Topography Modulates Effects of Nitrogen Deposition on Microbial Resource Limitation in a Nitrogen-Saturated Subtropical Forest

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Abstract

Background

Nitrogen (N) saturation theory proposes that an ecosystem might switch from N limitation to carbon (C), phosphorus (P), or other nutrient limitations if it receives continuous N input. Yet, after N limitation is removed, which nutrient is the most limited and whether topography modulates such change is rarely tested at a microbial level. Here, we conducted a two-year N addition experiment under two different topography positions (i.e. a slope and a valley) in a N-saturated subtropical forest. Soil enzyme activity was measured, and ecoenzymatic stoichiometry indexes were calculated as indicators of microbial resource limitation.

Results

In the valley, two-year N addition changed the activity of all studied enzymes to various degrees. As a result, microbial C limitation was aggravated in the valley, and consequently microbial decomposition of soil labile organic C increased, but microbial P limitation was alleviated due to the stoichiometry balance. On the slope, however, N addition did not significantly change the activity of the studied enzymes, and did not alter the status of microbial resource limitation.

Conclusions

These results indicate that C is a more limited element for microbial growth than P after removing N limitation, but we also highlight that topography can regulate the effect of N deposition on soil microbial resource limitation in subtropical forests. These findings provide useful supplements to the N saturation theory.

Introduction

Over the past few decades, atmospheric nitrogen (N) deposition increased rapidly, and changed the structure and function of terrestrial ecosystems (Galloway et al. 2008). To describe effects of N deposition on ecosystems, a N saturation theory was developed basing on studies from temperate forests (Aber et al. 1989). One view of this theory proposes that continuous N input may reduce system requirements for N, and finally the system may become N-saturated. In this case, carbon (C), phosphorus (P) or water limitation is expected to occur or be aggravated (Aber et al. 1989). This is a useful theory for predicting dynamics of terrestrial ecosystems in response to increased N deposition (Chen et al. 2018d).

This proposition, however, has yet to specify which nutrient would be the most limited after removing N limitation. In plants, this topic has been studied widely. Recent studies tend to suggest that N deposition may switch N limitation to P limitation (Braun et al. 2010; Crowley et al. 2012; Deng et al. 2017; Gress et

al. 2007; Li et al. 2016), although some studies find no effects (Finzi 2009; Weand et al. 2010) or other limitations, such as calcium (Ca) limitation (McNulty et al. 1996). In microbes, however, the discussion is relatively few, and the situation might differ from of plants. For microbes, C is often a more limited element compared to N, P or other nutrients (Soong et al. 2020). Thus, when N limitation is removed after continuous N input, C may be the most limited factor for microbial growth, rather than P or other nutrients. However, this expectation is not consistent with many recent studies, which suggest that N addition may aggravate microbial P limitation (Marklein and Houlton 2012). Since microbes are as important as plants in an ecosystem, more studies are undoubtedly needed to address how microbial resource limitation changes after increased N deposition.

The ecoenzymatic stoichiometry method provides a new tool to study such topic. Compared to traditional methods that measure effects of substrate additions on microbial biomass or respiration as indicators of microbial resource limitation (Traoré et al. 2016), ecoenzymatic stoichiometry has a number of advantages. First, it is much faster, because it measures activities of only four enzymes, including β -D-glucosidase (BG), L-leucine aminopeptidase (LAP), β -N-acetylglucosaminidase (NAG), and acid/alkaline phosphatase (AP). Second, using enzymes as proxy indicators of C, N, and P acquisition, it is much easier for us to understand which nutrient is more limited to an ecosystem (Sinsabaugh 1994). However, most previous studies reported effects of N addition on the activity of the single enzyme (Chen et al. 2016; Jian et al. 2016; Marklein and Houlton 2012), very few studies have assessed responses of ecoenzymatic stoichiometry to N additions (Wang et al. 2015). By collecting published data regarding single enzyme activity in response to N addition, a previous meta-analysis reported that nitrogen deposition may aggravate microbial C limitation (Chen et al. 2018d). However, this study has limitations: firstly, the selected published studies rarely reported C, N, and P acquisition enzymes at the same time, which largely limited the data's comparability and the conclusion's generality; secondly, most selected studies were conducted in N-limited systems rather than N-saturated systems, and thus cannot answer the above question regarding changes of resource limitation after a system has been N-saturated. These limitations highlight the importance of more field experiments conducted in N-saturated systems and more experiments measuring enzymes fully for calculating ecoenzymatic stoichiometry.

Topography can modulate many microbial processes, but is often ignored in the previous N-deposition studies. Most N-deposition simulation experiments were conducted in one topography position (mostly in flat ground) or did not distinguish topography positions (Zhang et al. 2021), while few experiments estimated whether and how topography regulates the responses of microbial processes to N deposition (Zhang et al. 2013). In a previous study, divergent responses of soil asymbiotic N_2 fixation to N addition has been found between the valley and slope, implying the important effect of topography on ecosystem processes (Wang et al. 2019). However, whether microbial resource limitation status has similar responses needs further exploration. In general, soil N level will be lower in the upslope (due to erosion) than in the downslope or valley (Weintraub et al. 2015), resulting in a higher sensitivity of microbes to the addition of N in the upslope than in the downslope or valley. Nevertheless, the N leaching will be higher in the upslope than in the downslope or valley (Wang et al. 2019), which in turn weakens the N addition

effects. These contrary mechanisms make the topography effects uncertain. Lacking knowledge of topography effects has become an important limitation for model prediction regarding responses of terrestrial ecosystem processes to atmospheric N deposition.

Therefore, in this study we conducted a two-year N addition experiment in a subtropical karst forest, where soil microbes have been proven to be N-saturated (Chen et al. 2018b), to test how N deposition changes the status of microbial resource limitation in such a N-saturated situation. In order to investigate whether topography regulates effects of N additions on microbial resource limitation status, the N-addition experiment was set up at two topography positions, i.e., a valley and a slope. To our knowledge, this is the first N-deposition simulation experiment site that considers the effects of topography in the subtropical forest. We hypothesized that 1) nitrogen deposition may aggravate microbial C limitation based on the previous studies; and 2) the response of microbial limitation to nitrogen additions may differ between valley and slope.

Materials And Methods

Site description

The studied forest locates in Mulun National Nature Reserve of Huanjiang County, southwest China (24°54′ – 24°07′ N, 107°56′ – 108°00′E; 299–686 m a.s.l) (Wang et al. 2019). This area has a monsoon climate. Mean annual temperature is about 19°C, with the lowest monthly mean in January and the highest in July. The mean annual precipitation is about 1389 mm, with a distinct seasonal pattern: the wet season is from April to October and the dry season is from November to March. The selected forest is about 35 years old after clear-cut, dominated by *Cryptocarya chinensis* (Hance) Hemsl., *Cinnamomum saxatile* H. W. Li, *Koeleruteria minor* Hemsl., *Pittosporum tobira* (Thunb.) Ait., *Bridelia tomentosa* Bl., *Murraya exotica* L. Mant. The soil is leptosols based on the FAO World Reference Base for Soil Resources (IUSS-Working-Group 2006). More detailed information of soil properties can be found in a previous study (Wang et al. 2019) .

Experimental design

The experiment was initiated in April 2016 (Wang et al. 2019). The experiment was conducted in the valley and on the slope, respectively, where the soil properties are different (Table 1): there are higher concentrations of soil organic C, total N, NO_3^- , and Ca and greater NO_3^- leaching rate on the slope than in the valley. In each position, a randomized block design was adopted with three N treatments and three blocks: control (CT, 0 kg N ha⁻¹ yr⁻¹), low-N addition (LN, 50 kg N ha⁻¹ yr⁻¹) and high-N addition (HN, 100 kg N ha⁻¹ yr⁻¹). Thus, there are 18 plots (10 m× 10 m each) in total. Each plot is surrounded by a 10 m wide buffer strip. NH_4NO_3 was weighed and mixed with 10 L of water (equals to 1.2 mm annual precipitation) for each N-treatment plot, and the NH_4NO_3 solution was sprayed every month to the forest floor with a backpack sprayer. The control plots received 10 L of water without fertilizer.

Table 1

The differences in soil properties between slope and valley. BD, SOC, TN, TP, NH_4^+ , NO_3^- , Ca and Mg are bulk density, soil organic carbon, total nitrogen, nitrate, ammonium, calcium and magnesium, respectively. Value is mean (standard error). Different letters denote the significant difference between treatments.

	Valley	Slope
BD (g cm^{-3})	0.90 (0.04)	0.95 (0.05)
pH	7.19 (0.17)	7.32 (0.54)
SOC (g kg^{-1})	40.46 (3.77) b	52.24 (5.01) a
TN (g kg^{-1})	3.74 (0.35) b	4.72 (0.50) a
TP (g kg^{-1})	2.26 (0.16)	1.91 (0.22)
$\text{NH}_4^+\text{-N}$ (mg kg^{-1})	7.60 (0.41)	5.68 (0.26)
$\text{NO}_3^-\text{-N}$ (mg kg^{-1})	19.91 (3.01) a	13.33 (1.07) b
Ca (g kg^{-1})	7.04 (0.70) b	14.80 (2.1) a
NO_3^- leaching rate ($\text{mg d}^{-1}\text{kg}^{-1}$ dry resin)	3.27 (0.01) b	5.01 (0.01) a

Soil sampling and lab experiments

Soil sampling was conducted twice in May, 2017 and 2018, respectively. In each plot, five soil cores were collected and mixed into a composite sample. Soils were passed through a 2 mm mesh sieve after picking out roots and stones. The sieved soil samples were divided into two portions for further processes. One portion was air-dried at room temperature for analyzing soil properties, including soil organic C (SOC), total N (TN), total P (TP), dissolved organic C (DOC), total dissolved N (TDN), and available P (AVP) (Carter and Gregorich 2006; Lasota and Błońska 2021). Another portion were kept on ice in the field and were stored at 4°C in the laboratory for analyses of soil NH_4^+ and NO_3^- concentrations, microbial biomass C (MBC), N (MBN), and P (MBP), and extracellular enzyme activity (Chen et al. 2018b). Soil NH_4^+ and NO_3^- concentrations were analyzed by an autoanalyzer (FIAstar 5000, FOSS, Sweden). MBC, MBN, and MBP were determined by the chloroform fumigation extraction method (Brookes et al. 1985; Brookes et al. 1982; Vance et al. 1987). The nitrification rate and NO_3^- leaching rate were measured and provided by Liang Zheng in 2018.

Enzyme activity assays were conducted within two weeks after soil sampling. β -D-glucosidase (BG), L-Leucine aminopeptidase (LAP), β -N-acetylglucosaminidase (NAG), and acid phosphatase (AP) were assayed with published microplate protocols. In addition, in order to model decomposition rates, we also measured the activity of ligninolytic enzyme (i.e. polyphenol oxidase [POX]) with a spectrophotometrical method. Detailed assay processes can be found in our previous studies (Chen et al. 2018b; Chen et al. 2017). Enzyme activity was expressed to units per g of soil organic carbon, i.e., $\mu\text{mol g}^{-1} \text{SOC h}^{-1}$.

Ecoenzymatic stoichiometry

Several methods were used to reflect microbial resource limitation status. First, simple ratios of enzyme activities were used. Higher BG/(LAP + NAG) and BG/AP indicate lower N and P limitation, respectively (Waring et al. 2014). Second, vector length and angle of ecoenzymatic stoichiometry was conducted as follows (Moorhead et al. 2013):

$$\text{Vector length (unitless)} = \sqrt{(\ln BG / \ln[NAG + LAP])^2 + (\ln BG / \ln AP)^2} \quad (1)$$

Vector angle (°) = Degrees(ATAN2(($\ln BG / \ln AP$), ($\ln BG / \ln[NAG + LAP]$))) (2) A longer
vector length indicates greater C limitation; vector angles of < 45° and > 45° indicate N and P limitation, respectively (Moorhead et al. 2013).

Third, C:N and C:P of the available resources (DOC/TDN ($R_{C:N}$) and DOC/AVP ($R_{C:P}$), respectively) were compared to the Threshold Elemental Ratios (TER) for C:N and C:P ($TER_{C:N}$ and $TER_{C:P}$, respectively). When $R_{C:N} - TER_{C:N}$ or $R_{C:P} - TER_{C:P}$ is less than zero, soil microbes are not limited by N or P. When the $R_{C:N} - TER_{C:N}$ or $R_{C:P} - TER_{C:P}$ is greater than zero, microbes are N or P limited. In the latter case, higher $R_{C:N} - TER_{C:N}$ or $R_{C:P} - TER_{C:P}$ indicate higher N or P limitation (Sternner and Elser 2002). $TER_{C:N}$ and $TER_{C:P}$ were calculated as follows (Sinsabaugh et al. 2009):

$$TER_{C:N} = (BG / (NAG + LAP)) \times B_{C:N} / n_0 \quad (3)$$

$$TER_{C:P} = (BG / AP) \times B_{C:P} / p_0 \quad (4)$$

where $B_{C:N}$ and $B_{C:P}$ are C:N and C:P ratios of microbial biomass, and n_0 and p_0 are the intercepts calculated from regressions of $\ln(BG)$ vs $\ln(NAG + LAP)$ and $\ln(BG)$ vs $\ln(AP)$, respectively.

We also modeled organic C decomposition (M , % day^{-1}) (Sinsabaugh and Moorhead 1994):

$$M = \left(CUE \times ENZ_{TOT} \right) / (1 + ([NAG + LAP] / BG) + (AP / BG)) \quad (6)$$

where ENZ_{TOT} is the sum of BG, POX, (BG + LAP), and AP normalized to their maximum values. CUE is carbon use efficiency, which was calculated according to previous studies (Chen et al. 2018b; Li et al. 2021; Sinsabaugh et al. 2013).

Data analysis

In this study, soil enzyme activity had no responses to one-year N addition, but had significant changes after two years of N addition. Thus, in the following analyses we only focused on the results after two years of N additions. One-way analysis of variance (ANOVA) with least significant difference (LSD) test was used to test the effects of the two-year N addition on soil properties, enzyme activities and ecoenzymatic stoichiometry indexes. A one-sample t-test was used to test the difference between $R_{C:N} - TER_{C:N}$ (or $R_{C:P} - TER_{C:P}$) and zero, and the difference between vector angle and 45° . All statistical analyses were conducted using SPSS 16.0 statistical software (SPSS Inc., Chicago, IL, USA). All reported significant differences are at $P < 0.05$.

Results

In the control, mean enzyme activities were 1.9 ± 0.1 , 31.7 ± 4 , 0.01 ± 0.01 , 0.6 ± 0.04 , and $1.8 \pm 0.1 \mu\text{mol g}^{-1} \text{SOC h}^{-1}$ for BG, POX, LAP, NAG, and AP, respectively, and there were no significant difference between slope and valley (Fig. 1). On the slope, N addition did not change activities of all studied enzymes (Fig. 1). In the valley, N addition changed activities of all studied enzymes after two years of N treatments (Fig. 1). The BG and NAG activities increased significantly in the high-N treatment compared to the control (Fig. 1a, d), and AP activity increased significantly in both high-N and low-N addition treatments (Fig. 1e), but POX and LAP activities decreased significantly in the high-N treatment (Fig. 1c, b).

As for ecoenzymatic stoichiometry, N addition did not change any indexes of ecoenzymatic stoichiometry on the slope position (Figs. 2 and 3). However, in the valley, N additions significantly increased BG/AP and vector length (Fig. 2a, b), but did not change the BG/(LAP + NAG) in both low and high N treatments compared to the control (Fig. 2c). $R_{C:N} - TER_{C:N}$ was significantly lower than zero in the control, and N addition significantly decreased $R_{C:N} - TER_{C:N}$ in both low-N and high-N treatments (Fig. 2d). Vector angle in the control was significantly greater than 45° , and N addition significantly decreased vector angle in the high-N treatment (not in the low-N treatment). $R_{C:P} - TER_{C:P}$ in the control was significantly greater than zero, and both low-N and high N treatments significantly increased $R_{C:P} - TER_{C:P}$ (Fig. 2e, f). In addition, N addition significantly increased the modeled decomposition rate (M) in both low-N and high-N treatments, and significantly decreased BG/POX in the high-N treatment (Fig. 3a, b).

Two-year N addition also changed soil properties (Fig. 4). In the valley, N addition significantly increased the concentrations of DOC and TDN in the high-N treatment (but not for the low-N treatment), and significantly increased nitrification rate and NO_3^- leaching rate in both low-N and high-N treatments, but did not changed other soil variables, including the concentrations of SOC, TN, TP, AVP, MBC, MBN, MBP, NH_4^+ and NO_3^- . On the slope, N addition significantly increased nitrification rate and NO_3^- leaching rate in both low-N and high-N treatments, but had no significant effects on other studied variables (Fig. 4 and Table S1).

Discussion

Responses of microbial enzyme activity to N additions

The mean enzyme activities measured in controls are similar to those measured in nearby forests (Chen et al. 2018a; Chen et al. 2018b). Nitrogen addition did not change activities of all studied enzymes after one year of N treatments in all topography positions (data not shown), but started to alter soil enzyme activity two years later in the valley, especially in high-N treatments, indicating that the duration and rate of N addition are important factors affecting soil enzyme activity. After two years of N addition, the responses of the individual enzyme activity to N addition are consistent with the patterns reported in the recent meta-analysis studies (Chen et al. 2018c; Jian et al. 2016): in the valley, the BG, NAG, and AP activity increased significantly, but POX and LAP activity decreased significantly with the elevated N addition (Fig. 1). This is interesting, because it may reflect a general change of enzyme activity in response to N addition. If so, the results of ecoenzymatic stoichiometry in this study can represent not only a regional pattern, but also for other regions.

Responses of microbial resource limitation to N additions in the valley

We confirmed that the studied forest is N-saturated, and found that N addition makes the symptom of N-saturation more obvious. There are several lines of evidence to indicate that the studied forest is N-saturated: $R_{C:N} - TER_{C:N}$ was less than zero in controls (in both valley and slope), indicating that the forest is not limited by N (Fig. 2d); by contrast, this forest may be P-limited because vector angle was greater than 45° and the $R_{C:P} - TER_{C:P}$ was greater than zero in controls (Fig. 2e and f). These evidence are also found in the previous studies (Chen et al. 2018b). Since this forest has been N-saturated, it is not surprising that continued N addition make the symptom more obvious, which is evident from that $R_{C:N} - TER_{C:N}$ was significantly lower in N treatments than in the control (Fig. 2d). In addition, increased nitrification rate and NO_3^- leaching rate (Table 1) also support the aggravated N-saturation according to N saturation theory (Aber et al. 1989).

We found that N aggravates microbial C limitation in this forest, which is consistent with the first hypothesis. The evidence is from the increased vector length in N-addition plots compared to the control (Fig. 2a). Similarly, we also found increased vector length in a previous meta-analysis by collecting enzyme activity data from 36 published N-addition studies (Chen et al. 2018d). Aggravated microbial C limitation has been suggested in many previous N-addition experiments (Treseder 2008). Here, we proposed two likely reasons for the aggravated microbial C limitation after N addition in this study. First, it is a result of nutrient balance. To reach a balance of C and N, microbes will increase decomposition of organic matter to get more available C as N increases. This is supported by modeled decomposition rates (M) in this study, which was increased by 43% and 54% in low-N and high-N addition, respectively, compared to the control (Fig. 3a). As a result, increase in soil DOC concentration was also found (Table 1). However, in this study, the increased total decomposition rate is mainly attributed to the increased decomposition of the labile C fraction, rather than the non-labile C fraction. This can be

Loading [MathJax]/jax/output/CommonHTML/jax.js used POX (as a result, the decreased BG/POX, Fig. 3b), since

BG and POX are two different kinds of enzymes to decompose labile C (such as cellulose) and non-labile C fraction (such as lignin), respectively (Chen et al. 2018c). Therefore, the reduced decomposition of non-labile C fraction may be another reason for the aggravated microbial C limitation in this study, because it decreases the sources of available C.

Compared to the aggravated C limitation, however, N addition may alleviate microbial P limitation, because vector angle decreased but $R_{C:P} - TER_{C:P}$ increased in N-treatments relative to controls (Fig. 2). This result rejects our general belief that N addition will lead to P limitation (Marklein and Houlton 2012). We note that increased AP may be a reason for the alleviated P limitation (Fig. 1e). However, this might be a paradox, because increased AP not only reflects the increased organic P decomposition (meaning alleviated P limitation), but also reflects the increased P demand of microbes (meaning aggravated P limitation). Instead, we propose that alleviated P limitation can be explained more directly by the increased BG/AP (Fig. 2b), which indicates that microbes need more C than P. According to Liebig's law of the minimum, the single scarcest resource in relation to plant demands is the most limiting; this means that only one nutrient can limit plant productivity at a time (Liebig 1842). Since C is more limiting relative to P given the result of BG/AP, N addition aggravated C limitation, rather than P limitation.

Topography modulates effects of N additions

The aggravated C limitation or alleviated P limitation, however, were not found on the slope after two years of N addition, indicating that topography plays a role in modulating the effects of N addition on microbial resource limitation. This result supports the second hypothesis. Most previous studies did not consider topography effects when they study the responses of ecosystem processes to atmospheric N deposition. This bias may over- or under-estimate effects of N deposition given that most nature ecosystems are not completely flat. In a previous study with the same experimental site, we have found the divergent responses of soil asymbiotic N_2 fixation to N additions between the valley and slope (Wang et al. 2019). Here, we also found similar pattern for microbial activity, further highlighting the importance of topography in regulating the effects of N deposition on ecosystem processes.

As mentioned before, there might be two contrary mechanisms (i.e., different soil N levels and N leaching potentials) controlling the effects of the topography. In the current study, a reason for the weaker impacts of N addition on the slope may be due to the higher NO_3^- leaching in this topography position (Table 1). Soil gross nitrification rate in the limestone soil of the karst forest is very fast (Li et al. 2017), so that deposited NH_4^+ would be rapidly transformed to NO_3^- upon entering the soil. Considering that soil NO_3^- is more susceptible to leaching than NH_4^+ , and that more rock outcrops and the greater inclination on the slope, more NO_3^- would be leached from the slope, which weakens effects of N addition on microbial activity on the slope.

It is surprising that soil N level is higher on the slope than in the valley in our studied forest (Table 1), which might be another mechanism making the valley position more sensitive to N addition relative to the slope. There is evidence that N additions change ecosystem processes, such as soil C and N availability,

more pronouncedly in N limited than in N rich ecosystems (Aber et al. 1998; Chen et al. 2015). Higher soil N status on the slope is not common in nature, but has been found in the karst region of south China (Liu et al. 2011). The divergent Ca contents between valley and slope might be responsible to this phenomenon. In karst forests, soil on the slope generally had a higher Ca content due to the higher rock explosion (Table 1). Because Ca has a protection effect in storing soil organic matter (SOM) (Wen et al. 2017; Wen et al. 2016), soil on the slope has a higher SOM content and thus higher soil N content relative to the valley.

Conclusions

The current study made two important discoveries that worth highlighting. First, our findings in the valley position provide an important supplement to the N saturation theory, which proposes that continued N addition to an ecosystem will switch N limitation to other limitations. In plants, most studies suggested that after N limitation is removed, P will be the most important element in limiting plant growth. However, this topic is rarely discussed at a microbial level. Carbon and P are two likely elements to limit microbial growth, and here we suggest that C will be the more limited element after N limitation is removed, which is different from the situation in plants. This finding has important implications for the global C cycling. It explains why N addition stimulates the early stages of litter decomposition but in general does not affect longer term decomposition rates (Knorr et al. 2005): during the early decomposition phase, excess labile C availability leads to N limitation, but in later stage, decomposers become C-limited as C:N of decomposing material decreases (Soong et al. 2020). Second, we also found that topography can modulate the effects of N addition on microbial resource limitation. Considering that most previous studies ignored the topography effects and thus over- or under-estimate N deposition effects, this study highlight that topography should be included in Earth system models in order to better predict the responses of ecosystem processes to atmospheric N deposition.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and material

The datasets used in the current study are available from the corresponding author on reasonable request.

Competing interests

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The authors declare that they have no competing interests

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Authors' contributions

Conceived and design the experiment: Hao Chen, Dejun Li. Obtained data and samples in the field: Junjie Tang. Processed samples in the lab: Junjie Tang, Xibin Sun. Analyzed the data and wrote the manuscript: Hao Chen. All authors read and approved the final manuscript.

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References

1. Aber J, McDowell W, Nadelhoffer K, Magill A, Berntson G, Kamakea M, McNulty S, Currie W, Rustad L, Fernandez I (1998) Nitrogen saturation in temperate forest ecosystems- Hypotheses revisited. *Bioscience* 48:921–934
2. Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM (1989) Nitrogen saturation in northern forest ecosystems. *Bioscience* 39:378–386
3. Braun S, Thomas VF, Quiring R, Fluckiger W (2010) Does nitrogen deposition increase forest production? The role of phosphorus. *Environ Pollut* 158:2043–2052
4. Brookes PC, Landman A, Pruden G, Jenkinson DS (1985) Chloroform fumigation and the release of soil-nitrogen - a rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Biol Biochem* 17:837–842
5. Brookes PC, Powlson DS, Jenkinson DS (1982) Measurement of microbial biomass phosphorus in soil. *Soil Biol Biochem* 14:319–329
6. Carter MR, Gregorich EG (2006) *Soil sampling and methods of analysis* (second edition). Taylor & Francis Group, Boca Raton, FL, USA
7. Chen H, Li D, Gurmessa GA, Yu G, Li L, Zhang W, Fang H, Mo J (2015) Effects of nitrogen deposition on carbon cycle in terrestrial ecosystems of China: A meta-analysis. *Environ Pollut* 206:352–360
8. Chen H, Li D, Mao Q, Xiao K, Wang K (2018a) Resource limitation of soil microbes in karst ecosystems. *Science of The Total Environment* 650:241–248
9. Chen H, Li D, Xiao K, Wang K (2018b) Soil microbial processes and resource limitation in karst and non-karst forests. *Funct Ecol* 329:61–64

10. Chen H, Li D, Zhao J, Xiao K, Wang K (2018c) Effects of nitrogen addition on activities of soil nitrogen acquisition enzymes: A meta-analysis. *Agr Ecosyst Environ* 252:126–131
11. Chen H, Li D, Zhao J, Zhang W, Xiao K, Wang K (2018d) Nitrogen addition aggravates microbial carbon limitation: Evidence from coenzymatic stoichiometry. *Geoderma* 329:61–64
12. Chen H, Luo P, Wen L, Yang L, Wang K, Li D (2017) Determinants of soil extracellular enzyme activity in a karst region, southwest China. *European Journal of Soil Biology* 80:69–76
13. Chen J, Luo YQ, Li JW, Zhou XH, Cao JJ, Wang RW, Wang YQ, Shelton S, Jin Z, Walker LM, Feng ZZ, Niu SS, Feng WT, Jian SY, Zhou LY (2016) Co-stimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Glob Change Biol* 23:1328–1337
14. Crowley K, McNeil B, Lovett G, Canham C, Driscoll C, Rustad L, Denny E, Hallett R, Arthur M, Boggs J (2012) Do nutrient limitation patterns shift from nitrogen toward phosphorus with increasing nitrogen deposition across the northeastern United States? *Ecosystems* 15:940–957
15. Deng Q, Hui D, Dennis S, Reddy KC (2017) Responses of terrestrial ecosystem phosphorus cycling to nitrogen addition: A meta-analysis. *Glob Ecol Biogeogr* 26:713–728
16. Finzi AC (2009) Decades of atmospheric deposition have not resulted in widespread phosphorus limitation or saturation of tree demand for nitrogen in southern New England. *Biogeochemistry* 92:217–229
17. Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai ZC, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA (2008) Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science* 320:889–892
18. Gress SE, Nichols TD, Northcraft CC, Peterjohn WT (2007) Nutrient limitation in soils exhibiting differing nitrogen availabilities: what lies beyond nitrogen saturation? *Ecology* 88:119–130
19. IUSS-Working-Group (2006) World reference base for soil resources 2006. FAO, Rome
20. Jian S, Li J, Chen J, Wang G, Mayes MA, Dzantor KE, Hui D, Luo Y (2016) Soil extracellular enzyme activities, soil carbon and nitrogen storage under nitrogen fertilization: A meta-analysis. *Soil Biol Biochem* 101:32–43
21. Knorr M, Frey SD, Curtis PS (2005) Nitrogen additions and litter decomposition: A meta-analysis. *Ecology* 86:3252–3257
22. Lasota J, Błońska E (2021) C:N:P stoichiometry as an indicator of Histosol drainage in lowland and mountain forest ecosystems. *Forest Ecosystems* 8:39
23. Li D, Yang Y, Chen H, Xiao K, Song T, Wang K (2017) Soil Gross Nitrogen Transformations in Typical Karst and Nonkarst Forests, Southwest China. *Journal of Geophysical Research Biogeosciences* 122:2831–2840
24. Li T, Wang R, Cai J, Meng Y, Wang Z, Feng X, Liu H, Turco RF, Jiang Y (2021) Enhanced carbon acquisition and use efficiency alleviate microbial carbon relative to nitrogen limitation under soil acidification. *Ecological Processes* 10:32. doi:10.1186/s13717-021-00309-1

25. Li Y, Niu S, Yu G (2016) Aggravated phosphorus limitation on biomass production under increasing nitrogen loading: a meta-analysis. *Glob Change Biol* 22:934–943
26. Liebig J (1842) *Animal chemistry, or organic chemistry in its application to physiology and pathologie*. Johnson Reprint Corporation, New York
27. Liu S, Zhang W, Wang K, Chen H, Shu S, Tan W (2011) Spatiotemporal heterogeneity of topsoil nutrients in Karst Peak-Cluster depression area of Northwest Guangxi, China. *Acta Ecol Sin* 31:3036–3043 (in Chinese)
28. Marklein AR, Houlton BZ (2012) Nitrogen inputs accelerate phosphorus cycling rates across a wide variety of terrestrial ecosystems. *New Phytol* 193:696–704
29. McNulty SG, Aber JD, Newman SD (1996) Nitrogen saturation in a high elevation New England spruce-fir stand. *For Ecol Manage* 84:109–121
30. Moorhead DL, Rinkes ZL, Sinsabaugh RL, Weintraub MN (2013) Dynamic relationships between microbial biomass, respiration, inorganic nutrients and enzyme activities: informing enzyme-based decomposition models. *Front Microbiol* 4:223
31. Sinsabaugh RL, Hill BH, Shah JJF (2009) Ecoenzymatic stoichiometry of microbial organic nutrient acquisition in soil and sediment. *Nature* 462:795–798
32. Sinsabaugh RL, Manzoni S, Moorhead DL, Richter A (2013) Carbon use efficiency of microbial communities: stoichiometry, methodology and modelling. *Ecol Lett* 16:930–939
33. Sinsabaugh RL, Moorhead DL (1994) Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. *Soil Biol Biochem* 26:1305–1311
34. Sinsabaugh RS (1994) Enzymic analysis of microbial pattern and process. *Biol Fertil Soils* 17:69–74
35. Soong JL, Fuchslueger L, Maraon-Jimenez S, Torn MS, Janssens IA, Penuelas J, Richter A (2020) Microbial carbon limitation: The need for integrating microorganisms into our understanding of ecosystem carbon cycling. *Glob Change Biol* 26:1952–1961
36. Sterner RW, Elser JJ (2002) *Ecological stoichiometry: the biology of elements from molecules to the biosphere*. Princeton University Press, Princeton
37. Traoré O, Kiba DI, Arnold M, Fliessbach A, Oberholzer HR, Nacro HB, Lompo F, Oberson A, Frossard E, Bünemann EK (2016) Fertilization practices alter microbial nutrient limitations after alleviation of carbon limitation in a Ferric Acrisol. *Biol Fertil Soils* 52:177–189
38. Treseder KK (2008) Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecol Lett* 11:1111–1120
39. Vance ED, Brookes PC, Jenkinson DS (1987) An extraction method for measuring soil microbial biomass-C. *Soil Biol Biochem* 19:703–707
40. Wang R, Dorodnikov M, Yang S, Zhang Y, Filley TR, Turco RF, Zhang Y, Xu Z, Li H, Jiang Y (2015) Responses of enzymatic activities within soil aggregates to 9-year nitrogen and water addition in a semi-arid grassland. *Soil Biol Biochem* 81:159–167

41. Wang Z, Li D, Zheng M, Chen H, Sun X, Wang K (2019) Topography modulates effects of nitrogen deposition on asymbiotic N₂ fixation in soil but not litter or moss in a secondary karst forest. *Journal of Geophysical Research: Biogeosciences* 124:3015–3023
42. Waring BG, Weintraub SR, Sinsabaugh RL (2014) Ecoenzymatic stoichiometry of microbial nutrient acquisition in tropical soils. *Biogeochemistry* 117:101–113
43. Weand MP, Arthur MA, Lovett GM, Sikora F, Weathers KC (2010) The phosphorus status of northern hardwoods differs by species but is unaffected by nitrogen fertilization. *Biogeochemistry* 97:159–181
44. Weintraub SR, Taylor PG, Porder S, Cleveland CC, Asner GP, Townsend AR (2015) Topographic controls on soil nitrogen availability in a lowland tropical forest. *Ecology* 96:1561–1574
45. Wen L, Li D, Chen H, Wang K (2017) Dynamics of soil organic carbon in density fractions during post-agricultural succession over two lithology types, southwest China. *J Environ Manage* 201:199–206
46. Wen L, Li D, Yang L, Luo P, Chen H, Xiao K, Song T, Zhang W, He X, Chen H (2016) Rapid recuperation of soil nitrogen following agricultural abandonment in a karst area, southwest China. *Biogeochemistry* 129:341–354
47. Zhang J, Li Y, Wang J, Chen W, Tian D, Niu S (2021) Different responses of soil respiration and its components to nitrogen and phosphorus addition in a subtropical secondary forest. *Forest Ecosystems* 8:37
48. Zhang N, Xu W, Yu X, Lin D, Wan S, Ma K (2013) Impact of topography, annual burning, and nitrogen addition on soil microbial communities in a semiarid grassland. *Soil Sci Soc Am J* 77:1214–1224

Figures

Figure 1

Changes of activities of studied enzymes (μmol g⁻¹ SOC h⁻¹) after two years of N addition. (a) BG (β-D-glucosidase) and (b) POX (polyphenol oxidase) are two C-acquisition enzymes; (c) LAP (L-leucine aminopeptidase) and (d) NAG (β-N-acetylglucosaminidase) are two N-acquisition enzymes; (e) AP is a P-acquisition enzyme. CT: control; LN: low-N addition; HN: high-N addition. Error bars denote the standard error (n= 3). Different letters indicate significant difference (P< 0.05) between treatments.

Figure 2

Changes of enzymatic stoichiometry indexes after two years of N addition. (a) Vector length and (b) BG/AP are two indicators of microbial C limitation. High vector length or BG/AP means greater C limitation. (c) BG/(LAP+ NAG) and (d) $R_{C:N} - TER_{C:N}$ are two indicators of microbial N limitation. High BG/(LAP+NAG) means lower N limitation, while higher $R_{C:N} - TER_{C:N}$ means higher N limitation. (e) Vector angle and (f) $R_{C:P} - TER_{C:P}$ are two indicators of microbial P limitation. Greater vector angle indicates greater P limitation when vector angles $> 45^\circ$; higher $R_{C:P} - TER_{C:P}$ means higher P limitation. CT: control; LN: low-N addition; HN: high-N addition. Error bars denote the standard error ($n=3$). Different letters indicate significant difference ($P < 0.05$) between treatments. The asterisks indicate significant difference between $R_{C:N} - TER_{C:N}$ (or $R_{C:P} - TER_{C:P}$) and zero.

Figure 3

Effects of N addition on (a) modeled decomposition rate (M) and (b) the ratio of BG (β -D-glucosidase) to POX (polyphenol oxidase). BG and PPO are two C-acquisition enzymes, decomposing labile and non-labile organic C, respectively. CT: control; LN: low-N addition; HN: high-N addition. Error bars denote the standard error ($n = 3$). Different letters indicate significant difference ($P < 0.05$) between treatments.

Figure 4

Effects of two-year N addition on the dissolved organic carbon (DOC), total dissolved nitrogen (TDN), available phosphorus (AVP), nitrification rate, and leaching rate of nitrate (NO₃⁻). The nitrification rate and NO₃⁻ leaching rate was provided by Liang Zheng, who measured them in 2018. Error bars denote the standard error (n= 3). Different letters indicate significant difference (P< 0.05) between treatments.

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