Eurasian J Soil Sci 2019, 8 (1) 54 - 59

Eurasian Journal of Soil Science

Journal homepage : http://ejss.fesss.org

# Does anthropogenic phosphorus input reduce soil microbial resource allocation to acquire nitrogen relative to carbon?

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## Abstract

We aimed to test if anthropogenic P input into ecosystems reduces microbial resource allocation to acquire N (and alleviate N shortage if any) because microbes no longer produce N-rich phosphatase for P acquisition. Literatures reporting the effect of P fertilization on C-acquiring (β-1,4-glucosidase, BG) and N-acquiring (β-1,4-N-acetylglucosaminidase, NAG, which also acquires C) enzymes were collected and synthesized. We predicted that P addition elevates BG:NAG especially in P-poor ecosystems because P addition alleviates N shortage and reduces the microbial resource allocation to acquire N relative to C. The synthesized data demonstrated that P fertilization occasionally reduced BG:NAG, which is inconsistent with the prediction. However, this might not mean that the initial hypothesis was rejected. Stimulated microbial activity and turnover by P fertilization could have caused microbes depend the C sources more on chitin (and peptidoglycan) compared with on cellulose because chitin (and peptidoglycan) is a main component of microbial body and re-provided through microbial turnover. The changes in C resources accompanied by the altered P availability may have largely influenced BG:NAG, masking the role of BG:NAG for indicating microbial resource allocation to C and N acquisitions.

**Keywords**: β-1,4-glucosidase (BG), β-1,4-N-acetylglucosaminidase (NAG), ecoenzymatic stoichiometry, phosphatase, phosphorus fertilization.

## Article Info

Received : 01.03.2018
Accepted : 10.12.2018

## Introduction

Marklein and Houlton (2012) proposed in their meta-analysis that anthropogenic nitrogen (N) elevation can compensate phosphorus (P) shortage of biota through elevating phosphatase production, because N-rich phosphatase (C:N ratio of protein is generally up to 4) synthesis requires a large amount of N (Olander and Vitousek; 2000; Treseder and Vitousek, 2001; Houlton et al., 2008). From their hypothesis, it can be indicated that P shortage elevates microbial N requirement in order to produce the N-rich phosphatase and accelerates N shortage (if any). Accordingly, anthropogenic P inputs into ecosystems can reduce microbial resource allocation to acquire N (relative to C) and alleviate N shortage because microbes reduce the production of the N-rich phosphatase in P-rich conditions.

Many previous studies assumed that the resource allocation of microbes to acquire nutrients and energy could be expressed as ecoenzymatic stoichiometry, i.e., ratios of extracellular enzymes targeting C, N and P (Sinsabaugh et al., 2008, 2009; Waring et al., 2014). Among a variety of extracellular enzymes targeting C, N, and P, β-1,4-glucosidase (BG, catalyzing the terminal reaction in cellulose degradation), β-1,4-N-acetylglucosaminidase (NAG, catalyzing the terminal reaction in chitin degradation), and acid (or alkaline) phosphatase (AP, hydrolyzing organic phosphorus) has been measured most widely (Olander and Vitousek,

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e-ISSN: 2147-4249
DOI: 10.18393/ejss.498039

2000; Allison and Vitousek, 2005; Sinsabaugh et al., 2009; Turner and Wright, 2014; Waring et al., 2014; Jian et al., 2016). Thus the ratio of BG and NAG (BG:NAG), and of BG and AP (BG:AP) are often used as indicators of microbial resource allocation to the acquisition of N and P relative to C, respectively (Sinsabaugh et al., 2008, 2009; Turner and Wright, 2014; Waring et al., 2014; Moorhead et al., 2016; Zhou et al., 2017; Rosinger et al., 2018; Tatariw et al., 2018), although few studies recently suggested that the BG:NAG may not always indicate the microbial resource allocation to the acquisition of N relative to C because NAG can be also produced for acquiring C as well as N (Mori et al., 2018a, b; Wang et al., 2018).

Based on the above hypothesis and assumption, it is predicted that P addition would elevate BG:NAG because P addition reduces the microbial resource allocation to acquire N relative to C through reducing the N requirement to synthesize phosphatase (note that P addition does not necessarily reduce the absolute resource investment on N acquisition). The response ratio of BG:NAG to P addition would be larger in P-poor ecosystems where BG:AP is smaller (Figure 1). We also need to consider P-poor but N-rich conditions (such as areas with extremely high N deposition or fertilized with N), where the reduced requirement of N in relation to P addition would not affect BG:NAG because NAG is probably produced targeting C acquisition rather than N (Mori et al. 2018a,b; Wang et al. 2018) (note that chitin contains both N and C) (Figure 2). In such conditions, P addition would not elevate BG:NAG (Figure 1). Overall, the relationship between BG:AP and the response ratio of BG:NAG to P addition is predicted as follows: (i) the response ratio of BG:NAG to P fertilization would be higher than 1 and become larger as BG:AP decreases; or (ii) BG:NAG does not change in response to P addition if the soil is rich in N (response ratio is around 1) (Figure 1).

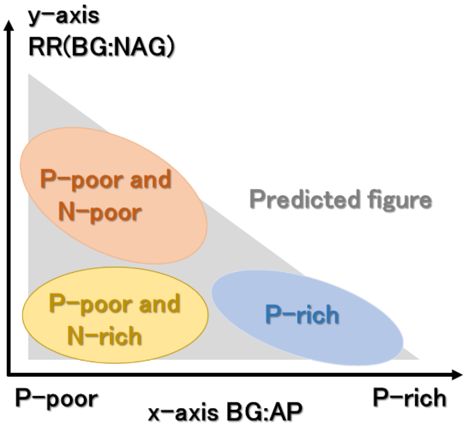

Figure 1. The predicted relationship between BG:AP and the response ratio of BG:NAG to P addition. RR(BG:NAG) represents the response ratio of BG:NAG to P addition.

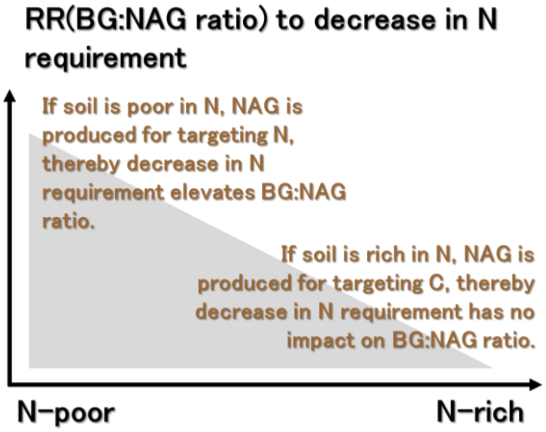

Figure 2. Predicted response of BG:NAG to a decrease in N requirement. RR(BG:NAG) represents the response ratio of BG:NAG to a decrease in N requirement. If soil is poor in N, NAG is produced for targeting organic N. In such a case, a decrease in N requirement reduces microbial allocation on N-acquiring enzyme (NAG) and as a result BG:NAG will be elevated. Meanwhile, if soil is rich in N, NAG is produced for targeting C, thereby a decrease in N requirement has no impact on BG:NAG.

In this study, we aimed to test if anthropogenic P input into ecosystems reduces microbial resource allocation to acquire N (and alleviate N shortage if any) by collecting the literatures reporting the effect of P fertilization on the activities of extracellular enzymes including BG, NAG, and AP.

## Material and Methods

We collected the literatures reporting the effects of P addition on the activities of NAG and BG. We used the search engine Web of Science to collect published literatures with the following combinations of key words for searching; ("phosph* add*" OR "P add*" OR "phosph* elevat*" OR "P elevat*" OR "phosph* fertiliz*" OR "P fertiliz*" OR "phosph* appl*" OR "P appl*"OR "phosph* enrich*" OR "P enrich*") AND (glucosidase OR β-glucosidase OR "β glucosidase" OR BG OR βG) AND (NAG OR chitinase OR β-1,4-N-acetyl-β-glucosaminidase OR "N-acetyl β-glucosaminidase" OR glucosaminidase). All papers collected in the above procedure reported AP activity as well as BG and NAG activities, which enabled us to evaluate the relationship between BG:AP and the response ratio of BG:NAG to P addition. We compensated the literature list by using other search engines, Google and Google Scholar, because several papers were not collected by the procedure. Since only a few number of papers were available for our research purpose, the data taken in the same site but with different types of nutrient addition (e.g., comparison between data in N-added and NP-added site) or different soil layer were counted as different data points (Table 1).

## Results and Discussion

We found 29 data points from 9 literatures (Table 1). At the first glance, the relationship between BG:AP and the response ratio of BG:NAG to P fertilization (Figure 3) seems consistent with the predicted pattern (Figure 1): As BG:AP increased up to 0.5, the response ratio of BG:NAG to P fertilization declined (Figure 3). However, there were critical differences between the result and the prediction. We observed that response ratios of BG:NAG to P addition were lower than 1 in several cases (i.e., P addition reduced BG:NAG). Originally, we assumed that (i) the response ratio of BG:NAG to P fertilization should be higher than 1 if P fertilization reduces microbial N requirement, or (ii) BG:NAG does not change in response to P addition if the soil is rich in N (response ratio is around 1). The lower response ratio than 1 may indicate that the P addition increased N requirement relative to C requirement by biota. Although we cannot completely deny the possibility, it is less likely because P fertilization reduces N-rich phosphatase production (Marklein and Houlton, 2012). Thus the present analysis failed to testify the hypothesis: anthropogenic P input into ecosystems reduces microbial resource allocation to acquire N.

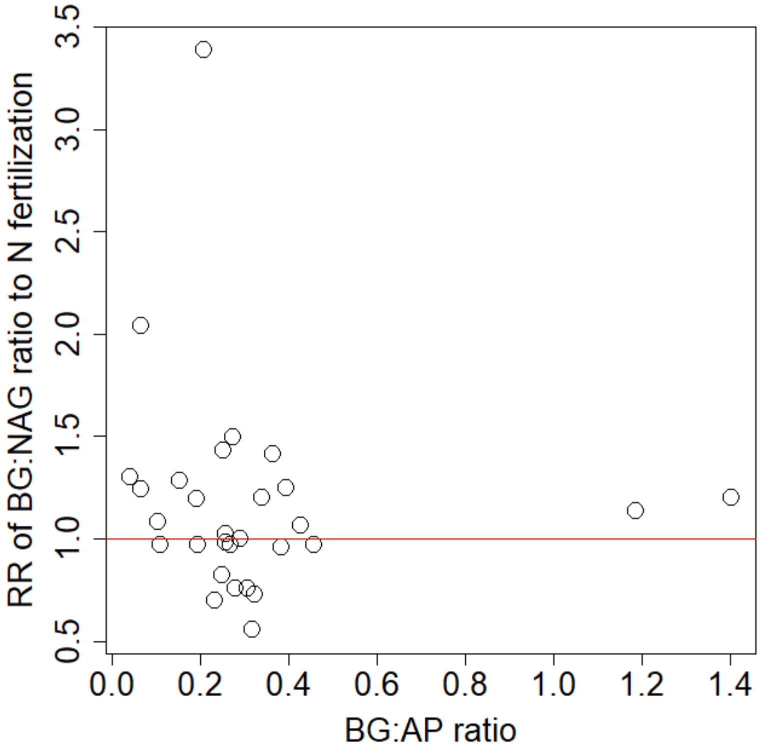

Figure 3. The relationship between BG:AP and the response ratio of BG:NAG to P addition in no P controls. This figure was drawn using data taken from literatures. RR(BG:NAG) represents the response ratio of BG:NAG to P addition. 1:1 line was drawn in the figure.

Table 1. Reference information

| No | Comments | Location | MAT (°C) | Annual precipitation (mm) | Reference |
|---|---|---|---|---|---|
| 1 | Tropical lowland forest | Barro Colorado, Panama | 26.0 | 2600 | Turner and Wright (2014) |
| 2 | O-layer without N fertilization in stream | The Bear Brook Watershed in Marine | 5.1 | 1320 | Mineau et al. (2014) |
| 3 | O-layer with N fertilization in stream | The Bear Brook Watershed in Marine | 5.1 | 1320 | Mineau et al. (2014) |
| 4 | B-layer without N fertilization in stream | The Bear Brook Watershed in Marine | 5.1 | 1320 | Mineau et al. (2014) |
| 5 | B-layer with N fertilization in stream | The Bear Brook Watershed in Marine | 5.1 | 1320 | Mineau et al. (2014) |
| 6 | Chinese fir plantation (without N addition) | Qianyanzhou Forest Experimental Site, China | 17.9 | 1471.2 | Dong et al. (2015) |
| 7 | Chinese fir plantation (50 kg N per ha was added) | Qianyanzhou Forest Experimental Site, China | 17.9 | 1471.2 | Dong et al. (2015) |
| 8 | Chinese fir plantation (100 kg N per ha was added) | Qianyanzhou Forest Experimental Site, China | 17.9 | 1471.2 | Dong et al. (2015) |
| 9 | Rice cropping system (N and K was added simultanously) | Jiangxi, China | 18.0 | 1470 | Zhang et al. (2015b) |
| 10 | Cropland (N was added simultanously) | South Lake station, China | >10.0 | 1300 | Zhang et al. (2015a) |
| 11 | Shortgrass prairie | Cedar point, Nebraska, USA | 9.3 | 454 | Riggs and Hobbie (2016) |
| 12 | Shortgrass prairie (N was added simultanously) | Cedar point, Nebraska, USA | 9.3 | 454 | Riggs and Hobbie (2016) |
| 13 | Shortgrass prairie (K was added simultanously) | Cedar point, Nebraska, USA | 9.3 | 454 | Riggs and Hobbie (2016) |
| 14 | Shortgrass prairie (N and K were added simultanously) | Cedar point, Nebraska, USA | 9.3 | 454 | Riggs and Hobbie (2016) |
| 15 | Tallgrass prairie | Konza Prairie, Kansas, USA | 12.0 | 872 | Riggs and Hobbie (2016) |
| 16 | Tallgrass prairie (N was added simultanously) | Konza Prairie, Kansas, USA | 12.0 | 872 | Riggs and Hobbie (2016) |
| 17 | Tallgrass prairie (K was added simultanously) | Konza Prairie, Kansas, USA | 12.0 | 872 | Riggs and Hobbie (2016) |
| 18 | Tallgrass prairie (N and K were added simultanously) | Konza Prairie, Kansas, USA | 12.0 | 872 | Riggs and Hobbie (2016) |
| 19 | Shortgrass prairie | Shortgrass Steppe, Colorado, USA | 8.4 | 364 | Riggs and Hobbie (2016) |
| 20 | Shortgrass prairie (N was added simultanously) | Shortgrass Steppe, Colorado, USA | 8.4 | 364 | Riggs and Hobbie (2016) |
| 21 | Shortgrass prairie (K was added simultanously) | Shortgrass Steppe, Colorado, USA | 8.4 | 364 | Riggs and Hobbie (2016) |
| 22 | Shortgrass prairie (N and K were added simultanously) | Shortgrass Steppe, Colorado, USA | 8.4 | 364 | Riggs and Hobbie (2016) |
| 23 | Glaciated mixed hardwood forest | Ohio, USA | 8.1 | 1200 | Carrino-Kyker et al. (2016) |
| 24 | Glaciated mixed hardwood forest (elevated pH) | Ohio, USA | 8.1 | 1200 | Carrino-Kyker et al. (2016) |
| 25 | Unglaciated mixed hardwood forest | Ohio, USA | 10.7 | 1000 | Carrino-Kyker et al. (2016) |
| 26 | Unglaciated mixed hardwood forest (elevated pH) | Ohio, USA | 10.7 | 1000 | Carrino-Kyker et al. (2016) |
| 27 | Broadleaf forest | Maoershan Forest Ecosystem Research Station, China | −18.5 to 22.0 | 629 | Zhou et al. (2017) |
| 28 | Pine forest | Maoershan Forest Ecosystem Research Station, China | −18.5 to 22.0 | 629 | Zhou et al. (2017) |
| 29 | Watershed | Lead Mountain in Maine, USA | - | - | Tatariw et al. (2018) |

The discrepancy between our predictions and the result could be explained by an altered C resource composition. It was reported that cellulose decomposition was stimulated by P fertilization (Kaspari et al., 2008; Fanin et al., 2015), which leads a decrease in BG-targeting C in soils (i.e., cellulose). Although the stimulated cellulose decomposition is associated with elevated BG activity, the BG:NAG ratio could not be altered if decomposition of chitin and peptidoglycan (NAG is involved in the degradation of peptidoglycan, as well as chitin) is equally stimulated by P fertilization (which needs to be tested). Instead, the cellulose:chitin (and peptidoglycan) ratio could be lowered because chitin and peptidoglycan is re-provided in soils through microbial turnover as as chitin and peptidoglycan are main components of microbial body. By contrast, cellulose is basically not provided from the microbial body. Accordingly, microbial activity could be more chitin (and peptidoglycan)-dependent under P-added conditions, leading to a larger NAG activity relative to BG (lower BG:NAG) because microbes shift enzyme activity for targeting more-abundant substrates. If this is true, at least in some cases, changes in BG:NAG represent the progress of decomposition stage or the strength of microbial activity rather than a microbial allocation on C and N acquisition, which challenges the idea suggested in previous studies (e.g., Waring et al., 2014). Although this new hypothesis still lacks definitive evidences, it potentially explains the synthesized pattern. Monitoring the BG:NAG ratios as well as decomposition ratios of cellulose and chitin both in manipulated N-shortage and N-rich conditions in a laboratory experiment may provide a chance to examine what actually controls the pattern of BG:NAG.

The present analysis failed to demonstrate the reduced N requirement by P fertilization, but this might not mean that the initial hypothesis was rejected. As discussed above, changes in C resources accompanied by the altered P availability may have largely influenced BG:NAG, masking the role of BG:NAG for indicating microbial resource allocation to C and N acquisitions. Another indicator to access microbial N acquisition, which is not affected by the changes in C resources, is necessary for testing if P input into ecosystems reduces microbial resource allocation to acquire N and alleviate N shortage.

## Acknowledgement

This study was financially supported by a grant from environmental research aid, The Sumitomo Foundation 153082 and Grant-in-Aid for JSPS Postdoctoral Fellowships for Research Abroad (28.601) to T Mori.

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