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Research Paper

Initial responses of grass litter tissue chemistry and N:P stoichiometry to varied N and P input rates and ratios in Inner Mongolia

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ABSTRACT

Anthropogenic nitrogen (N) and phosphorus (P) inputs can alter the stoichiometry of senesced plant tissues, a key trait controlling nutrient cycling. However, it is unclear how fertilization rate affects plant litter tissue chemistry under varied N:P supply ratios. In a 2-year study, we investigated the effects of N and P supply rates at three N:P input ratios (4:1, 16:1, and 60:1) on the chemical constitution and N:P stoichiometry of the litter of two grasses: *Leymus chinensis* and *Stipa krylovii*. We further evaluated the differential responses of chemical constitution and N:P stoichiometry in leaf and culm litter of *L. chinensis*. Combined N and P fertilization increased soil acidity and plant-available N, but decreased plant-available P, especially when fertilization occurred at N:P ratio = 60:1. Litter N and P concentrations showed positive response to N and P inputs, and N concentration increased with fertilization rate under N:P ratio = 4:1, but P concentration decreased under N:P ratio = 60:1. Furthermore, we found stronger responses of N and P in *L. chinensis* and culms than in *S. krylovii* and leaves. Stoichiometric responses became more positive with increasing N and P fertilization level at each ratio. Nitrogen and P inputs also significantly improved potassium, copper, and sodium concentrations in senesced shoots independent of fertilization rates except for sodium at N:P ratio = 16:1, which had weaker responses in *L. chinensis* and leaves than in *S. krylovii* and culms. The effects of N and P inputs on other elements were primarily influenced by species and organs, but were also idiosyncratically affected by input levels at each ratio. These results indicate that decreasing evenness of N and P inputs may have increasingly severe non-linear impacts on nutrient cycling and that these impacts will be greater in *L. chinensis*-dominated ecosystems compared to those dominated by *S. krylovii*.

1. Introduction

Global nitrogen (N) availability is continually increasing due to anthropogenic N inputs (Galloway et al., 2004). This increase has decreased plant diversity, influenced nutrient cycling, and reduced ecosystem stability (Berg and Matzner, 1997; Peñuelas et al., 2012, 2013; Roth et al., 2013). Concurrently, anthropogenic phosphorus (P) inputs are also increasing due to agricultural P fertilization and growing deposition of P in dust (Okin et al., 2004; Mahowald et al., 2010). However, P is not increasing at the same rate as N in many systems, leading to progressively greater P-limitation. In response to these changes in soil N and P supply, plants often alter the stoichiometry of living tissues (Ågren and Weih, 2012; Yuan and Chen, 2015a) and adopt modified nutrient conservation strategies (See et al., 2015; Yuan and Chen, 2015a). Consequently, stoichiometry of senesced plant tissues (i.e. litter) is also changed by continued anthropogenic increases in N and P supplies (Kobe et al., 2005). Litter stoichiometry strongly influences microbial growth and litter decomposition rates, thereby partially regulating soil nutrient availability and carbon sequestration (Hessen et al., 2004; Mooshammer et al., 2012). Therefore, exploring the effects of varied N and P enrichment rates at different ratios on litter stoichiometry is critical to understanding the influence of anthropogenic activities on nutrient cycling in grassland ecosystems.

Terrestrial ecosystems are commonly limited by either N or P or co-limited by N and P. Many studies have investigated the influence of anthropogenic N and P inputs on leaf litter stoichiometry individually, but simultaneous N and P fertilization is less commonly examined (Yuan and Chen, 2015a). Generally, the concentrations of N ([N]) and P

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([P]) in senesced leaves increase with respective nutrient fertilization (Lü et al., 2013; Mao et al., 2013; Yuan and Chen, 2015a). Similarly, senesced leaves often exhibit increased [N] and [P] in response to combined N and P fertilization (Yuan and Chen, 2015a; Lü et al., 2016), but homeostatic [N] and [P] have also been observed (Mayor et al., 2014; Lü et al., 2016). The general increase of [N] and [P] in response to individual or combined N and P fertilization can promote faster litter decomposition early on by stimulating microbial activity (Berg and Matzner, 1997; Liu et al., 2010). However, the fine-scale responses of litter N:P ratio to simultaneous N and P addition is more complex, since the responses of litter chemistry and decomposition rates vary greatly across organs and species (Lü et al., 2012a). These inconsistent results may arise from differences between species' physiology and demand of nutrients, as well as differences in the relative magnitude and stoichiometry of fertilization amongst experiments (; Lü et al., 2012a; Mayor et al., 2014; Yuan and Chen, 2015a).

The effects of N and P fertilization on [N], [P], and nutrient cycling may be also partially regulated by the response of other elements to fertilization (Kaspari et al., 2008) and may manifest through diverse pathways. For example, invertebrates and other detritivores can be limited by low sodium ([Na]) and calcium ([Ca]) concentrations in litter (Kaspari et al., 2009; Ott et al., 2014), and phosphatase activity at the root surface can also be regulated by available Ca and Mg in soil (Gabbrielli et al., 2010), thus making the response of these elements important to the overall responses of ecosystem function to fertilization. Similarly, manganese (Mn) is essential to lignin biosynthesis in plants and lignin degradation by microbes (Perez and Jeffries, 1992), and available magnesium (Mg) and zinc (Zn) in soil are also important for litter decomposition (Powers and Salute, 2011).

A limited number of studies have considered the concentrations of elements beyond N and P to N and P fertilization. In an early review, Berg and Matzner (1997) suggested that N addition results in a species or elements-specific increased rate of nutrient uptake in plants, leading to increases in other elements with [N]. For example, potassium concentration ([K]) increases with N fertilization, whereas [Ca] decreases in freshly formed litter of *Pinus sylvestris* (Berg and Staaf, 1980). In litter of *Picea abies*, the [Ca] increases, whereas Mn concentration ([Mn]) decreases (Berg and Tamm, 1991a, 1991b). The differences observed in the response of [Ca] to fertilization, as well as the limited number of investigations into the response of other elements beyond N and P, suggest that the response of litter stoichiometry to fertilization is variable between species and that stoichiometric responses are poorly understood.

Some previous studies have explored the effects of N and P fertilization on other mineral elements in green leaves, which can inform our expectations of litter responses to fertilization. Ågren and Weih (2012) found that the green-leaf [Mn] increases in concert with [N] and [P] in response to N and P fertilization to support continued plant growth, but that the concentrations of other elements (e.g. [K] and [Mg]) remained relatively constant under the same conditions. In contrast, only a fixed amount of some other elements is needed by plants, causing their concentrations in green leaves to decrease as biomass increases via a dilution effect. However, excessive N inputs also cause soil acidification, which leaches base cations out of soils and decreases [K], [Ca] and [Mg] in living plants (Bowman et al., 2008; Sardans and Peñuelas, 2015). By contrast, N fertilization is also associated with increased [Mn] due to increased Mn-availability with soil acidification (Tian et al., 2016). The effects of N inputs on soil acidification may also be affected by available P (Mao et al., 2017). Therefore, the responses of mineral elements in plants are likely influenced by different N input rates and the available P. While the stoichiometry of green leaves is often reflected in that of litter (Kobe et al., 2005), indirect effects of fertilization on the availability of other nutrients in the soil can affect resorption in unexpected ways (See et al., 2015). Therefore, elemental responses in green leaves to fertilization are not always indicative of the response of decomposition and associated biogeochemical cycling.

Culms represent 37.5 to 58.2% of above-ground biomass in grasses (Freschet et al., 2010; Lü et al., 2012b). Although culm litter is often more nutrient-poor than leaf litter, and therefore decomposes more slowly (Freschet et al., 2010, 2013), it also plays an important role in nutrient cycling (Freschet et al., 2010; Mao et al., 2013). Thus, the contribution of culm litter to carbon storage and nutrient cycling in ecosystems is potentially substantial and distinct from that of leaf litter (Lü et al., 2012b; Mao et al., 2013; Hobbie, 2015). Despite this, few studies examine the effect of N and P inputs on grass culm stoichiometry. Those that have been done have demonstrated both increases and no response in [N] of culm litter (Lü et al., 2012b; Chen et al., 2015). Thus, compared to leaf litter, the impacts of N and P inputs on stoichiometric traits of culm litter is critically understudied.

We conducted a series of field experiments to investigate the net effects of different N and P addition rates at three different N:P ratios on elemental concentrations and N:P stoichiometry in grass litter, focusing on the responses of under-reported nutrients and plant tissues (Chen et al., 2015; Hobbie, 2015; Yuan and Chen, 2015a). By varying the N:P stoichiometry (supply ratio) and application rate (input level) of fertilizer, we simulated a progressively more N-rich/P-limited world due to differential anthropogenic inputs of N and P. We first measured the influence of three different fertilization rates on soil [N], [P], [K], [Ca], [Mg], [Na], [Mn], iron concentration([Fe]), and copper concentration ([Cu]) in three separate experiments, each using a fertilizer with a different N:P ratio. Then, we evaluated the concentration of each element within the shoot litter of a dominant perennial species (*Leymus chinensis*), and its companion species (*Stipa krylovii*) under the same fertilization treatments in the grasslands of Inner Mongolia. Finally, we evaluated differences in the response of these elemental concentrations between culm and leaf litter of *L. chinensis*. *S. krylovii* was omitted from the organ analysis due to sampling limitations.

2. Materials and methods

2.1. Study area

The study was conducted at the Ecological Station of Inner Mongolia University, located in the center of an area of Mongolian grassland (44°10′00.2″ N 116°28′53.9″ E). The region has a semi-arid continental climate with a mean annual temperature and precipitation of 0.6 °C and 346 mm, respectively, and a plant growing season from May to September (80% of precipitation occurs during this time). The ecosystem is a temperate steppe mainly dominated by *L. chinensis*, a perennial C3 grass. *S. krylovii* is a common companion species to *L. chinesis* and is also a perennial C3 grass. Because these two species have important ecological and economic value, we selected them as our focal species for this study. Notably, *L. chinensis* is a rhizomatous grass, which often have weaker short-term responses to N inputs compared to tussock-forming grasses like *S. krylovii* (Han and Han, 2014). The soil in this area is classified as a Calcium Chestnut soil according to the ISSS Working Group RB (1998). The total C, N, P, K, Ca, Mg, Fe, Mn, Cu, Zn, and Na concentrations in the 0–10 cm soil layer were 27.66, 1.2, 0.35, 2.76, 29.85, 5.46, 14.19, 0.29, 0.011, 0.054, and 0.24 mg g^{-1}, respectively, with an average pH of 8.15. This site was heavily grazed before 2013, at which point it was fenced to exclude grazing by large animals.

2.2. Experimental design

We conducted three concurrent fertilization experiments, each testing the effect of increasing fertilization rates (henceforth "level": "low," "intermediate," and "high") at a different fertilizer N:P input ratio. Specifically, we tested the effect of fertilization level at either N:P = 4:1, N:P = 16:1, and N:P = 60:1 (mass-based), which corresponded to N-limited conditions, approximately balanced N and P supply, and P-limited conditions, respectively (Güsewell, 2004).