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Effects of Nitrogen Addition on Soil Enzyme Activity and Enzyme Stoichiometric Characteristics in the Tianshan Alpine Grassland: Postprint

Authors: Le Jiajia, Su Yuan, Peng Qingwen, Geng Fengzhan, Han Wenxuan, Li Wenli, Li Kaihui, Liu Xuejun, Li Kaihui, Liu Xuejun

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Abstract

To investigate the effects of nitrogen addition on soil enzyme activities in alpine grassland ecosystems, a study was conducted in 2018 at the Bayinbuluke Grassland Ecosystem Research Station, Chinese Academy of Sciences. Four nitrogen addition levels were established (control, N0, 0 kg · hm ^{2} · a ^{1}; low nitrogen, N1, 10 kg · hm ^{2} · a ^{1}; medium nitrogen, N3, 30 kg · hm ^{2} · a ^{1}; high nitrogen, N9, 90 kg · hm ^{2} · a ^{1}) to examine the response characteristics of soil enzyme activities to nitrogen addition, soil enzyme stoichiometric ratios, and the relationships between soil enzyme activities and soil environmental factors. The results showed that, compared with the control, nitrogen addition at the N3 level significantly increased the activities of -1,4-glucosidase (G), -D-cellobiohydrolase (CBH), and -1,4-xylosidase (X) ($P < 0.05$); the N1 and N3 levels significantly increased alkaline phosphatase (AKP) activity ($P < 0.05$); the N3 level significantly decreased polyphenol oxidase (PPO) activity ($P < 0.05$); nitrogen addition had no significant effect on leucine aminopeptidase (LAP) activity; and N-acetyl- -D-glucosaminidase (NAG) activity was significantly increased at the N3 level ($P < 0.05$). Correlation analysis revealed that the activities of all eight soil enzymes were significantly correlated with soil organic carbon (except for NAG) and total phosphorus (TP), but not with soil total nitrogen (TN). The stoichiometric ratio of soil enzyme activity C N P in the study area was 1 1 1.2, which deviated from the global ecosystem ratio of 1 1 1, indicating that soil microbial growth in this study area was limited by phosphorus. Redundancy analysis (RDA) further revealed that soil organic carbon and soil total phosphorus content were the main factors influencing soil enzyme activities.

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Full Text

Preamble

Effects of Nitrogen Addition on Soil Enzyme Activities and Ecoenzymatic Stoichiometry in Alpine Grassland

LEI Jia-jia[1,2], SU Yuan[1,2], PENG Qing-wen[1,2], GENG Feng-zhan[1,2], HAN Wen-xuan[1,3], LI Wen-li , LI Kai-hui[1], , LIU Xue-jun[1,3]

(1. Key Laboratory of Biogeography and Bioresource in Arid Land, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, Xinjiang, China;
2. University of the Chinese Academy of Sciences, Beijing 100049, China;
3. College of Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China;
4. Grassland Station of Bayingol Mongolian Autonomous Prefecture of Xinjiang, Korla 841000, Xinjiang, China;
5. Bayinbuluk Grassland Ecosystem Research Station, Chinese Academy of Sciences, Bayinbuluk 841314, Xinjiang, China)

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Funding: National Natural Science Foundation of China (41673079)
Corresponding author: LIU Xue-jun (1993-), male, Ph.D., research focus on grassland ecology. E-mail: liu310@cau.edu.cn

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Abstract

The effects of nitrogen (N) addition on activities of soil carbon (C), N, and phosphorus (P) circulation-related enzymes were investigated in an alpine grassland ecosystem in 2018. A long-term simulated N addition experiment was initiated at the Bayinbuluk Grassland Ecological Research Station of the Chinese Academy of Sciences in 2009; four N fertilizer treatments were applied at different sites: control (N0, 0 kg · hm ^{2} · a ^{1}), low nitrogen (N1, 10 kg · hm ^{2} · a ^{1}), moderate nitrogen (N3, 30 kg · hm ^{2} · a ^{1}), and high nitrogen (N9, 90 kg · hm ^{2} · a ^{1}). In the present study, stoichiometric ratios of soil ecological enzymes and the relationships between soil enzyme activities and environmental factors were analyzed. Our results revealed the following: Compared with N0, N3 treatment significantly increased -1,4-glucosidase (BG), cellobiohydrolase (CBH) and -1,4-xylosidase activities (x) ($P < 0.05$). N1 and N3 treatments obviously increased phenoloxidase (PPO) activity ($P < 0.05$). N3 treatment obviously increased -1,4-N-acetylglucosaminidase (NAG) activity ($P < 0.05$). No N treatment affected L-leucine aminopeptidase activity (LAP). Correlation analysis showed that activities of all soil enzymes were positively correlated to soil organic C (SOC) content (except NAG) and to total phosphorus (TP) content but were not to total nitrogen content. Soil ecoenzymatic C:N:P stoichiometry in the

study area was 1:1:1.2, which is inconsistent with the global 1:1:1 pattern. This result suggests that soil microbial growth in the studied grasslands is limited by P content. Canonical redundancy analysis indicated that SOC and TP were the dominant factors affecting soil enzyme activity.

Keywords: nitrogen addition; soil enzyme activity; soil ecoenzymatic stoichiometry; nutrient limitation; alpine grassland; Bayinbuluk

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1.2.2 Soil Physicochemical Properties

Soil organic carbon (SOC) was measured using the potassium dichromate oxidation method, total nitrogen (TN) was determined by the Kjeldahl method, total phosphorus (TP) was measured using the H SO -HClO digestion method, available phosphorus (AP) was extracted with 0.5 mol · L ¹ NaHCO and analyzed by molybdenum-blue colorimetry, and available nitrogen (AN) was determined using the FOSSTecator Kjeltec system [15].

1.2.3 Soil Enzyme Activities

The activities of -1,4-glucosidase (G), cellobiohydrolase (CBH), -1,4-xylosidase (X), L-leucine aminopeptidase (LAP), -1,4-N-acetylglucosaminidase (NAG), and alkaline phosphatase (AKP) were measured using a microplate fluorometric assay [16]. Phenoloxidase (PPO) activity was determined using a spectrophotometric method [17]. All measurements were performed with three replicates.

1.3 Statistical Analysis

One-way ANOVA was used to analyze the effects of N addition on soil enzyme activities and stoichiometry, followed by LSD or Dunnett' s T3 post-hoc tests (= 0.05). Principal component analysis (PCA) was performed to examine the relationships between enzyme activities and environmental factors. Redundancy analysis (RDA) was conducted to identify the key environmental factors influencing enzyme activities. All statistical analyses were performed using SPSS 23.0 and R software.

2.2 Effects of Nitrogen Addition on Soil Enzyme Activities

Compared with the control (N0), moderate nitrogen addition (N3) significantly increased the activities of carbon-acquiring enzymes (G, CBH, and X) ($P < 0.05$) (Fig. 1a, 1b, 1c). N1 and N3 treatments significantly enhanced PPO activity ($P < 0.05$), while N3 treatment also significantly increased NAG activity ($P < 0.05$). No significant effects were observed on LAP activity across all N treatments (Fig. 1d, 1e). AKP activity was significantly higher under N1 and N3 treatments compared to N0 ($P < 0.05$), with N3 showing the greatest increase

(Fig. 1f). Vector analysis revealed that the vector lengths for enzyme activities under N3 treatment were 0.47, 1.44, and 1.24 for G, CBH, and X respectively, indicating significant differences from N0 ($P < 0.05$). The angle between vectors (Vector A) was less than 45° for C-acquiring enzymes and greater than 45° for N- and P-acquiring enzymes, suggesting microbial nutrient limitation patterns.

2.3 Soil Ecoenzymatic Stoichiometry

The ratios of enzyme activities showed consistent patterns across treatments (Table 2). The ln(G):ln(LAP+NAG) ratio ranged from 0.81 to 1.05, while ln(G):ln(AKP) ranged from 0.83 to 1.36. The vector lengths and angles did not differ significantly among N treatments ($P > 0.05$), indicating that N addition did not alter the overall ecoenzymatic stoichiometry. Correlation analysis revealed that enzyme activities were positively correlated with SOC and TP contents ($R^2 = 0.33$ and 0.38 respectively, $P < 0.05$) but not with TN content.

3.1 Relationships Between Enzyme Activities and Nutrient Limitation

The C:N ratio and enzyme stoichiometry indicated that microbial growth was primarily limited by P availability. The vector analysis approach [18] showed that N addition, particularly at moderate rates, shifted microbial resource allocation toward C acquisition. The significant positive correlation between PPO activity and TP content ($r = -0.725$, $P < 0.05$) suggests that P availability regulates oxidative enzyme production. These findings align with previous studies showing P limitation in alpine grassland ecosystems [19, 27].

3.2 Stoichiometric Characteristics of Soil Enzymes

The observed ecoenzymatic stoichiometry of 1:1:1.2 (C:N:P) deviates from the global average of 1:1:1 [10], indicating stronger P limitation in this alpine grassland. This pattern reflects the adaptation of microbial communities to low P availability in high-altitude ecosystems. The consistent stoichiometric ratios across N treatments suggest that microbial nutrient demands are constrained by resource availability rather than by N addition rates.

3.3 Conclusions

This study demonstrates that: (1) Moderate N addition (N3) significantly increased activities of C-acquiring enzymes (G, CBH, X) and PPO, while also enhancing NAG activity, but did not affect LAP activity. All enzyme activities were positively correlated with SOC and TP contents. (2) Soil ecoenzymatic stoichiometry remained stable at approximately 1:1:1.2 across all treatments,

indicating persistent P limitation. The differential responses of enzyme activities to N addition reflect microbial strategies to maintain stoichiometric balance under nutrient constraints.

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