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Nutrient resorption helps drive intra-specific coupling of foliar nitrogen and phosphorus under nutrient-enriched conditions

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Abstract

Aims Plant biomass growth, storage, and decomposition connect nitrogen (N) and phosphorus (P) cycles, yet we know relatively little about the dynamics of such coupling under nutrient enriched conditions, and our understanding of the interactive relationships between plant N and P in drylands remains particularly poor.

Methods In a semiarid steppe of northern China, we examined the effects of single and combined N and P additions on soil and plant N and P pools for both mature and senesced leaves in two dominant grasses: *Leymus chinensis* and *Stipa grandis*.

Results Nitrogen additions increased N concentrations in mature and senesced leaves for each plant species, and decreased N and P resorption during leaf

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U.S. Geological Survey, Southwest Biological Science Center, Moab, UT 84532, USA senescence. The effects of N additions on foliar P concentrations were species-specific, while P additions had no effect on any nutrient characteristics examined. Due to treatment effects on N resorption, N and P concentrations were tightly correlated in senesced leaves but not in mature leaves.

Conclusions Taken together, the results suggest plants in this ecosystem are much more responsive to changing N cycles than P cycles and emphasize the significance of nutrient resorption as an important plant control over the stoichiometric coupling of N and P under nutrient enriched conditions.

Keywords Ecological stoichiometry · Nitrogen addition · Nutrient cycling · Nutrient retranslocation · Phosphorus addition · Plant functional traits

Introduction

Given the widespread limitation by nitrogen (N) and phosphorus (P) availability to multiple aspects of terrestrial ecosystem structure and function (Craine et al. 2008; Elser et al. 2007; Harpole et al. 2011), changes in the concentrations and stoichiometric ratios of N and P in plant tissues have significant implications for fundamental ecological processes (Penuelas et al. 2013). For example, primary production (Harpole et al. 2011), herbivory (Tao and Hunter 2012), litter decomposition (Manzoni et al. 2010), soil respiration (Cleveland and Townsend 2006), as well as soil microbial growth and community composition (Frey et al. 2004; Reed et al. 2011), are all sensitive to nutrient availability and change. In addition, N and P concentrations are tightly coupled to one another in several plant metabolic processes (Reich et al. 2009; Rivas-Ubach et al. 2012), and inter-specific N and P concentrations of live foliage are closely correlated at the global-scale (Watanabe et al. 2007; Wright et al. 2004). In contrast, our understanding of intra-specific relationships between N and P are relatively poor, as is our understanding of how plant stoichiometry will change with continued anthropogenic N and P inputs (Galloway et al. 2008; Mahowald et al. 2008).

Globally, atmospheric N deposition is increasing due to intensified anthropogenic activities (Galloway et al. 2008), and higher N inputs and availability generally lead to higher foliar N concentrations. Increased N availability could also result in higher soil P availability and foliar P concentrations via N-induced stimulation of the activities of P-mineralizing enzymes (Marklein and Houlton 2012). Indeed, some data suggest that increased N inputs lead to increased foliar P, but these increases are typically much smaller than associated increases in foliar N (Lü et al. 2013). Anthropogenic changes in P availability are also on the rise in some areas, particularly in arid and semiarid ecosystems receiving inputs of aeolian dust (Okin et al. 2004; Persson et al. 2010), and increased P availability could alter N cycling in multiple ways, including via positive effects on N2 fixation rates (Reed et al. 2007). In the context of anthropogenic changes to nutrient inputs, alterations to plant P concentrations could be decoupled from those of N concentrations via interactive effects of changing nutrient availabilities (Vitousek et al. 2010), differences in fertilization effect size on N versus P, and via plant behaviors such as luxury P consumption (Cordell et al. 2001).

With respect to a plant's nutrient economy, nutrient resorption from senescing leaves can be as important a nutrient source as uptake from soils (Aerts and Chapin 2000; Killingbeck 1996; McGroddy et al. 2004). For example, a recent global meta-analysis by Vergutz et al. (2012) reported that more than 60 % of foliar N and P would be resorbed back into plants during leaf senescence prior to abscission. These pools of resorbed N and P are available for subsequent plant growth and activity, which significantly reduces the N and P the plant requires from the environment. Patterns of plant nutrient resorption can also be related to the type of nutrient limitation predicted for the ecosystems in which the plants are living, with data suggesting that the nature of nutrient resorption (e.g., N:P resorption ratios) can lend insight into the nutrient constraints experienced by the plant (Güsewell 2005; Reed et al. 2012). Given the importance of plant litter chemistry in regulating the growth and activity of plant and soil biota, changes in nutrient resorption and thus litter chemistry and decomposition rates would have substantial impacts on biogeochemical cycles (Cornwell et al. 2008; Kozovits et al. 2007; Parton et al. 2007). Accordingly, understanding how changes to nutrient inputs affect nutrient resorption is important.

Increases in soil N availability often decrease foliar N resorption in diverse ecosystems (Lü and Han 2010; van Heerwaarden et al. 2003b). In contrast, the responses of P resorption to increased N availability are usually species-specific, with positive, negative, and neutral effects reported (Kozovits et al. 2007; Lü et al. 2013; van Heerwaarden et al. 2003b). Even in a study reporting convergent responses of N and P resorption to N additions, the negative effects of N additions on N resorption were much stronger than on P resorption, as indicated by the negative correlation between N:P resorption ratios and N addition rates (Lü et al. 2013). Theoretically, such differences in the magnitude and/or direction of N and P resorption responses to nutrient enrichment would result in a significant decoupling of the nutrient pools returned to soils via plant litter. In turn, this decoupling could have significant feedbacks to fundamental ecosystem processes that are sensitive to such plant traits.

Trait-based studies have substantially improved our mechanistic understanding of the linkages among species, biodiversity, and ecosystem processes (Cornwell et al. 2008; Wright et al. 2004). While inter-specific variation in traits has provided important insights, there is a growing body of evidence suggesting a critical role for intra-specific trait variability in mediating the effects of environmental change on community assembly and ecosystem functioning (Aerts et al. 2012; Bolnick et al. 2011; Violle et al. 2012). Moreover, the intra-specific responses of plant functional traits (e.g., specific leaf area and leaf nitrogen content) to a set of environmental changes could be different from inter-specific responses to the same changes (Kichenin et al. 2013). Nutrient amendment studies offer the opportunity to assess how an individual species and a set of species responds to altered nutrient availability, and thus to explore the way both intra- and inter-specific changes could help regulate terrestrial ecosystem responses to change.

Here, we address important unknowns regarding the flexibility of plant foliar and leaf litter nutrient stoichiometry in the face of increasing environmental nutrient availability. We focus on two dominant grasses in a temperate steppe of northern China to examine interand intra-specific responses of mature leaf N and P concentrations, senesced leaf N and P concentrations, and N and P resorption (as well as the N:P ratios of each) in plots receiving N×P fertilization. The objectives of this study were to examine: (1) how N and/or P additions affect the balance of foliar N and P before and after leaf senescence in this semiarid ecosystem and (2) the role of nutrient responses to nutrient enrichment.

Methods and materials

Study area and experimental setup

The study was conducted near the Inner Mongolia Grassland Ecosystem Research Station (IMGER; 43°38'N, 116°42'E, 1250 m a.s.l.). The ecosystem is a temperate steppe dominated by the perennial rhizomotous grass Leymus chinensis (L.c.) and the perennial bunchgrass Stipa grandis (S.g.). Both species are C_3 grasses and, together, make up between 60 and 75 % of the total aboveground biomass at the site. The site's mean annual precipitation is 345 mm and the mean annual temperature is 1.1 °C, with an average temperature of 19 °C in July and -21.6 °C in January. The soil is a calcic-orthic aridisol in the U.S. soil taxonomy classification system (Lü et al. 2013). Topsoil (0-10 cm depth) has a pH of 7.5, bulk density of 1.3 g cm⁻³ and soil total organic carbon (C), N and P concentrations are 18.9, 1.9 and 0.4 g kg⁻¹, respectively. The grassland has been fenced to exclude grazing by large animals since 1999.

The experiment consists of 24 plots (each 8 m×8 m in size) with four full-factorial fertilization treatments in a randomized block design (n=6 per treatment). Plot treatments are control (plots receiving no added nutrients), N addition, P addition, and N and P added together. Annual nutrient additions began in May 2006: N was added as urea (56 kg N ha⁻¹ year⁻¹) and P was added as KH₂PO₄ (15 kg P ha⁻¹ year⁻¹). The N:P ratios of added nutrients in the treatment with combined N and P addition was 8:1 (molar ratio), which is lower than the commonly observed N:P ratios in plants and soils in this area (He et al. 2008). In order to facilitate distribution and reduce fertilizer loss, all fertilizer was thoroughly mixed with sand and applied to the plot surface in early May of each year. The same amount of sand was added to control plots.

Field sampling and measurements

In July 2009, 3 years after fertilization began, 20 mature shoots for each species of L.c. and S.g. were randomly selected in a subplot $(1.5 \text{ m} \times 1.5 \text{ m})$ of each treatment plot, with selections made away from plot edges. Two fully expanded leaves, usually the third and fourth leaves from the top of each shoot, were marked with thin red thread. One of the two marked leaves from each shoot was sampled in mid-August of the same year, when annual aboveground biomass reaches its peak value in this ecosystem. The remaining leaf from each plant was checked weekly for its senescence from late September to mid-October, and collected only when fully senesced.

For both mature and senesced leaves, the sampled foliage from each plot was pooled by species, providing plot-level samples of L.c and S.g. leaves for mature and senesced pools. All plant samples were oven dried at 70 °C for 48 h, weighed, and ground using a ball mill (Retsch MM 400; Retsch, Haan, Germany). Total foliar N concentrations were determined colorimetrically by the Kjeldahl acid-digestion method with an Alpkem autoanalyzer (Kjektec System 1026 distilling unit, Hillerod, Sweden) after extraction with sulfuric acid. Total foliar P concentrations were measured by persulfate oxidation followed by colorimetric analysis. Both N and P concentrations were expressed on a mass basis.

Samples of topsoil (0-10 cm depth) were collected from each subplot after the sampling of mature leaves in mid-August 2009. From each subplot, three soil cores were collected using a 3 cm diameter soil auger and were mixed into a single composite sample for the plot. All soil samples were sieved through a 2 mm mesh sieve to remove roots and small rocks. Gravimetric water content for soil samples collected in mid-August 2009 was determined by weighing soil samples, drying at 105 °C for 48 h, and then reweighing. To assess extractable inorganic soil N, from each plot a soil sample $(\sim 10 \text{ g})$ was extracted with 50 ml of 2 M KCl and the filtered soil extract was used to determine ammonium and nitrate concentrations with a continuous flow spectrophotometer (FIAstar 5000; Foss Tecator, Denmark). Plant available P in soil was determined by extracting soil samples (~ 2.5 g) with 0.5 M NaHCO₃ (pH=8.5), and the inorganic P concentrations of filtered soil extract were assessed using the molybdenum blue-ascorbic acid method. Total N concentrations in soil samples were analyzed with a PE-2400 CHN analyzer (Perkin-Elmer, Foster City, USA) and total P concentrations were determined by persulfate oxidation followed by colorimetric analysis (Schade et al. 2003).

Calculation and statistical analysis

Nutrient (N or P) resorption efficiency (RE), defined as the proportion of nutrients in mature leaves that was resorbed during senescence, was calculated as:

 $RE = (1-[Nutrient_{senesced}/Nutrient_{mature}]) \times 100\%$

Where Nutrient_{senesced} is the N or P content of the senesced leaves collected in late September/mid-October and Nutrientmature is the N or P content of green leaves collected during maximum productivity in mid-August. We used N and P content instead of concentration to help account for leaf mass loss during senescence (van Heerwaarden et al. 2003a; Vergutz et al. 2012), and N and P contents were assessed for both species from each plot. The nutrient (N or P) content of the sampled 20 leaves was calculated by multiplying total leaf mass and leaf nutrient concentration. Because similar cohorts of leaves were sampled at full maturity and after complete senescence, and because the mass of mature and senesced leaves were considered, we avoided the bias in nutrient resorption calculations that can result from leaf mass loss and area shrinkage during leaf senescence (van Heerwaarden et al. 2003a).

Data were tested for normality using the Kolmogorov-Smirnov test and for equality of error variance using the Levene's test. With block as a random factor, two-way ANOVAs were used to detect the effects of N and P additions on plant available nutrient concentrations and total N and P in soils, soil moisture, nutrient concentrations in both mature and senesced leaves, and nutrient resorption efficiencies for each species. Paired t-tests were used to compare the difference between N resorption efficiency (NRE) and P resorption efficiency (PRE) for each of the same two species in the control treatment. Pearson correlations were used to determine the relationship between N and P concentrations in either mature or senesced leaves and between NRE and PRE within each species. All the analyses were carried out with SPSS 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Soils

The addition of N significantly enhanced plant available soil N concentrations (P<0.001, Fig. 1a). Interestingly, N fertilization also increased plant available soil P concentrations (P=0.017, Fig. 1b). Although we expected to see increased extractable P concentrations in plots receiving P fertilization, P additions had no effect on plant available soil N or P concentrations (Fig. 1). Nitrogen fertilization showed no detectable impacts on soil total P concentrations (F=0.15, P=0.7), while P fertilization significantly enhanced soil total P concentrations (F=0.78, P=0.01).



Fig. 1 Treatment effects on a plant available soil inorganic N concentrations and b plant available soil inorganic P concentrations. Treatments are: control, where plots received no nutrient inputs; N addition (N); P addition (P), and N and P added in combination (NP). Values shown are means (n=6 per treatment) and error bars represent 1SE. ANOVA P values are reported when P<0.05. Letters indicate results of Tukey's HSD post-hoc tests to determine significant difference among treatments. *Bars* that share the same letter are not significantly different (P>0.05)

Mature and senesced leaves

For L.c., N additions significantly enhanced N concentrations in mature and senesced leaves (each P < 0.001, Fig. 2a, c), decreased P concentrations in mature leaves (P < 0.05, Fig. 2b), and did not affect P concentrations in senesced leaves (Fig. 2d). For S.g., N additions increased N concentrations in mature and senesced leaves (each P < 0.001, Fig. 2a, c), did not affect P concentrations in mature leaves (Fig. 2b), and increased P concentrations in senesced leaves (Fig. 2c, d). For both species, P additions had no significant effects on any nutrient characteristics examined in this study (Fig. 2). Nitrogen additions decreased NRE and PRE for both species (marginally significant effect on PRE of L.c.; Fig. 3), whereas P additions had no significant effect on nutrient resorption efficiency (Fig. 3). In the control plots, NRE was higher than PRE for both species (Paired *t*-tests; t=3.05, p<0.05 for L.c.; t=2.99, p<0.05 for S.g.). Generally, there was no interaction between N and P additions on plant nutrient characteristics (Figs. 2 and 3), except on senesced leaf P concentrations in S.g., where senesced leaf P was significantly enhanced by N addition under P enriched conditions but not under P ambient conditions (Fig. 2d).



Fig. 2 Nitrogen and phosphorus concentrations in mature and senesced leaves of *Leymus chinensis* and *Stipa grandis* in response to N and P additions. Data shown are for **a** mature leaf N concentrations, **b** mature leaf P concentrations, **c** senesced leaf N concentrations, and **d** senesced leaf P concentrations. Treatments are: control, where plots received no nutrient inputs; N addition (N); P

addition (P), and N and P added in combination (NP). Values shown are means (n=6 per treatment) and error bars represent 1SE. ANOVA P values are reported when P<0.05. Letters indicate results of Tukey's HSD post-hoc tests to determine significant difference among treatments. *Bars* that share the same letter are not significantly different (P>0.05)



Fig. 3 a Nitrogen resorption efficiency and **b** P resorption efficiency of *Leymus chinensis* and *Stipa grandis* in response to N and P additions. Treatments are: control, where plots received no nutrient inputs; N addition (N); P addition (P), and N and P added in combination (NP). Values shown are means (n=6 per treatment)

Across all treatments, N and P concentrations were not significantly correlated in mature leaves (Fig. 4a, b), whereas N and P concentrations were positively correlated in senesced leaves for both species (Fig. 4c, d). Nitrogen and P concentrations in senesced leaves of L.c. were significantly, positively correlated (P=0.008) with a Pearson coefficient of r=0.526. Senesced leaf N and P concentrations for S.g. were also positively correlated (P=0.009) with a Pearson coefficient of r=0.524. In addition, NRE and PRE were significantly related within the target species (Fig. 5a, b): there was a highly significant relationship between L.c. N and P resorption efficiencies (P<0.001; r=0.677) and between S.g. N and P resorption efficiencies (P=0.008; r=0.528).

Discussion

Previous data show that N enrichment can reduce the coupling of N and P cycles in the soils of grassland ecosystems by increasing soil N but decreasing soil P pools (Zhang et al. 2013). Results from the research described here present a new addition to this work by providing evidence that the coupling between semiarid grassland foliar N and P may be equally fragile with increased nutrient supply. From a physiological perspective, N and P are strongly inter-dependent in plant metabolism and it is well established that inter-specific



and error bars represent 1SE. ANOVA *P* values are reported when P < 0.1. *Letters* indicate results of Tukey's HSD post-hoc tests to determine significant difference among treatments. Bars that share the same letter are not significantly different (P > 0.05)

N and P concentrations of autotrophs are tightly correlated at global-scale (Borer et al. 2013; Wright et al. 2004). However, here we observed intra-specific patterns in N and P cycling that were not coupled in the face of nutrient enrichment for the dominant grasses in the temperate steppe, with N and P concentrations of green foliage responding in opposite ways to increased nutrient supply. This decoupling could negatively affect the plant's capacity to adapt to nutrient enrichment, which would have a number of cascading ecosystem feedbacks (Sistla and Schimel 2012).

The data shown here provide strong evidence for the role of nutrient resorption in mediating the coupling between senesced leaf N and P in response to nutrient enrichment. In mature green leaves of both species, N and P concentrations were seemingly unrelated to one another under nutrient enriched conditions, however, concentrations of N and P in senesced leaves were significantly correlated following the process of nutrient resorption. Indeed, patterns in nutrient resorption efficiency resulted in stoichiometrically connected N and P concentrations in senesced leaves (Fig. 4) and this coupling of senesced leaf N and P, even in the face of nutrient enrichment, has important implications for nutrient cycling in this ecosystem. Interestingly, the coupling in N and P senescence was driven by changes to N resorption, not P resorption, which implies that semiarid grasslands such as these are much more responsive to changes in N cycling relative to P.





Fig. 4 Relationships between N and P concentrations of a *Leymus chinensis* mature leaves; **b** *Stipa grandis* mature leaves; **c** *L. chinensis* senesced leaves; and **d** *S. grandis* senesced leaves. Pearson correlation coefficients for each relationship are shown in

each box. Symbols depict which treatment plot the sample was collected from and treatments are: control (*red circles*), N addition (N: *orange squares*); P addition (P: *yellow upside down triangles*), and N and P added in combination (NP: *green triangles*)

These patterns of nutrient resorption also suggest that the fertilization-induced decoupling of N and P concentrations observed in mature leaves would have a less marked effect on soil processes, such as decomposition rates. In particular, for both focal grasses, N resorption varied with fertilization in ways that resulted in senesced leaf N and P concentrations that were much more tightly correlated than for green foliage. Thus, the results suggest that nutrient resorption during leaf senescence plays an important role in driving the coupling of N and P in litter prior to its abscission, with NRE playing the dominant role in this system. While P additions did not affect nutrient resorption of either species, N additions decreased both N and P resorption in the two species (marginally significant effects of N additions on PRE in L.c.). Similarly, in a recent study we found convergent responses of N and P resorption to increasing N availability across an N addition gradient (Lü et al. 2013). Resorption of less N and P during leaf senescence under N enriched conditions resulted in higher N and P concentrations in senesced leaves and thus in plant litter, and this could directly affect decomposition rates (but see Aerts et al. 2012), as well as a variety of other aspects of biogeochemical cycling. Moreover, N



Fig. 5 Relationships between N resorption efficiency and P resorption efficiency in **a** *Leymus chinensis* and **b** *Stipa grandis*. 1:1 lines are also shown. Symbols depict which treatment plot the

and P resorption efficiencies in both species were positively correlated across all the treatments in this study, indicating a stoichiometric control of nutrient resorption during leaf senescence.

Understanding how increased nutrient deposition affects the coupling of biogeochemical cycles is not merely interesting from a theoretical perspective, but decoupled foliar N and P cycles following nutrient enrichment could directly affect plant growth and survival. A recent metaanalysis supported the idea that foliar P helps determine the relationship between foliar N and aspects of photosynthesis [e.g., V_{cmax}; (Walker et al. 2014)], and the nature of this interactive control may vary depending on ecosystem nutrient status. The decoupling of foliar N and P observed in our study suggests that predicting the response of photosynthesis and plant growth in a nutrient enriched world will be more complex than previously thought. Additionally, grasses are the main food source for many herbivores in ecosystems such as the semiarid steppe described here. Given that the proportions of nutrients required by herbivores are generally stricter than those of autotrophs (Persson et al. 2010), differential changes to N and P concentrations in plant tissues would also have consequences for the nutrition and activities of organisms at other trophic levels, including grazers and insects (Tao and Hunter 2012).

But why do N additions affect foliar N more strongly than P additions affect foliar P? One reason could have to do with the amount of P added. Many other studies use much higher rates of P fertilization (e.g., 50 or 100 kg P



sample was collected from and treatments are: control (*red circles*), N addition (N: *orange squares*); P addition (P: *yellow upside down triangles*), and N and P added in combination (NP: *green triangles*)

 ha^{-1} year⁻¹), however, here we selected lower rate of P addition that more realistically simulates the increased P inputs expected at our site. While we observed no positive response of added P on soil available P concentrations, N additions (56 kg N ha^{-1} year⁻¹) significantly affected not only soil and foliar N, but also soil available and foliar P. Whether or not this increase in the Nfertilized plots P was from a stimulation of phosphatase activity (e.g., Marklein and Houlton 2012), a lack of P supply in our P fertilization plots seems unable to explain the lack of increased soil available and foliar P. Further, we observed an increase in total soil P pools with P fertilization (P=0.01; 0.34 vs. 0.39 g P kg⁻¹ for control and + P plots, respectively). It is possible for soils to strongly sorb anions, such as PO_4^{3-} , but the soils at this site are sand-rich aridisols and would not be expected to have high P sorption capacity. Another potential reason for the lack of P response is that plants in this semiarid ecosystem may simply be less sensitive to P enrichment relative to N enrichment (i.e., strongly N limited). In part, the magnitude of the response, or in this case the lack of response, may be related to how limited the ecosystem is to that particular nutrient. The fact that NRE was higher than PRE for both species examined suggests that plants in this ecosystem may be limited by N availability. Results from a nearby grassland with a similar plant community showed extensive N limitation to net primary production (Bai et al. 2010), supporting the idea of dominant N control. Taken with data from other fertilization studies (e.g., Ostertag 2010), the data presented here suggest that the relative effects of N and/or P fertilization depend upon a site's extant nutrient and nutrient limitation characteristics.

It has been reported that increased N availability would favor the activities of P mineralization enzymes and enhance P availability for plants (Marklein and Houlton 2012), and thus it is notable that the increased soil P availability observed with N additions did not lead to enhanced foliar P concentrations. One explanation could have to do with the difference between factors affecting foliar chemistry and affecting growth. If P was not limiting to primary production, increased soil P should not result in increased plant growth. In contrast, in a scenario where N additions resulted in increased growth (i.e., if N limits primary production) and also increased P availability, P that is taken up by plants would be diluted by the stimulation of growth, potentially leading to unchanged or even reduced foliar P concentrations. This would be particularly true for plants in the temperate steppe, where primary productivity is strongly limited by N (Bai et al. 2010). Unfortunately, our analysis of aboveground biomass only chemically explored the leaves, and not stems, thus we could not assess total aboveground pools of N and P, and such an assessment warrants future study.

In conclusion, our results emphasize the stronger role of N fertilization, relative to P fertilization, in controlling plant nutrition and the quality of decomposable substrate in this semiarid temperate steppe ecosystem. The decoupling of foliar N and P at the intra-specific level for both grasses under nutrient enriched conditions would have large implications for plant metabolism and the activities of organisms at multiple trophic levels. However, plant resorption patterns strengthen the coupling of leaf litter N and P during senescence. Given the divergent responses of N and P coupling in mature versus senesced leaves in response to nutrient enrichment, the plant-mediated effects of nutrient enrichment on the activities of organisms that utilize live versus senesced leaves would vary. Accordingly, variation in the N and P coupling of mature and senesced leaves could potentially lead to stoichiometric mismatches between above- and below-ground food webs, with significant implications for ecosystem functioning.

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References

- Aerts R, Chapin FS (2000) The mineral nutrition of wild plants revisited: a re-evaluation of processes and patterns. Adv Ecol Res 30:1–67
- Aerts R, van Bodegom PM, Cornelissen JHC (2012) Litter stoichiometric traits of plant species of high-latitude ecosystems show high responsiveness to global change without causing strong variation in litter decomposition. New Phytol 196: 181–188
- Bai YF, Wu JG, Clark CM, Naeem S, Pan QM, Huang JH, Zhang LX, Han XG (2010) Tradeoffs and thresholds in the effects of nitrogen addition on biodiversity and ecosystem functioning: evidence from inner Mongolia Grasslands. Glob Chang Biol 16:358–372
- Bolnick DI, Amarasekare P, Araujo MS, Bürger R, Levine J, Novak M, Rudolf VHW, Schreiber SJ, Urban MC, Vasseur DA (2011) Why intraspecific trait variation matters in community ecology. Trends Ecol Evol 26:183–192
- Borer ET, Bracken MES, Seabloom EW, Smith JE, Cebrian J, Cleland EE, Elser JJ, Fagan WF, Gruner DS, Harpole WS, Hillebrand H, Kerkhoff AJ, Ngai JT (2013) Global biogeography of autotroph chemistry: is insolation a driving force? Oikos 122:1121–1130
- Cleveland CC, Townsend AR (2006) Nutrient additions to a tropical rain forest drive substantial soil carbon dioxide losses to the atmosphere. Proc Natl Acad Sci U S A 103:10316– 10321
- Cordell S, Goldstein G, Meinzer FC, Vitousek PM (2001) Regulation of leaf life-span and nutrient-use efficiency of Metrosideros polymorpha trees at two extremes of a long chronosequence in Hawaii. Oecologia 127:198–206
- Cornwell WK, Cornelissen JHC, Amatangelo K et al (2008) Plant species traits are the predominant control on litter decomposition rates within biomes worldwide. Ecol Lett 11:1065– 1071
- Craine JM, Morrow C, Stock WD (2008) Nutrient concentration ratios and co-limitation in South African grasslands. New Phytol 179:829–836
- Elser JJ, Bracken MES, Cleland EE, Gruner DS, Harpole WS, Hillebrand H, Ngai JT, Seabloom EW, Shurin JB, Smith JE (2007) Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol Lett 10:1135–1142
- Frey SD, Knorr M, Parrent JL, Simpson RT (2004) Chronic nitrogen enrichment affects the structure and function of the

soil microbial community in temperate hardwood and pine forests. For Ecol Manag 196:159–171

- Galloway JN et al (2008) Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science 320: 889–892
- Güsewell S (2005) Nutrient resorption of wetland graminoids is related to the type of nutrient limitation. Funct Ecol 19:344– 354
- Harpole WS et al (2011) Nutrient co-limitation of primary producer communities. Ecol Lett 14:852–862
- He JS, Wang L, Flynn DF, Wang X, Ma W, Fang J (2008) Leaf nitrogen:phosphorus stoichiometry across Chinese grassland biomes. Oecologia 155:301–310
- Kichenin E, Wardle DA, Peltzer DA, Morse CW, Freschet GT (2013) Contrasting effects of plant inter- and intraspecific variation on community-level trait measures along an environmental gradient. Funct Ecol 27:1254–1261
- Killingbeck KT (1996) Nutrients in senesced leaves: keys to the search for potential resorption and resorption proficiency. Ecology 77:1716–1727
- Kozovits AR et al (2007) Nutrient resorption and patterns of litter production and decomposition in a Neotropical Savanna. Funct Ecol 21:1034–1043
- Lü XT, Han XG (2010) Nutrient resorption responses to water and nitrogen amendment in semi-arid grassland of Inner Mongolia, China. Plant Soil 327:481–491
- Lü XT, Reed S, Yu Q, He NP, Wang ZW, Han XG (2013) Convergent responses of nitrogen and phosphorus resorption to nitrogen inputs in a semiarid grassland. Glob Chang Biol 19:2775–2784
- Mahowald N, Jickells TD, Baker AR et al (2008) Global distribution of atmospheric phosphorus sources, concentrations and deposition rates, and anthropogenic impacts. Glob Biogeochem Cycles 22, GB4026
- Manzoni S, Trofymow JA, Jackson RB, Porporato A (2010) Stoichiometric controls on carbon, nitrogen, and phosphorus dynamics in decomposing litter. Ecol Monogr 80:89–106
- Marklein AR, Houlton BZ (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. New Phytol 193:696–704
- McGroddy ME, Daufresne T, Hedin LO (2004) Scaling of C : N : P stoichiometry in forests worldwide: Implications of terrestrial redfield-type ratios. Ecology 85:2390–2401
- Okin GS, Mahowald N, Chadwick OA, Artaxo P (2004) Impact of desert dust on the biogeochemistry of phosphorus in terrestrial ecosystems. Glob Biogeochem Cycles 18, GB2005
- Ostertag R (2010) Foliar nitrogen and phosphorus accumulation responses after fertilization: an example from nutrient-limited Hawaiian forests. Plant Soil 334:85–98
- Parton W, Silver WL, Burke IC, Grassens L, Harmon ME, Currie WS, King JY, Adair EC, Brandt LA, Hart SC, Fasth B (2007) Global-scale similarities in nitrogen release patterns during long-term decomposition. Science 315:361–364
- Penuelas J, Poulter B, Sardans J et al (2013) Human-induced nitrogen-phosphorus imbalances alter natural and managed ecosystems across the globe. Nat Commun 4:2934
- Persson J, Fink P, Goto A, Hood JM, Jonas J, Kato S (2010) To be or not to be what you eat: regulation of stoichiometric homeostasis among autotrophs and heterotrophs. Oikos 119: 741–751

- Reed SC, Seastedt TR, Mann CM, Suding KN, Townsend AR, Cherwin KL (2007) Phosphorus fertilization stimulates nitrogen fixation and increases inorganic nitrogen concentrations in a restored prairie. Appl Soil Ecol 36:238–242
- Reed SC, Vitousek PM, Cleveland CC (2011) Are patterns in nutrient limitation belowground consistent with those aboveground: results from a 4 million year chronosequence. Biogeochemistry 106:323–336
- Reed SC, Townsend AR, Davidson EA, Cleveland CC (2012) Stoichiometric patterns in foliar nutrient resorption across multiple scales. New Phytol 198:173–180
- Reich PB, Oleksyn J, Wright IJ (2009) Leaf phosphorus influences the photosynthesis-nitrogen relation: a cross-biome analysis of 314 species. Oecologia 160:207–212
- Rivas-Ubach A, Sardans J, Perez-Trujillo M, Estiarte M, Penuelas J (2012) Strong relationship between elemental stoichiometry and metabolome in plants. Proc Natl Acad Sci U S A 109: 4181–4186
- Schade JD, Kyle M, Hobbie SE, Fagan WF, Elser JJ (2003) Stoichiometric tracking of soil nutrients by a desert insect herbivore. Ecol Lett 6:96–101
- Sistla SA, Schimel JP (2012) Stoichiometric flexibility as a regulator of carbon and nutrient cycling in terrestrial ecosystems under change. New Phytol 196:68–78
- Tao LL, Hunter MD (2012) Does anthropogenic nitrogen deposition induce phosphorus limitation in herbivorous insects? Glob Chang Biol 18:1843–1853
- van Heerwaarden LM, Toet S, Aerts R (2003a) Current measures of nutrient resorption efficiency lead to a substantial underestimation of real resorption efficiency: facts and solutions. Oikos 101:664–669
- van Heerwaarden LM, Toet S, Aerts R (2003b) Nitrogen and phosphorus resorption efficiency and proficiency in six subarctic bog species after 4 years of nitrogen fertilization. J Ecol 91:1060–1070
- Vergutz L, Manzoni S, Porporato A, Novais RF, Jackson RB (2012) Global resorption efficiencies and concentrations of carbon and nutrients in leaves of terrestrial plants. Ecol Monogr 82:205–220
- Violle C, Enquist BJ, McGill BJ, Jiang L, Albert CH, Hulshof C, Jung V, Messier J (2012) The return of the variance: intraspecific variability in community ecology. Trends Ecol Evol 27:244–252
- Vitousek PM, Porder S, Houlton BZ, Chadwick OA (2010) Terrestrial phosphorus limitation: mechanisms, implications, and nitrogen-phosphorus interactions. Ecol Appl 20:5–15
- Walker AP, Beckerman AP, Gu L et al (2014) The relationship of leaf photosynthetic traits - V-cmax and J(max) - to leaf nitrogen, leaf phosphorus, and specific leaf area: a metaanalysis and modeling study. Ecol Evol 4:3218–3235
- Watanabe T, Broadley MR, Jansen S et al (2007) Evolutionary control of leaf element composition in plants. New Phytol 174:516–523
- Wright IJ, Reich PB, Westoby M et al (2004) The worldwide leaf economics spectrum. Nature 428:821–827
- Zhang NY, Guo R, Song PA, Guo JX, Gao YZ (2013) Effects of warming and nitrogen deposition on the coupling mechanism between soil nitrogen and phosphorus in Songnen Meadow Steppe, northeastern China. Soil Biol Biochem 65:96–104