen et al. 2007). However, the quantitative relationship between the magnitude of changes in N deposition, changes in community composition and community litter quality has never been empirically quantified, which may retard our ability to predict the changes of biogeochemical cycles under global change scenarios.

Litter quality, in the context of litter decomposition, is generally best defined by its chemical traits (Melillo et al. 1982) and is sensitive to N enrichment (Zhang et al. 2016). Lignin, cellulose and hemicellulose are the most abundant carbon components in plant litters that feed the soil carbon cycle (Fioretto et al. 2005). Lignin has been repeatedly shown to be a central driver of litter decomposition rate for most types of litter (Melillo et al. 1982; Freschet et al. 2012), and thus its degradation is an important step in terrestrial carbon cycling (Fioretto et al. 2005). Water and acid hydrolyzable polysaccharides, such as cellulose and hemicellulose, are also important natural polymers that retard litter decomposition (Cornwell et al. 2008), although less critically than lignin (Sjöberg et al. 2004). Regarding nutrient concentrations, litter N appears as the predominant driver of litter decomposition rates (Hobbie 2000; Cornwell et al. 2008). As a consequence, the lignin:N ratio is often found to be the single best predictor of litter decomposition (Melillo et al. 1982). While other litter traits, such as tannins, phosphorus, calcium, manganese, or the quality of leachates, have also been shown to affect

decomposition, their influence appear more context dependent and remain to be tested more comprehensively across biomes (e.g., Fanin et al. 2011; Hobbie 2015).

Litter quality is highly sensitive to global change factors, and particularly to N deposition (Henry et al. 2005). Higher N deposition drives for instance decreased lignin and lignin:N ratios at the species level, which causes litters to decompose more quickly throughout the decomposition process (Hobbie 2000). It also leads to increased litter N which tends to accelerate the initial decomposition rate while slowing down the later decomposition phases (Berg et al. 2010; Berg 2014). Such plastic changes of litter chemical traits following N deposition would influence C and nutrient cycling in terrestrial ecosystems.

Community mean litter quality, as quantified by community-weighted means of litter traits, is the principal factor controlling litter decomposition and nutrient release (Aerts et al. 2003; Garnier et al. 2004). The community-weighted means of functional traits is influenced by both species turnover and intraspecific trait variation, and their relative influence has been shown to vary across spatial scales (Siefert et al. 2015). While there is growing evidence of the importance of intraspecific trait variation in driving the responses of community mean trait values to environmental variation across moderate gradients of environmental changes (Kichenin et al. 2013), species turnover is likely to predominate in case of more drastic changes (Albert et al. 2011; Siefert et al. 2015). In the context of N deposition, previous work has indeed shown that the changes of species dominance and community composition are highly dependent on the total amount of deposited N (Duprè et al. 2010), suggesting that the effect of species turnover on the changes of community litter quality would increase with N addition rates as compared to that of intraspecific variation. In a similar way, the hierarchical-response framework (HRF) predicts that, within any N deposition treatment, ecological change under chronic resource alterations would temporally begin with individual physiological changes, followed by species reordering in communities and then species loss and immigration (Smith et al. 2009).

To more clearly identify the impacts of atmospheric N deposition on litter chemical traits in terrestrial ecosystems, we carried out a long-term N addition experiment that started in 2008 in a semi-arid

grassland in northern China, with six N addition rates of 0, 2, 5, 10, 20, and 50 g m⁻² year⁻¹. We examined litter N, lignin, cellulose, and hemicellulose concentrations at both species- and community-level in the semi-arid grassland. Considering the positive effect of N addition on litter quality in terrestrial ecosystems (Hobbie 2000; Henry et al. 2005) and that increased N availability generally facilitates the growth of species and individuals with higher litter quality, we hypothesized that: (1) species turnover and intraspecific trait variation effects should reinforce each other as higher N availability generally leads to higher plant N content, lower concentrations of structural C components, and thus higher litter quality on the one hand and facilitates species with a N acquisitive strategy on the other hand; and (2) the relative impact of plant turnover versus intraspecific trait variation on changes in total ecosystem litter quality inputs would shift from low N deposition to high N deposition treatments, with species turnover being higher in high N deposition treatments.

Materials and methods

Study site

This study was carried out in a semi-arid grassland, fenced since 1999 to exclude large animals, in Inner Mongolia Autonomous Region of China (43°13'N, 116°14'E). Mean annual temperature is 0.9 °C, and mean annual precipitation is 355 mm with approximately 70% falling from May to August. Most of plants in the semi-arid grassland are grasses, and a few others are forbs and sedges. The dominant species of this area are two grasses, *L. chinensis* and *Stipa grandis*. The soil is classified as Calcic-Orthic Aridisol by the US soil classification system. This site had not received fertilizer before our experiment. The natural atmosphere N deposition is about 1–2 g N m⁻² year⁻¹ in this region for the 1990s and 2000s (Jia et al. 2014), and the primary productivity of this grassland has been shown to be N-limited (Bai et al. 2010).

Experimental design

This N deposition experiment began in September 2008, with nine rates of N addition (0, 1, 2, 3, 5, 10, 15, 20, and 50 g N m⁻² year⁻¹). All treatments were

randomized to plots within each of ten replicate blocks, which were separated by 2 m walkways. The size of each plot was 8 m × 8 m. All plots were separated by 1 m walkways in each of the replicate blocks. Purified NH₄NO₃ (> 99%) addition occurred two times each year and started on 1 November 2008 and continued on the first day of June and November for each year. Fertilizer was mixed with purified water, and then was sprinkled evenly to each plot to simulate wet N deposition. It was estimated that less than 1 mm of water was added to each N addition plot in the experiments. In November, fertilizer was mixed with the fine sand, and then spread evenly by hand to each plot to simulate dry N deposition. The sand was sieved to 1 mm size, dipped in hydrochloric acid, washed by purified water, and then heated in an oven at 120 °C for 24 h. In this study, we randomly selected six replicate plots from a selection of six N addition treatments (0, 2, 5, 10, 20, and 50 g N m⁻² year⁻¹; two N additions year⁻¹) amounting to a total of 36 plots.

Sampling and chemical analysis

Plant aboveground biomass was collected in mid-August 2015 by clipping all plants above the soil surface using a 1 m × 1 m quadrat, which was randomly placed in each plot at least 50 cm inside each plot to avoid edge effects. All living aboveground plants were sorted to species, oven-dried at 65 °C for 48 h, and then weighed.

Based on the community composition data measured in August 2015 during the community biomass sampling, we sampled the standing litter (senesced in 2015) for all the species in each plot in October 2015, when all the plants were senesced. Standing litters were sampled above the soil surface with scissors. The plants were considered ready to abscise, when they were completely dry and yellow with no signs of deterioration (Wright and Westoby 2003). After sampling, the senesced shoots were taken to the laboratory, oven dried for 48 h at 65 °C, then cut into pieces, and finally ground with a ball mill (Retsch MM 400, Retsch GmbH and Co KG, Haan, Germany) to be used in subsequent litter chemical analyses. We choose seven dominant species, including *L. chinensis* (L.c.), *S. grandis* (S.g.), *Achnatherum sibiricum* (A.s.), *Agropyron cristatum* (A.c.), *Carex korshinskii* (C.k.), *Cleistogenes squarrosa* (C.s.) and *Koeleria cristata*

(K.c.). Together, those species contributed > 98% of the total biomass averaged across the whole gradient of N addition treatments.

Total N concentration of plant samples was measured colorimetrically by the Kjeldahl acid-digestion method with an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden). For lignin measurement, 50 mg subsamples were extracted with phenol: acetic acid: water (1.1:1.0:0.9), separated from confounding low molecular weight phenolics with 72% dilute H₂SO₄, digested in concentrated H₂SO₄, and then estimated as the proportion of acid-unhydrolyzable residue remaining from the initial sample (Booker et al. 1996). Cellulose and hemicellulose were determined as glucose equivalents with a two-step hydrolysis procedure. The first hydrolysis step was treated with concentrated H₂SO₄, and the second hydrolysis step was performed with 72% dilute H₂SO₄ at 575 °C to release the glucose monomers (Taylor 1995).

Calculation and statistical analysis

We estimated the observed values (O) of average concentrations of N, lignin, cellulose, and hemicellulose at the community level, within each of the 36 plots, by calculating the average shoot chemical concentrations of all seven of the most abundant species weighted by their total aboveground biomass. The observed values of the litter chemical characteristics were calculated with the equation:

$$O = \sum_{i=1}^n LoiRoi, \quad (1)$$

where *Loi* is the litter chemical characteristics of species *i* in each plot, and *Roi* is the relative contribution of species *i* to total aboveground biomass in each plot.

In order to quantify the relative contribution of community composition changes in driving the alteration of community litter quality, we also calculated the expected value (E) for each of the five characteristics of litter quality (N, lignin, cellulose, hemicellulose, and lignin:N) at the community level. The calculation of the expected value was based on the assumptions that (1) N addition did not alter the community composition though it increased community aboveground biomass, that is, the relative

contribution of each species to the community biomass in the N addition treatments was consistent with that in the control treatment; and that (2) N addition changed each of those five litter quality characteristics (based on the observed litter chemistry of each species). The expected values (E) of N, lignin, cellulose, hemicellulose and lignin:N ratio were calculated with the equation:

$$E = \sum_{i=1}^n LiRi, \quad (2)$$

where Li is the mean value of litter chemical characteristics of species i in each plot, and Ri is the mean value of the relative contribution of species i to total aboveground biomass across all six plots of the control treatment. Thus, we got one E value for each litter chemical characters in each plot.

The relative contribution (RC) of changes in community composition to the total changes of community litter chemical characteristics in response to N addition was calculated with the equation:

$$RC = \frac{ON - E}{ON - OC}, \quad (3)$$

where ON is the observed value in each N-enriched community and OC is the observed value in the control community.

The equality of error variance was verified for all data using Levene's test, and normality was tested for all data using Kolmogorov–Smirnov test. Data for N addition rates were \log_{10} transformed to meet the assumption of normality and homogeneity for plant chemistry analysis. A principal component analysis (PCA) was performed to detect the primary axes of variation among different species with N deposition, using litter chemistry of N, lignin, cellulose, hemicellulose and lignin:N. Two-way analysis of variances (ANOVAs) were used to analyze the effects of species identity, N addition rate, and their possible interactions on aboveground biomass and relative biomass of each species, the concentration of N, lignin, cellulose, and hemicellulose, lignin:N ratio, and PCA axis 1 scores for all species. One-way ANOVA was used to examine the impacts of N addition rate on aboveground biomass and relative biomass of each species. Tukey's multiple comparison post hoc test was employed to detect significant differences among treatment means. One-way analysis of covariance (ANCOVA) was used to distinguish the two slopes

between the expected and observed values of litter N, lignin, cellulose, hemicellulose and lignin:N ratio at the community level. All statistical analyses were performed with SPSS version 17.0 (SPSS, Chicago, IL, USA).

Results

Differences in species litter quality

Considering all litter traits together, the variation in litter quality across species and treatment was relatively well summarized by the first axis of the PCA (67.1% of the total variation in litter chemical traits; Fig. 1), which depicted an overall change from litter with low N and high structural C components on the left hand side, to litter with high N and low structural C components on the right hand side. Large differences were found among species regarding PCA axis 1 scores (Fig. 1; $P < 0.001$).

Responses of community composition to N addition

Nitrogen addition substantially altered community composition with significant impacts on biomass and relative biomass of individuals species, and those impacts were dependent upon species identity ($P < 0.001$; Fig. 2a, b). Across all treatments, N

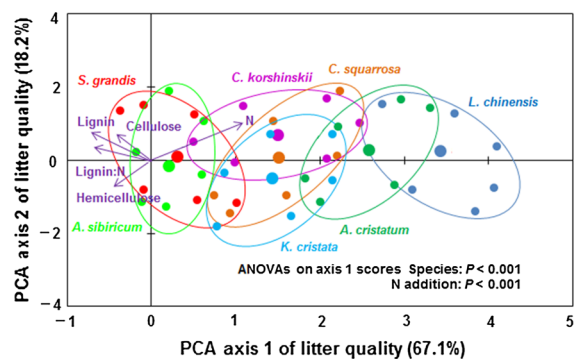


Fig. 1 Principal component analysis (PCA) of five litter chemical properties representative of litter quality across the seven most abundant species and six N addition rates. Each species is displayed by a different color and ellipse under different rate of N addition rates (0, 2, 5, 10, 20, and 50 g N m⁻² year⁻¹, small dots), together with their centroid (large dot). Vectors represent litter chemistry variables

addition increased the aboveground biomass of *L. chinensis* (Fig. 2a), the species displaying the highest litter quality (Fig. 1). This increase was most pronounced for the 20 and 50 g N m⁻² year⁻¹ addition rates with up to 77% increase (Fig. 2a). The biomass of *S. grandis* and *A. cristatum* was slightly increased by N addition at rates of 5 and 10 g N m⁻² year⁻¹, but strongly decreased at rates of 20 and 50 g N m⁻² year⁻¹ (Fig. 2a). The biomass of *A. sibiricum* was slightly increased at N addition rates of 2, 5, and 10 g N m⁻² year⁻¹, but slightly decreased at high rates of N addition (20 and 50 g N m⁻² year⁻¹; Fig. 2a). Among the seven species, *S. grandis* and *A. sibiricum* are the two species that produce litter with the lowest quality, whereas *A. cristatum* produces litter of relatively high quality (Fig. 1). Nitrogen addition had no impact on the aboveground biomass of *C. korshinskii*, *C. squarrosa*, and *K. cristata* (Fig. 2a), three species with average litter quality (Fig. 1).

As a result of these changes, N deposition rates of 2, 5 and 10 g N m⁻² year⁻¹ induced only minor changes in the relative biomass of species within the community, with only *L. chinensis* taking a slightly higher importance (Fig. 2b). In contrast, at N deposition rates of 20 and 50 g N m⁻² year⁻¹, *L. chinensis* became highly dominant relative to all other species, representing up to > 70% of the biomass of the community (Fig. 2b). While the relative biomass of all other species tended to decrease at these high N deposition rates, those of *S. grandis*, *A. sibiricum* and *A. cristatum* showed the strongest decrease (Fig. 2b).

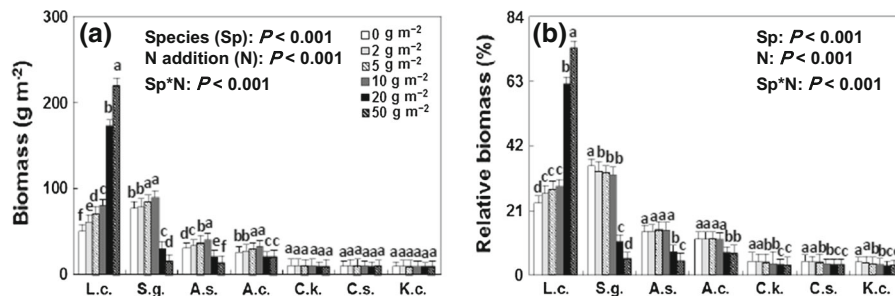


Fig. 2 Effects of nitrogen addition (0, 2, 5, 10, 20, and 50 g N m⁻² year⁻¹) on aboveground biomass and relative aboveground biomass of the seven most abundant species. Data are shown as means ± 1 SE. Different letters indicate

Responses of litter chemistry at the species level

Litter N, lignin, cellulose and hemicellulose concentrations, and the lignin:N ratio varied greatly among the seven species ($P < 0.01$; Fig. 3a–e). Averaged across all N addition treatments, litter N concentrations ranged from 0.77% in *A. cristatum* to 1.26% in *L. chinensis* (Fig. 3a). Lignin concentration was highest in *S. grandis* and lowest in *A. cristatum* (Fig. 3b). Litter N concentration generally increased with increasing N addition rates ($P < 0.01$; Fig. 3a), whereas litter lignin, cellulose, hemicellulose, and lignin:N ratio decreased ($P < 0.001$; Fig. 3b–e). Interactions were detected between species and N addition rate for all litter traits examined here (all $P < 0.001$; Fig. 3), indicating that litter traits of some species were more influenced by N deposition than these of other species. Considering the treatment with the highest addition rate, *L. chinensis* was the species that showed the strongest proportional change in most litter traits, including N (+ 45%), lignin (– 14%) and hemicellulose (– 11%) concentrations, and the lignin:N ratio (– 43%).

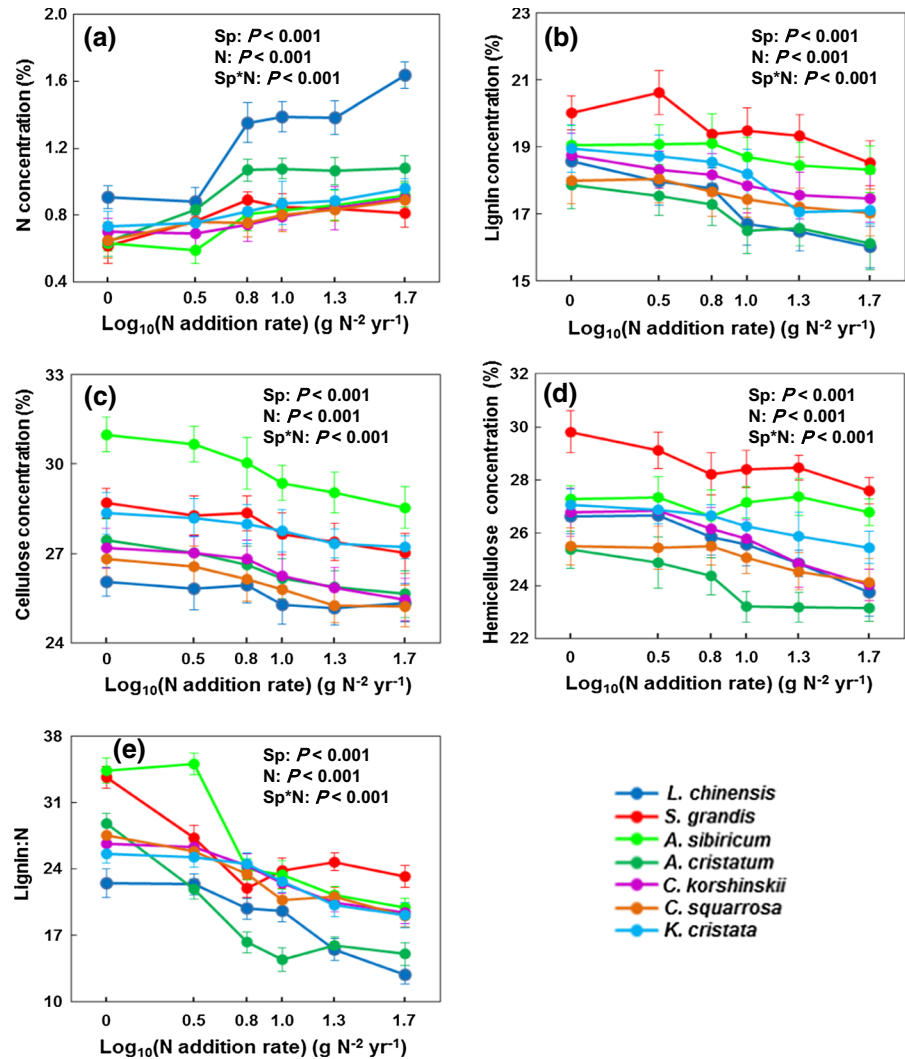
Consistent with results from traits taken separately, N addition and species identity both explained a significant part of the variation in overall litter quality, as represented by the PCA axis 1 ($P < 0.001$ in both cases, Fig. 1).

Responses of litter chemistry at the community level

Nitrogen addition rates had a positive influence on both the expected and observed community litter N concentrations (both $P < 0.001$; Fig. 4a) and a

significant differences ($P < 0.05$) among treatments for each species. *L.c.* *Leymus chinensis*, *S.g.* *Stipa grandis*, *A.s.* *Achnatherum sibiricum*, *A.c.* *Agropyron cristatum*, *C.k.* *Carex korshinskii*, *C.s.* *Cleistogenes squarrosa*, *K.c.* *Koeleria cristata*

Fig. 3 Effects of nitrogen addition (0, 2, 5, 10, 20, and 50 g N m⁻² year⁻¹) on litter N, lignin, cellulose, hemicellulose, and lignin:N ratio of the seven most abundant species. Data are shown as means \pm 1 SE



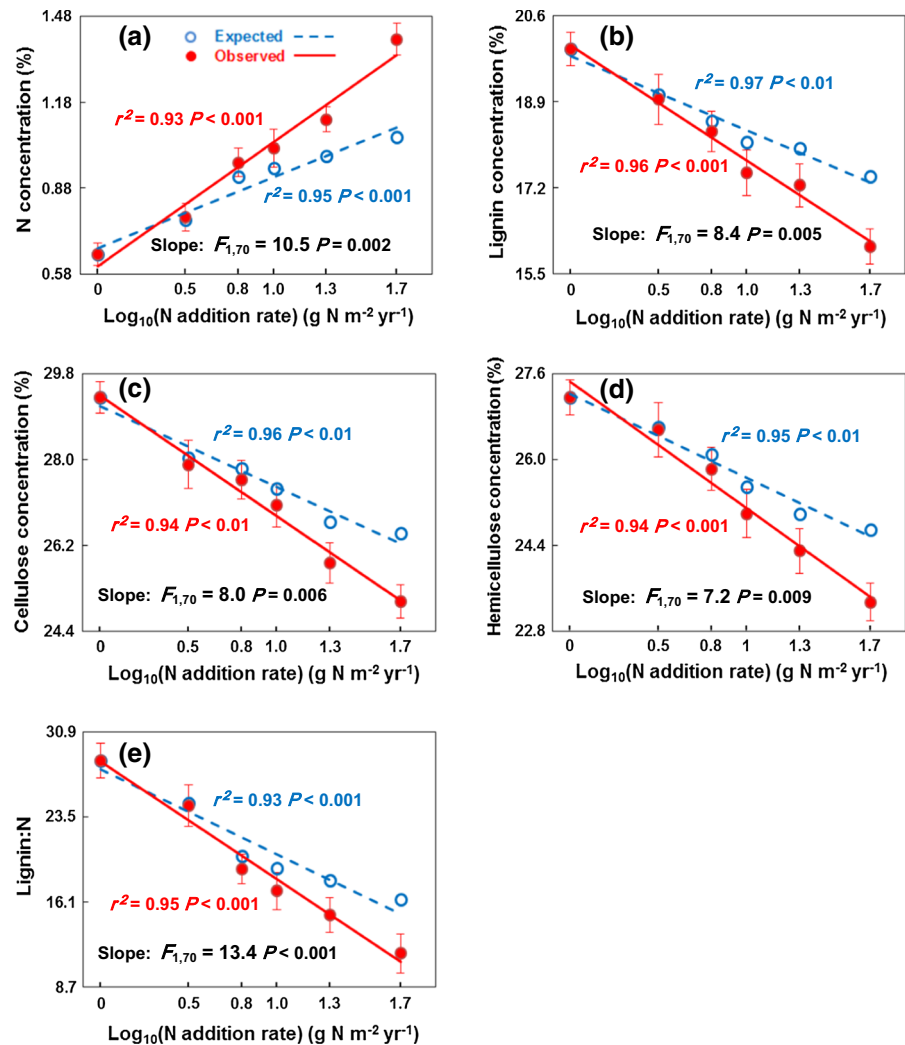
negative effect on the expected and observed values of community litter C related chemistry, including lignin, cellulose, hemicellulose, and lignin:N ratio (all $P < 0.001$; Fig. 4b–e). The observed changes of community litter N and structural C chemistry were more sensitive to the increases of N addition rates than that of the expected values, as indicated by the significant differences between slopes of observed and expected regression lines (Fig. 4a–e). The relative contribution of changes in species abundance to the changes in all aspects of community litter C and N chemistry increased with increasing N addition rates (Fig. 5a–e), but was always lower than the contribution of intraspecific changes. Across all the N addition

treatments, change in species abundance contributed to 5–40% for litter N and 2–30% for litter C chemistry.

Discussion

Nitrogen addition could change aboveground biomass and relative biomass of individual species, and consequently alter community composition in grassland ecosystems (Clark and Tilman 2008). The different responses of individual species to N deposition in term of biomass may have been the result of their specific strategy of nutrient use, which can change competitive interactions and dominance patterns, and consequently alter community composition

Fig. 4 Effects of nitrogen addition on the expected (only accounting for intraspecific trait changes) and observed (accounting for both intraspecific and interspecific driven changes) values of litter N, lignin, cellulose, hemicellulose, and lignin:N ratio at the community level. Data are shown as means \pm 1 SE

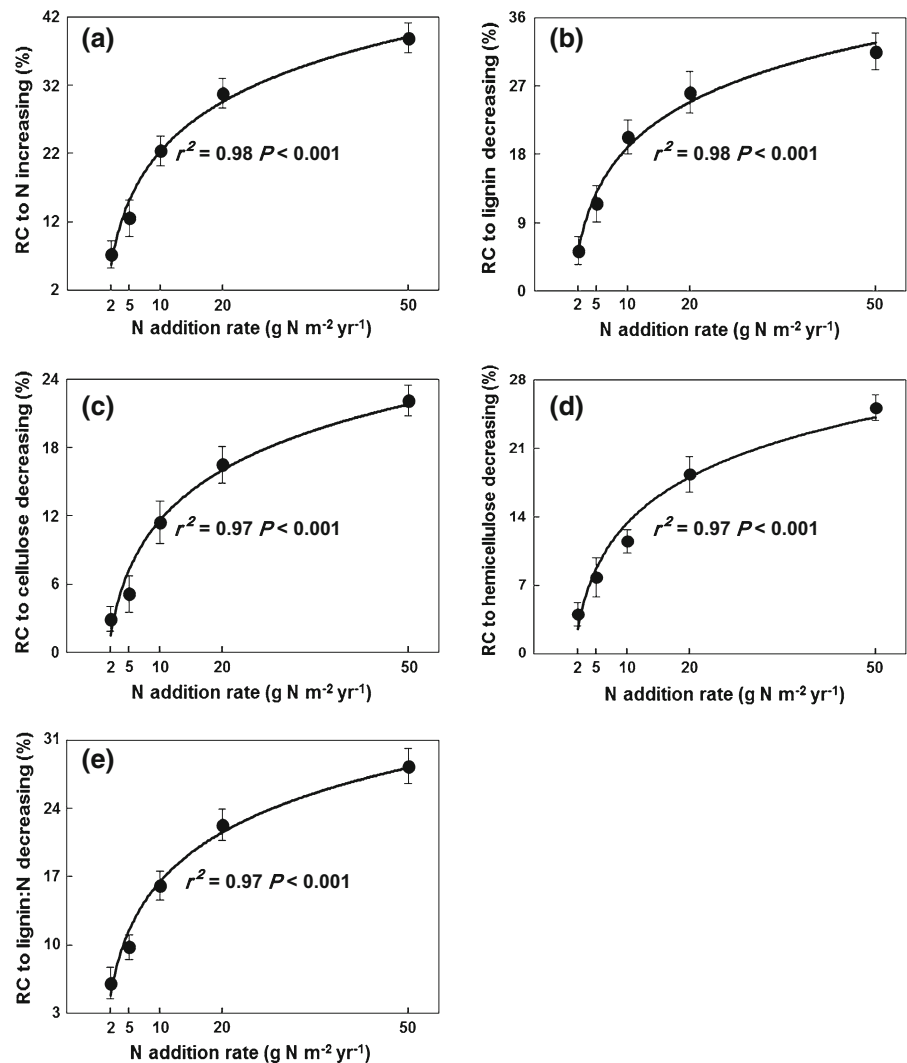


in terrestrial ecosystem (Yang et al. 2011). In our semi-arid grassland, high rates of N addition lead to the dominance of *L. chinensis*, the species with highest litter quality and generally decreased the abundance of other species, particularly these producing lower litter quality. Previous study showed that *L. chinensis* usually sustains high levels of foliar N to improve water use efficiency (Chen et al. 2005). In conditions of increased N deposition and therefore soil N availability (Zhang et al. 2014a, b), this likely contributes to *L. chinensis* dominance pattern in this semi-arid grassland, where water availability strongly limits primary productivity (Li et al. 2011). In contrast, *S. grandis*, another dominant species in this ecosystem that shows strong decline in abundance at moderate to high rates of N deposition, can achieve

high water use efficiency at low levels of foliar N (Chen et al. 2005) and may benefit less from improved soil N availability.

While our results showed significant interspecific variation of litter quality across the seven most abundant species, this variation was rather moderate and generally lower than observed within many ecosystems with more diverse sets of species (Hobbie 2000). Such moderate variation in litter quality across species, potentially due to the low diversity of plant functional types present in our grass-dominated ecosystem, implies that species turnover could play here a less important role in driving the responses of community-level litter quality to N deposition than in more diverse communities (e.g., Hobbie 2000).

Fig. 5 Relative contribution (RC) of changes in community composition in the overall community-level changes in litter N, lignin, cellulose, hemicellulose, and lignin:N ratio under N addition treatments (0, 2, 5, 10, 20, and 50 g N m⁻² year⁻¹). Data are shown as means \pm 1 SE



In parallel, the increasing rate of N addition substantially and consistently increased litter N and decreased lignin, cellulose, hemicellulose, and lignin:N ratio for all seven species in this semi-arid grassland, suggesting that intraspecific changes in litter traits may play a critical role in overall change in community litter quality. Previous studies also found that N enrichment had positive effects on litter N and negative effects on lignin and lignin:N, consequently with positive impacts on litter decomposability (Henry et al. 2005; Zhang et al. 2016). Nitrogen addition would enhance litter N concentration not only through increasing plant N uptake and accumulation (Chapin 1980) but also through decreasing N retranslocation from senescing organs (Kobe et al. 2005; Vergutz et al.

2012). It has also been demonstrated that N addition can sometimes decrease lignin, cellulose and hemicellulose concentrations in green or senesced plants, particularly for those with lower initial structural C concentration (Cotrufo and Ineson 1996), although the global trend shows a general increase in these chemical compounds (Liu et al. 2016). Nitrogen addition may promote plant photosynthesis through consuming more C components, with a trades-off in the metabolic pathways for the production of photosynthetic versus defense compounds (Jones and Hartley 1999). Plant chemical and structural compounds, such as lignin, could resist herbivory damage through reducing palatability and digestibility (De Long et al. 2016). Thus, the lower concentrations of C chemicals

under N enrichment would decrease plant chemical defense with consequences for soil nutrient dynamics in ecosystems.

Consistent with our first hypothesis, species turnover and intraspecific variation clearly reinforced each other across the N addition gradient. Indeed, most importantly, N addition facilitates the growth of *L. chinensis*, which is both the species producing the highest litter quality and that appears to increase most strongly its N concentration with increasing N deposition. Schuster et al. (2016) also demonstrated that N addition could decrease plant community C:N ratio in a restored tallgrass prairie, due to shifts in plant community composition. This and our results together suggest that the plant-mediated biogeochemical processes would be more sensitive to N deposition than that previously expected from a species-level perspective only (e.g., Knorr et al. 2005; Liu et al. 2016). Nonetheless, considering the large range of N deposition rates examined in this study (0–50 g N m⁻² year⁻¹) and the limited projected N deposition rates in the semi-arid grassland over the coming decades (Dentener et al. 2006), the indirect way via changes in species composition may be relatively less important than that of the direct way to alter community litter quality under realistic scenarios of N enrichment.

The relative contribution of species turnover to the changes of community litter quality increased with increasing N addition, which is consistent with our second hypothesis. Specifically, species turnover contributed to increases in litter N from 5 to 40%, and decreased lignin, cellulose and hemicellulose from 2 to 30% along N addition gradient, consistent with the global pattern of changes in intraspecific versus interspecific contribution with increasing environmental change described by Siefert et al. (2015). Our results further suggest that the dynamics of litter quality variation across a N addition gradient would parallel those predicted for the temporal dynamics of ecosystem changes in response to chronic resource alterations as predicted by the hierarchical-response framework (Smith et al. 2009). The hierarchical-response framework predicts that the temporal responses of ecosystem to chronic changes of resource availability begins with plant physiological changes followed by community structure change, which is what we observe with increasing magnitude of N addition rates across the gradient. In the treatments with lower N addition rates, we observed intraspecific

physiological changes related to the first step of the hierarchical-response framework, whereas for treatments with higher N addition rates, 7 years appear enough to achieve the second step of the framework: species reordering. But the time-frame examined here seems not enough to attain the third step of the hierarchical-response framework where species immigration would play a role. The increasingly strong importance of species turnover in ecosystem-level properties with the magnitude of the change observed here (or potentially with time after the change) emphasizes the critical importance of considering plant trait syndromes rather than individual traits when linking the responses of plant communities to their effects on ecosystem properties (Albert et al. 2011; Kichenin et al. 2013). Indeed, species turnover implies changes in multiple community-average trait values that can potentially drive greater changes in ecosystem properties than does intraspecific variation (e.g., Cornelissen et al. 2007). Mixing effects, caused by differences in the initial litter chemistry, play an important role in driving litter decomposition in natural ecosystems (Hättenschwiler and Vitousek 2000; Hector et al. 2000). Nitrogen addition may affect litter mixture effects through changing community composition, and consequently influence litter decomposition.

The substantial changes of community litter quality reported here across the N addition rate gradient has potentially great ecological implications for litter decomposition and ecosystem C and N cycling (Cornelissen et al. 2007; Hobbie 2015). Nitrogen deposition could alter litter decomposition directly via changing environmental conditions and indirectly via altering intraspecific litter quality (Berg and Matzner 1997). Our results extend this framework by showing the positive effects of community composition resulting from N deposition on plant litter quality at the community level. Current knowledge of the effect of litter quality on decay dynamics indicate that higher N availability for decomposers during the decomposition process would stimulate litter decomposition at initial stages but may retard it at later stages owing to an inhibition of lignin degradation (Berg and Matzner 1997; Berg 2014). Consistent with this, a global meta-analysis suggested that soil N enrichment would generally stimulate litter decomposition for litters of low lignin concentrations while inhibiting decomposition of high lignin litters (Knorr et al. 2005). The

range of shoot lignin concentrations observed in our grasslands, irrespective of species and treatment, corresponds to the intermediate litter quality class defined by Knorr et al. (2005) and suggest generally little effect of N deposition on litter decomposition rate. Nonetheless, this, in combination with the increase in litter quality due to both species turnover and intraspecific responses to N deposition, may theoretically lead to slightly less negative to slightly positive direct effect of N deposition on decomposability. Overall, our expectations of higher decomposition rate through both direct and indirect effects of N deposition would have implications for ecosystem C sequestration as substrates with higher quality could facilitate soil microbial enzyme activities and lead to soil C losses (Waldrop et al. 2004). It should be noted that such effects would likely occur in the O horizon following our current understanding of soil organic matter dynamics (Conant et al. 2011).

Conclusion

Our results demonstrate that long-term N deposition would increase standing litter quality directly through influencing litter C and N chemistry at the species level and indirectly through changing plant community composition, which has many potential consequences for litter decomposition and soil organic matter content. The contribution of changes in species abundance to the enhancement of community litter quality was positively correlated with the rates of N deposition, and amounted up to 40% of the observed change. Furthermore, plant litter quality was more sensitive to N enrichment when the changes of community composition were considered, owing to an asymmetric impacts of N enrichments on different plant species. While the role of plastic changes of litter chemical composition in response to N addition in driving ecosystem C cycling is relatively well known (Liu et al. 2016), our results shed new light on the dual role of species turnover, both directly and in interaction with intraspecific responses, in mediating the ecosystem biogeochemical responses to N deposition.

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