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Research article

Intensity and frequency of nitrogen addition alter soil chemical properties depending on mowing management in a temperate steppe

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ABSTRACT

Anthropogenic nitrogen (N) enrichment can significantly alter soil chemical properties in various ecosystems. Previous manipulative N experiments mainly focused on the intensity of N addition on soil properties by changing N input rates. It remains unclear, however, whether frequency of N addition can affect soil chemical properties. We examined the effects of frequency (2 versus 12 applications yr^{-1}) and rate (ranging from 0 to $50\,g\,N\,m^{-2}\,yr^{-1}$) of N addition on soil chemical properties of pH, base cations, soil pH buffering capacity (pHBC), and soil available micronutrients in a temperate steppe with and without mowing. Mowing significantly increased the effective cation exchange capacity (ECEC), soil exchangeable Ca and Na, available Fe, and soil pHBC when N was applied at low frequency. Low frequency of N addition significantly decreased soil pH and exchangeable Na but increased soil exchangeable Mg without mowing; however, it increased soil exchangeable Na and available Zn with mowing, while available Fe and Mn increased both with and without mowing. Higher rates of N addition ($\geq 20\,g\,N\,m^{-2}\,yr^{-1}$) decreased soil pH, ECEC and exchangeable Ca but increased soil available Fe, Mn and Cu regardless of the mowing treatment and frequency of N addition. Changes in soil organic matter, pHBC and ECEC were the main reasons affecting soil pH across mowing and N application treatments. Our results indicate that frequency of N addition played an essential role in altering soil chemical properties. Simulating N deposition via large and infrequent N additions can underestimate (exchangeable Mg and available Fe and Mn) or overestimate (soil pH and exchangeable Na) changes in soil properties. Our results further suggest that the effects of frequency of N addition on soil chemical attributes in semi-arid grassland ecosystems can be regulated by appropriate mowing management.

1. Introduction

Nitrogen (N) addition is well-documented to have considerable effects on above- and belowground processes of terrestrial ecosystems (Wei et al., 2013). Higher N availability can alleviate plant N limitation and enhance net primary productivity of terrestrial ecosystems (Bai et al., 2010). However, excessive N inputs promote loss of plant diversity via competitive exclusion by nitrophilic species (Simkin et al., 2016). Nitrogen enrichment directly increases soil N availability, but also alters soil microbial activity and composition that can accelerate N cycling rates resulting in soil acidification and changes in the availability of other nutrients (Guo et al., 2010). Interactions between plants and microbes could further cause complex effects on biogeochemical cycling processes and soil chemical properties.

The effect of N addition on ecosystem components depends on both intensity and frequency of N inputs (Zhang et al., 2014a, 2016a). In semi-arid grasslands, field experiments simulating atmospheric N deposition generally apply N once or twice during the growing season (Zhang et al., 2015; Ren et al., 2017). However, atmospheric N deposition occurs in more frequent events of smaller doses of N to ecosystems (Smith et al., 2009). Therefore, assessment of N enrichment effects could be biased when N is added in one or two large doses without considering N frequency impacts on ecosystem properties. For example, an N addition rate of $10\,g\,N\,m^{-2}\,yr^{-1}$ decreased soil pH by 8.9% and 4.4% when N was applied two and twelve times per year, respectively, after 4 years of N addition (Ning et al., 2015). Addition of large doses of N (as NH_4NO_3) at low frequency (two times per year) significantly increased ammonium toxicity to plant species as compared

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to small doses of N at high frequency (twelve times per year) (Zhang et al., 2016b). Plant species richness and colonization of new species were lower when N was added at low frequency than at high frequency (Zhang et al., 2014a, 2016b). Therefore, simulating N deposition via large and infrequent N inputs can cause significant alterations in ecosystem properties that are unrealistic. Given that many studies rely on large and infrequent N doses to simulate atmospheric N deposition, a better understanding of frequency effects of N addition on ecosystem processes is necessary.

Prolonged N addition can substantially influence soil chemical properties, including soil pH, phosphorus (P) sorption-desorption dynamics, base cations, micronutrients and pH buffering capacity (pHBC) (Högberg et al., 2006; Cai et al., 2017; Wang et al., 2017). Base cations are one of the main mechanisms of maintaining a soil pHBC (Bowman et al., 2008) and are essential nutrients for plant growth and metabolism (Lucas et al., 2011). The content of base cations is generally considered as a good indicator of soil fertility (Zhang et al., 2013). Micronutrients deficiency and toxicity are problems constraining ecosystem productivity, threatening food safety and micronutrient malnutrition (Jones et al., 2013). Losses of exchangeable base cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}), induced by N addition, is widely reported in agro-, forest and grassland ecosystems (Högberg et al., 2006; Lu et al., 2014, 2015; Wang et al., 2017). Soil acidification caused by excess N addition can result in complex micronutrient dynamics in soil-plant systems. For instance, high rates of N addition enhanced Fe deficiency, but also caused Mn toxicity in plant species of *Artemisia frigida* (Tian et al., 2016; Cai et al., 2017). Enhanced N inputs to ecosystems decrease soil pHBC as a result of base cation losses and increased solubility of Al^{3+} (Lu et al., 2015). However, these studies have mainly focused on impact of N dose or intensity, while the effect of frequency of N addition remains largely unknown.

Mowing is a common management practice to remove plant biomass, and is generally used by ecologists to mimic grazing in grassland ecosystems (Collins et al., 1998). Mowing can increase plant N concentrations, influence N addition effects on plant C:N:P stoichiometry (Han et al., 2014), and maintain plant species diversity under N addition (Collins et al., 1998). Mowing can increase soil C sequestration by enhancing the production of short-lived lignin-rich roots (Ziter and Macdougall, 2013) or reduce soil C sequestration by reducing photosynthetic C allocation to belowground pools (Wei et al., 2016). Studies on mowing effects in grasslands have mostly focused on plant productivity, diversity and soil C sequestration (Ziter and Macdougall, 2013; Han et al., 2014; Kotas et al., 2017). Grazing decreased soil cation exchange capacity and exchangeable Ca and increased soil pH in a tallgrass prairie (Teague et al., 2011), but related evidence in temperate steppe grasslands is scarce. Furthermore, while there is some evidence that mowing effects on plant biomass allocation, species richness and soil C and N contents depend on N availability (Kitchen et al., 2009; Blüthgen et al., 2012; Socher et al., 2012), it remains unclear how interactions of mowing and N addition (both in intensity and frequency) will alter soil chemical properties such as pH, base cations, micronutrients and pHBC. Determining these interactive effects on soil chemical properties are important to predict long-term sustainability of grasslands.

In a semi-arid steppe of Inner Mongolia of northern China, a long-term field experiment has been established since 2008 to monitor effects of intensity and frequency of N addition as well as mowing on ecosystem functioning (Zhang et al., 2015). Low background N deposition of $0.4\ g\ m^{-2}\ yr^{-1}$ makes this area an ideal place to investigate ecosystem responses to enriched N scenarios as caused by atmospheric deposition and fertilization (Zhu et al., 2015; Zhang et al., 2016a). In this experiment, aboveground processes have been extensively studied in terms of plant productivity, species diversity, ecosystem stability and colonization of new species (Zhang et al., 2014a, 2015; 2016a; b). Belowground N cycling parameters have also been reported, such as ammonia volatilization (Zhang et al., 2014b) and measurement of functional genes involved in N cycling (Ning et al., 2015). However, a mechanistic understanding of above- and belowground biological responses would be underpinned by knowing the changes in soil chemical properties as affected by mowing and N addition applied in various frequencies and intensities. In this case study, we hypothesized that 1) mowing would decrease soil base cations and micronutrient availability as a result of aboveground-biomass removal; 2) low frequency of N addition would result in lower soil pH, base cations and soil pHBC but higher available micronutrients due to input of a large N dose in the short term as compared to high frequency of N addition; 3) likewise, increasing the intensity of N addition would lead to lower soil base cations and pHBC but higher soil available micronutrients in concurrence with soil acidification. Previous studies conducted in grassland ecosystems have mostly neglected the effects of N addition and mowing on soil chemical attributes of pH, base cations, micronutrients and pHBC. Nevertheless, soil chemical changes could have important consequences for plant biomass productivity, species richness and soil C and N contents (Kitchen et al., 2009; Blüthgen et al., 2012; Socher et al., 2012). These changes may largely depend on if N is added with only one or two applications during the growing season or with monthly additions, but to our knowledge this has never been investigated. Therefore, the novelty of this study lies in addressing frequency of N addition and its interactive effects with mowing on soil chemical properties.

2. Materials and methods

2.1. Study site and experimental design

The case study is conducted near the Inner Mongolia Grassland Ecosystem Research Station (IMGERS; 116°14′E, 43°13′N, elevation 1255–1260 m a.s.l.) in a temperate steppe. The site has a semi-arid climate with mean annual precipitation of 351.4 mm and mean annual temperature of 0.9 °C, ranging from −21.4 °C in January to 19.7 °C in July. In this area, the growing season extends from May to August with approximately 72.8% precipitation falling during this time. The soil is described as a chestnut (Chinese classification) or Haplic Calcisols (the FAO Soil Taxonomy classification). The plant species *Leymus chinensis* and *Stipa grandis* account for over 60% of total peak aboveground biomass in the community. Background atmospheric N deposition in this area is less than $1.0\ g\ N\ m^{-2}\ yr^{-1}$ (Zhu et al., 2015).

The experimental design is described in full in Zhang et al. (2017). The experimental plots were fenced in 1999 to exclude large animal grazing. In September 2008, thirty-eight 8 m × 8 m plots were established in each of ten treatment blocks (total of 380 plots) with a 1 m buffer zone between any two adjacent plots arranged in a randomized block design. Nitrogen was applied as NH_4NO_3 in nine rates or intensities (0, 1, 2, 3, 5, 10, 15, 20, and $50\ g\ N\ m^{-2}\ yr^{-1}$, N_0, N_1, N_2, N_3, N_5, N_{10}, N_{15}, N_{20} and N_{50}, respectively) with two frequencies (2 times per year vs. 12 times per year). The high-frequency N addition (12 times per year) started on September 1st, 2008 and N was added on the first day of each month thereafter. The low-frequency N treatment (2 times per year) started on November 1st, 2008 and N was added on the first day of June and November thereafter. In this context, total N loadings between the two N-addition frequencies were equal in August and February. From May to October, N was applied in wet form by mixing with purified water (9.0 L total for all treatments: either 9.0 L once in June or 1.5 L monthly from May to October), and sprayed evenly to each plot to simulate wet N deposition. Annually, less than 1 mm of water was added to each plot. From November to April (winter season), N was applied in dry form by mixing NH_4NO_3 with sand to ensure an even distribution of additional N. In total, 0.5 kg sand, either 0.5 kg once in November or 0.08 kg monthly from November to April mixed with additional N was uniformly spread in each plot by hand. The sand was sieved through a 1 mm screen, dipped in hydrochloric acid, washed in purified water, and oven-dried at 120 °C to constant