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Long-term mowing did not alter the impacts of nitrogen deposition on litter quality in a temperate steppe

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ABSTRACT

Litter quality plays an important role in determining litter decomposition and nutrient cycling in terrestrial ecosystems. We investigated the main and interactive effects of nitrogen (N) addition and mowing on litter quality (%N, lignin, cellulose, and hemicellulose concentrations and lignin:N ratio) of four dominant species in a temperate steppe in northern China. In addition to species-specific impacts, both N addition and mowing can alter community composition by changing relative dominance of individual species, with consequences on litter decomposition. Nitrogen addition significantly increased N concentration of shoot litters at species- and community-level, while mowing did not affect N concentration. Nitrogen addition decreased concentrations of lignin, cellulose, hemicellulose, and lignin:N ratio at both species- and community-level. Mowing decreased lignin and cellulose concentrations, increased hemicellulose concentrations, and did not affect lignin:N ratio at species-level. Mowing decreased cellulose concentrations but did not alter lignin and hemicellulose concentrations, and lignin:N ratio at the community level. Furthermore, mowing did not alter the impacts of N addition on litter quality at both organization levels. Our results suggested that both N addition and mowing can influence litter quality by affecting individual species litter quality and community structure, and thus regulate the processes of litter decomposition and nutrient cycling in grassland ecosystems through a plant-mediated pathway. Importantly, our results showed that the role of annual mowing as a widely-used ecosystem management strategy globally appears to be limited in mediating the impacts of N deposition on plant-mediated nutrient cycling.

1. Introduction

Plant litter quality plays an important role in mediating the impacts of plants on nutrient cycling in terrestrial ecosystems (Vitousek, 1982; Hobbie, 1992; Knops et al., 2002). Lignin, cellulose, and hemicellulose are the major structural carbon (C) compounds in plant litters that retard decomposition (Hättenschwiler and Jørgensen, 2010; Henry et al., 2005). The inhibitory effects of cellulose and hemicellulose on litter decomposition mainly occur in the early stage, while lignin degradation often occurs in the late stage. Further, the ratio of initial nitrogen (N) to lignin contents often closely correlates with litter decomposition rates (Melillo et al., 1982). Litter quality—defined by both structural C components and N content—is expected to be sensitive to abiotic global change factors such as N enrichment (Liu et al., 2016; Zhang et al., 2016a,b) and ecosystem management strategies such as haymaking by mowing (Mikola et al., 2009). However, the interactive impacts of those factors on litter quality and its implications for decomposition dynamics remain largely unknown in grassland systems.

Nitrogen deposition has rapidly increased due to intensive anthropogenic activities, such as fossil fuel combustion, fertilizer use, and the cultivation of N-fixing crops (Galloway et al., 1995; Gruber and Galloway, 2008; Vitousek et al., 1997). Liu et al. (2013) found that the average annual N deposition increased by about 8 kg N ha^{-1} between 1980 and 2010 in China. Plant foliar N concentration in natural ecosystems and crop N uptake from long-term

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unfertilized croplands were significantly increased by N deposition (Liu et al., 2013). Changes in the soil environment following N deposition can influence litter chemistry, for example, by decreasing lignin concentration and thus accelerating decomposition, particularly for low-lignin litter (Norby, 1998; Hobbie, 2000). The effects of N deposition on nutrient status (Lü et al., 2012a; Wang et al., 2014) and community composition (Collins et al., 1998; Isbell et al., 2013) have been studied extensively in grassland ecosystems; however, the response of their C chemistry to N deposition is less clear at both species- and community-level. Furthermore, the effects of N deposition on phytochemistry characteristics are expected to be simultaneously modulated by other factors, such as ecosystem management strategy.

Grassland management strategies can also affect nutrient availability through their impacts on nutrient cycling and community structure in semi-arid ecosystems (Han et al., 2014; Niu et al., 2009; Xia et al., 2009). Mowing, a widely-used grassland management strategy for haymaking across northern China and other parts of the world, removes a majority of current year's aboveground biomass from the ecosystem, and thus has significant consequences on ecosystem composition (Collins et al., 1998; Isbell et al., 2013) and nutrient cycling (Giese et al., 2013; Klumpp et al., 2011). One of the dominant pathway through which mowing can alter nutrient cycling is by reducing plant C supply to soil and decreasing plant nutrient availability by the chronic removal of litter and thus nutrients within litter (Turner et al., 1993). However, short-term annual mowing can also increase nutrient concentrations as a result of increasing soil temperature and moisture values (Bardgett et al., 1998; Tix et al., 2006), changing relative allocation of nutrient, or increasing plant nutrient uptake after defoliation (Green and Detling, 2000; Mikola et al., 2009).

In contrast to our understanding of the impacts of mowing on plant nutrient status, whether mowing affects litter chemistry is less well understood at both species and community levels. Positive (Hiernaux and Turner, 1996; Mikola et al., 2009; Han et al., 2014), neutral (Lü et al., 2012b), and negative (Mikola et al., 2009) effects of mowing on plant N concentration has been reported, which were highly dependent on species identity and ecosystem types. Nutrient availability may also regulate ecosystem responses to mowing (Lü et al., 2012b), but it is not clear whether mowing interacts with N deposition to impact litter quality.

Here, we examined the effects of seven years of N deposition and mowing on litter quality, including N, lignin, cellulose, and hemicellulose concentrations of four dominant grasses at species and community levels in a temperate steppe in northern China. These grasslands are generally mown once anually at the end of the growing season for the forage harvest. We hypothesized that: (1) N addition would increase litter N concentration and decrease the concentrations of lignin, cellulose, and hemicellulose in litters and thus increase litter quality; (2) mowing would decrease N concentration but increase concentrations of lignin, cellulose and hemicellulose, and consequently decrease litter quality through the chronic removal of nutrients with plant biomass. Furthermore, given the divergent impacts of long-term N addition and mowing on nutrients in both soil and plants previously observed (Giese et al., 2009; Han et al., 2014), we hypothesized (3) mowing would decrease the effects of N addition on litter quality, and thus have an interactive effect of mowing and N addition.

2. Materials and methods

2.1. Study site

This study was carried out in a temperate steppe that located in Xilin River Basin, Inner Mongolia in northern China, which is dominated by *Leymus chinensis* and *Stipa grandis* (Zhang et al., 2014). Mean annual precipitation in this area is approximately 355 mm, with 80% falling during the growing season from May to September, and mean annual temperature is 0.9 °C. The soil is classified as Calcic-Orthic Aridisol (Zhang et al., 2016a,b). Plant primary productivity in this semi-arid grassland is N-limited (Bai et al., 2010; Zhang et al., 2015).

2.2. Experimental design

In September 2008, 24 plots measuring 8 m × 8 m were established with four treatments (control, N addition, mowing, and both N addition and mowing), and each treatment was replicated six times separated by 1 m buffer. Treatments were randomly assigned to plots within each block. Purified NH_4NO_3 (>99%) was added two times each year for N addition treatment, with a rate of 10 g N m^{-2} yr^{-1}. Each year in June, fertilizer was mixed with purified water, and then was sprinkled evenly to each plot to simulate wet N deposition. In November, fertilizer was mixed with sand (which was sieved to 1 mm size, dipped by hydrochloric acid, washed by purified water, and then heated by an oven at 120 °C for 24 h), and then spread evenly by hand to each plot to simulate dry N deposition. Less than 1 mm of water was added to the N addition plots. The ambient atmosphere N deposition rate is estimated as 1–2 g N m^{-2} yr^{-1} (Jia et al., 2014), and no additional fertilizer was added in this area before our experiment began. Plots were mown with a mower about 10 cm above the soil surface after the growing season each year, then mowing litter was removed to the edge of each plot.

2.3. Sampling and chemical analysis

Plant aboveground biomass was sampled in mid-August 2015 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 green aboveground plants were classified to species, and then weighed after oven-drying at 70 °C for 48 h.

Representative senesced aboveground tissues of the four perennial grasses, *L. chinensis* (L.c.), *S. grandis* (S.g.), *Achnatherum sibiricum* (A.s.) and *Agropyron cristatum* (A.c.) of the current year, were sampled in the plots in October 2015 (seven years after the start of the experimental treatments), when all the grasses were senesced and before the mowing treatment was carried out in that year. We considered the tissues ready to abscise when they were completely dry and yellow without signs of deterioration (Wright and Westoby, 2003). Together, these four species contributed >70% of the total aboveground biomass in this ecosystem.

The senesced shoots were transported to the laboratory, oven dried for 48 h at 70 °C, and then finely ground with a ball mill (Retsch MM 400, Retsch GmbH & Co KG, Haan, Germany). Total N concentration were analyzed by an Alpkem autoanalyzer (Kjektec System 1026 Distilling Unit, Sweden). Lignin was determined directly as the acid-insoluble residue after samples (50 mg) were extracted with phenol: acetic acid: water (1.1:1.0:0.9) and 72% dilute H_2SO_4 to remove confounding low molecular weight phenolics, followed by digestion in concentrated H_2SO_4 (Booker et al., 1996). Cellulose and hemicellulose were measured as glucose equivalents (Taylor, 1995) following a two-stage hydrolysis procedure consisting of a treatment with concentrated H_2SO_4 followed by a second hydrolysis step with 72% dilute H_2SO_4 at elevated temperature to release the glucose monomers.

2.4. Statistical analysis

Levene's test was used to test for the equality of error variance and the Kolmogorov-Smirnov test was used to test data normality. The effects of species identity, N addition, mowing and their