Effects of Nitrogen and Phosphorus Additions on Soil Microbial Community Structure and Ecological Processes in the Farmland of the Chinese Loess Plateau (Postprint)

Authors: KOU Zhaoyang, Chunyue Li, CHANG Shun, MIAO Yu, Zhang Wenting, LI Qianxue, DANG Tinghui, WANG Yi, LI Chunyue

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Abstract

Microorganisms regulate the responses of terrestrial ecosystems to anthropogenic nutrient inputs. The escalation of anthropogenic activities has resulted in a rise in the primary terrestrial constraining elements, namely nitrogen (N) and phosphorus (P). Nevertheless, the specific mechanisms governing the influence of soil microbial community structure and ecological processes in ecologically vulnerable and delicate semi-arid loess agroecosystems remain inadequately understood. Therefore, we explored the effects of different N and P additions on soil microbial community structure and its associated ecological processes in the farmland of Chinese Loess Plateau based on a 36-a long-term experiment. Nine fertilization treatments with complete interactions of high, medium, and low N and P gradients were set up. Soil physical and chemical properties, along with the microbial community structure were measured in this study. Additionally, relevant ecological processes such as microbial biomass, respiration, N mineralization, and enzyme activity were quantified. To elucidate the relationships between these variables, we examined correlation-mediated processes using statistical techniques, including redundancy analysis (RDA) and structural equation modeling (SEM). The results showed that the addition of N alone had a detrimental effect on soil microbial biomass, mineralized N accumulation, and β-1,4-glucosidase activity. Conversely, the addition of P exhibited an opposing effect, leading to positive influences on these soil parameters. The interactive addition of N and P significantly changed the microbial community structure, increasing microbial activity (microbial biomass and soil respiration), but decreasing the accumulation of mineralized N. Among them, N24P12 treatment showed the greatest increase in the soil nutrient content and respiration. N12P12 treatment increased the overall enzyme activity and total phospholipid fatty acid (PLFA) content by 70.93%. N and P nutrient contents of the soil dominate the microbial community structure and the corresponding changes in hydrolytic enzymes. Soil microbial biomass, respiration, and overall enzyme activity are driven by mineralized N. Our study provides a theoretical basis for exploring energy conversion processes of soil microbial community and environmental sustainability under long-term N and P additions in semi-arid loess areas.

Full Text

Preamble

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Effects of nitrogen and phosphorus additions on soil microbial community structure and ecological processes in the farmland of Chinese Loess Plateau

KOU Zhaoyang[1], LI Chunyue[1]*, CHANG Shun[1], MIAO Yu[1], ZHANG Wenting[1], LI Qianxue[1], DANG Tinghui[2], WANG Yi[3]

[1]Institute of Soil and Water Conservation, Northwest A&F University, Yangling 712100, China
[2]State Key Laboratory of Loess and Quaternary Geology, Institute of Earth Environment, Chinese Academy of Sciences, Xi' an 710061, China

Abstract: Microorganisms regulate the responses of terrestrial ecosystems to anthropogenic nutrient inputs. The escalation of human activities has increased the primary terrestrial limiting elements, nitrogen (N) and phosphorus (P). However, the specific mechanisms governing how soil microbial community structure and ecological processes respond in ecologically vulnerable and delicate semi-arid loess agroecosystems remain inadequately understood. Therefore, we explored the effects of different N and P additions on soil microbial community structure and associated ecological processes in farmland on the Chinese Loess Plateau based on a 36-year long-term experiment. Nine fertilization treatments with complete interactions of high, medium, and low N and P gradients were established. Soil physical and chemical properties, along with microbial community structure, were measured. Additionally, relevant ecological processes such as microbial biomass, respiration, N mineralization, and enzyme activity were quantified. To elucidate relationships between these variables, we examined correlation-mediated processes using statistical techniques including redundancy analysis (RDA) and structural equation modeling (SEM). The results showed that N addition alone had a detrimental effect on soil microbial biomass, mineralized N accumulation, and β-1,4-glucosidase activity. Conversely, P addition exhibited an opposing effect, positively influencing these soil parameters. The interactive addition of N and P significantly altered microbial community structure, increasing microbial activity (microbial biomass and soil respiration) but decreasing mineralized N accumulation. Among these, the N24P12 treatment showed the greatest increase in soil nutrient content and respiration. The N12P12 treatment increased overall enzyme activity and total phospholipid fatty acid (PLFA) content by 70.93%. Soil N and P nutrient contents dominated microbial community structure and corresponding changes in hydrolytic enzymes. Microbial biomass, respiration, and overall enzyme activity were driven by mineralized N. Our study provides a theoretical basis for exploring energy conversion processes of soil microbial communities and environmental sustainability under long-term N and P additions in semi-arid loess areas.

Keywords: nitrogen and phosphorus additions; microbial community structure; farmland ecosystem; nitrogen mineralization; soil enzyme activity

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*Corresponding author: LI Chunyue (E-mail: chunyue_{li}@snnu.edu.cn)

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1 Introduction

Soil microorganisms are major components of terrestrial ecosystems that play important roles in regulating organic matter decomposition and plant growth (Palansooriya et al., 2019). Microbes mediate nutrient migration in soil and respond to nutrient inputs through changes in community structure and biomass. Studying the functions and responses of soil microbial communities to nutrient changes can help predict ecosystem responses to natural and human-induced global environmental changes (Chen et al., 2019). Over the past decades, human activities have released large amounts of nitrogen (N) and phosphorus (P) into terrestrial ecosystems (Galloway et al., 2008; Wang et al., 2015), profoundly affecting soil microbial community functions and geochemical cycles (He et al., 2016).

Although N enrichment can increase N availability and alleviate N limitation in ecosystems, it can also cause soil acidification and alter microbial community structure (Widdig et al., 2020; Siegenthaler et al., 2022), affecting soil carbon (C) and N cycles (Nannipieri et al., 2018). Studies have shown that long-term N addition in farmlands (Liu et al., 2018), grasslands (Cruz et al., 2009), and forest ecosystems (He et al., 2021) changes soil bacterial and fungal communities by altering soil physicochemical properties (pH, ammonium, and nitrate N)

(Niu et al., 2021). Scholars have suggested that changes in microbial community structure relate to soil chemical properties (Zhang et al., 2022). Zhang et al. (2019) demonstrated that in N-limited semi-arid grasslands, reasonable N addition could enhance microbial biomass and respiration, though this effect was inhibited at N saturation. Changes in microbial activity and community structure can further alter soil extracellular enzyme activity, which drives soil organic matter decomposition and nutrient cycling (Chen et al., 2019). Based on the resource allocation theory of enzyme production (Allison and Vitousek, 2005), N addition can inhibit N-cycle enzyme activity while enhancing hydrolase activity involved in C and P cycles. However, effects of nutrient addition on soil enzyme activity cannot be explained solely by resource allocation theory, as enzyme activity is also closely related to factors such as microbial biomass (Wang et al., 2020).

Most recent studies have focused on soil microbial responses to N addition in forest and grassland ecosystems, whereas few have examined microbial community structure and function in farmland ecosystems under P addition and its interaction with N in ecologically fragile loess areas. N and P inputs are often not synchronized, and unbalanced N:P ratios lead to changes in microbial community structure and function (microbial communities are homeostatic, maintaining C:N:P ratios within a narrow range) (Schleuss et al., 2019), highlighting the urgent need to study independent and interactive effects of N and P additions on farmland ecosystems. P fertilization has no noticeable effects on microbial community composition and load in agricultural soils (Shi et al., 2012, 2013), but increases microbial biomass and fungal:bacterial (F:B) ratio in tropical forests (Li et al., 2015), while significantly reducing fungal abundance and altering fungal community composition in alpine meadow soils (He et al., 2016). Thus, P addition effects on soil microbial communities vary with climate and ecosystem. Interestingly, Liu et al. (2021) found that P addition alone had no effect on soil respiration in grasslands, but amplified respiration when N was added, with both nutrients jointly affecting soil enzyme patterns through soil eutrophication pathways.

The Loess Plateau is an important dry-farming area in northwestern China, but fragile ecological conditions, arid climate, and uneven precipitation distribution are primary obstacles to agricultural development. The Chinese government has implemented policies addressing environmental issues, such as converting natural grasslands and croplands to forest or afforested lands (Wu et al., 2019). Vegetation restoration has significantly increased surface soil microbial biomass in the loess arid region (determined by phospholipid fatty acid (PLFA)), maintaining F:B ratio due to similar increases in bacterial and fungal communities (Cai et al., 2022). Bacterial communities dominate overall soil microbial populations, while their composition proves more sensitive to soil pH variations than fungal communities (Zeng et al., 2021).

Preservation and restoration of land productivity are influenced not only by direct and indirect involvement of soil microorganisms in material and energy flow but also by nutrient input interventions (Han et al., 2021). Reasonable fertilizer application is important for achieving high crop yield and efficiency (Kracmarova et al., 2020). To increase crop production and agricultural income, local farmers use large amounts of chemical fertilizers, especially N and P fertilizers, far exceeding crop needs. Irrational fertilizer application limits crop yield, leads to soil pollution and water body eutrophication, and damages the ecological environment (Krauss et al., 2020). Enhancing field management practices and optimizing soil nutrient levels and microbial communities pose significant challenges in improving arable land utilization (Han et al., 2021). Therefore, determining effects of different N:P ratios on soil biological traits is important for efficient agricultural soil use in semi-arid areas.

In this study, we investigated changes in soil microbial community structure and ecological processes mediated by N and P additions over 36 years, further exploring driving mechanisms between microbial community structure and ecological processes. We also elucidated direct and indirect effects of nutrient addition on microbial communities and ecological processes. Based on previous findings, we hypothesized that: (1) N and P addition would induce alterations in soil microbial community structure, possibly attributable to changes in the nutritional environment for soil microorganisms; and (2) N addition alone affects soil ecological processes, including soil respiration, N mineralization, and enzyme activity, while interactive addition with P alleviates or amplifies this effect.

2.1 Study area and experimental design

The experimental soil was obtained from the Changwu Ecological Experiment Station of the Chinese Academy of Sciences (35°14 N, 107°41 E), located in the central-southern Loess Plateau, China. The station is situated at 1200 m a.s.l. with flat terrain and a warm temperate semi-humid continental monsoon climate, characterized by rainy summers and autumns and dry winters and springs, with an average annual precipitation of 580 mm and a frost-free period of 171 days. It represents a typical dry-farming area. The regional soil is black loam, with winter wheat as the main crop.

The long-term fertilization trial began in 1984 with eighteen treatments and three replications per treatment in a randomized block design, with plots arranged in three rows. Each trial plot measured 4 m × 8 m with a 1-m buffer zone. In this study, we selected nine treatments representing high, medium, and low interactions of N and P for sampling. The nine treatments were: CK (control, no fertilizer); N12 (N fertilizer at 90 kg/hm^2); N24 (N fertilizer at 180 kg/hm^2); P12 (P fertilizer at 90 kg/hm^2); N12P12 (N and P fertilizer at 90 and 90 kg/hm^2, respectively); N24P12 (N and P fertilizer at 180 and 90 kg/hm^2, respectively); P24 (P fertilizer at 180 kg/hm^2); N12P24 (N and P fertilizer at 90 and 180 kg/hm^2, respectively); and N24P24 (N and P fertilizer at 180 and 180 kg/hm^2, respectively). N and P fertilizers used were urea (46.4% N) and superphosphate (46.0% P_2O_5), respectively.

2.2 Sample collection

Soil samples were collected in May 2021 using five-point sampling across nine treatments with three replications each. After collection, samples were transported to the laboratory where plant residues and fallen roots were removed. Subsequently, samples were passed through a 2-mm sieve and air-dried.

2.3 Soil physical-chemical analysis

Soil clay content was measured with a Mastersizer 3000 laser analyzer (Malvern Instruments Ltd., Malvern, UK), with data output conforming to the Kaczynski soil texture classification standard. Soil moisture content (SMC) was determined using the oven-drying method. Soil organic matter (SOM) content was analyzed using the potassium dichromate external heating method. Soil pH was measured using a PHS-3C acidity meter. Soil total carbon (TC) and soil organic carbon (SOC) contents were analyzed using an elemental analyzer (Vario TOC, Elementar, Hanau, Germany). Soil total phosphorus (TP) was determined using the concentrated sulfuric acid-perchloric acid digestion molybdenum-antimony anticolorimetric method. Soil available phosphorus (AP) was determined using the sodium bicarbonate leaching molybdenum antimony resistance colorimetric method. Soil organic phosphorus (SOP) was determined by the high-temperature burning method. Soil total nitrogen (TN) content was determined using the Kjeldahl method. Soil nitrate nitrogen (NO_3^--N) and ammonium nitrogen (NH_4^+-N) were extracted with 0.5 mol/L K_2SO_4 solution after leaching, then determined by a continuous flow analyzer (Autoanalyzer 3, Bran-Luebbe, Hamburg, Germany). Experimental procedures followed Bao (2000).

2.4 Soil microbial community and enzymatic activity analysis

Soil microbial community structure was determined using the dipotassium hydrogen phosphate-trichloromethane method (Bunemann et al., 2004; Grayston et al., 2004). Each fatty acid component was identified using a gas chromatograph equipped with MIDI software. Bacteria were characterized using PLFAs i14:0, i15:0, a15:0, 15:0, i16:0, 10Me16:0, i17:0, a17:0, cy17:0, 17:0, br18:0, 10Me17:0, 18:1\$ $7, 10Me18 : 0, andcy19 : 0; fungiusingPLFAs18 : 2\ 6and18 : 2\ 9(BaathandAnderson, 2003); andactinomycetesusingPLFAs10Me17 : 1\ 7c, 10Me18 : 0, and10Me16 : 0(Zelles, 1997), respectively.PLFAsi14 : 0, i15 : 0, a15 : 0, i16 : 0, i17 : 0, anda17 : 0servedasindicatorsofGram - positivebacteria(GP); and16 : 1\ 7c, cy17 : 0, 18 : 1\ 7c, andcy19 : 0asindicatorsofGram - negativebacteria(GN)(Faninetal., 2019).Anaerobeswerecharacterizedusing14 : 0DMA, 15 : 0DMA, 16 : 0DMA, i15 : 0DMA, 16 : 1$ \$9c DAM, and cy19:0 DMA (Bossio et al., 1998).

Soil enzyme activities were analyzed using the microplate fluorometric method (Saiya-Cork et al., 2002). Umbelliferone was used as a substrate for β-1,4-glucosidase (BG), β-N-acetylglucosaminidase (NAG), and alkaline phosphatase

(AKP) activity. L-dihydroxyphenylalanine was used as a substrate for peroxidase (POD) and polyphenol oxidase (PPO) activity.

2.5 Soil microbial biomass, soil respiration, and mineralized nitrogen analysis

Soil microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were measured using the chloroform fumigation-potassium sulfate extraction method (Saiya-Cork et al., 2002). Soil respiration was determined using the alkali absorption titration method (He et al., 2022). Twenty-five grams of soil sample were placed at the bottom of a 250-mL glass tissue culture bottle containing a small glass bottle with 5 mL of 0.5 mol/L NaOH. The flasks were incubated at 25°C for 7 days. After CO_2 absorption by NaOH, the small beaker was removed, and 5 mL of 0.5 mol/L $BaCl_2$ was immediately added. The beaker was sealed and titrated with 0.125 mol/L hydrochloric acid. After continuous incubation for 28 days, soil NO_3^--N and NH_4^+-N contents before and after incubation were measured by K_2SO_4 leaching-flow analyzer, and soil nitrogen mineralization was calculated.

2.6 Statistical analysis

Soil respiration rate was determined by Equation 1:

$$R_t = \frac{(V_0 - V_t) \times \rho \times 44}{m \times t}$$

where R_t is the soil respiration rate (mg/(kg·d)); t is time (d); V_0 is the amount of hydrochloric acid used in the titration blank (mL); V_t is the amount of hydrochloric acid used in the titration (mL); ρ is the hydrochloric acid concentration (mol/L); and m is the weighed soil mass (g).

Respiratory entropy = R_t/MBC. Data are expressed as mean ± standard deviation of three parallel sample determinations and histograms were drawn using Origin 2017. Statistical analysis was performed using SPSS v.25.0, and one-way analysis of variance (ANOVA) and least significant difference (LSD) methods were used for comparisons among soil samples with a significance level of 0.05. Redundancy analysis (RDA) was performed using CANOCO v.5.0. Additionally, Amos Graphics software was used to construct structural equation models (SEM) to investigate relationships between soil ecological processes following N and P additions.

3.1 Changes of soil physical-chemical properties

There was no significant difference in soil particle size composition among treatments (Table 1). Compared with CK, high N addition treatments (N24, N24P12, and N24P24) significantly reduced soil pH. Soil TP content in P addition treatments (P and NP) was significantly higher than other treatments and showed positive correlation with P addition concentration. The overall change in soil AP content was consistent with TP change. N addition significantly increased soil NO_3^--N, NH_4^+-N, SMC, and SOM content. The N24P12 treatment increased these parameters by 16.47%, 55.17%, 10.43%, and 22.54%, respectively, compared with CK, and were significantly higher than other treatments.

3.2.1 Soil microbial community structure

PLFA content of soil microorganisms after long-term nutrient addition is shown in Table 2. Total soil PLFA was lower in N input treatments than in CK, and higher in P addition treatments than in CK, though differences were not significant. Total soil PLFA was significantly higher in combined N and P treatments than other treatments ($P<0.05$), with N12P12, N24P12, N12P24, and N24P24 treatments exhibiting 70.93%, 30.32%, 41.86%, and 42.23% higher PLFA contents than CK, respectively. Bacterial changes across treatments were consistent with total PLFA trends. Soil actinomycete contents in N24P12 and N24P24 treatments were significantly higher than other treatments, with 24.50% and 34.90% higher content than CK, respectively. N12 treatment had the lowest actinomycete content. Soil anaerobic bacteria levels were significantly higher in N12P24 and N24P24 treatments, with 103.85% and 50.00% increases compared with CK, while other treatments showed no significant differences from CK.

3.2.2 Soil microbial biomass

Results of soil microbial biomass analysis after long-term nutrient addition are shown in Figure 1 [Figure 1: see original paper]. Soil MBC content after fertilization was lower than CK. The highest MBC content was 415.32 mg/kg in P12 treatment and the lowest was 125.52 mg/kg in N24 treatment (Fig. 1a). Soil MBN content was significantly higher in combined N and P treatments than other treatments, with N24P12 showing the highest MBN content, followed by N24P24 (increased by 55.11% and 46.42%, respectively, compared with CK). Soil MBN content in N fertilizer treatments was significantly lower than CK, with N12 and N24 treatments decreasing by 44.72% and 71.81%, respectively (Fig. 1b).

3.3.1 Soil respiration and N mineralization

Long-term nutrient addition had a positive effect on soil respiration levels (Fig. 2 [Figure 2: see original paper]a and b). Soil respiration rate was higher in combined N and P treatments compared with single N or P treatments. Fertilization increased soil microbial respiration entropy to varying degrees, which was significantly lower in CK than other treatments ($P<0.05$), with the highest value of 0.37/d in N24 treatment. Accumulation of N mineralization in P-fertilized soils

(Fig. 2c) was significantly higher than CK, increasing by 10.21% and 16.89% in P12 and P24 treatments, respectively. In contrast, N fertilizer application had a significant inhibitory effect on N mineralization, which decreased with increasing N treatment, declining by 22.04% and 53.45% in N12 and N24 treatments, respectively. N mineralization levels in combined N and P treatments were lower than CK.

3.3.2 Soil enzyme activity

As shown in Figure 3 [Figure 3: see original paper], BG activity was significantly increased by P addition, with activity increasing by 111.08% and 59.63% in P12 and P24 treatments, respectively, compared with CK (Fig. 3a). N24 treatment had the lowest activity (0.10 nmol/(g · h)). NAG activity was highest in N24P12 treatment (5.54 nmol/(g · h)), followed by N12P24 treatment (4.30 nmol/(g · h)), and lowest in N24 treatment (46.48% reduction compared with CK, Fig. 3b). Under combined N and P treatments, AKP activity decreased with increasing fertilizer concentration. The highest activity occurred in N12P12 treatment (42.83 nmol/(g · h)), and the lowest in N24P24 treatment (11.75 nmol/(g · h)) (Fig. 3c).

N12 and N12P12 treatments significantly increased activities of two soil oxidases, PPO and POD, by 49.13% and 98.84%, and 44.21% and 47.37%, respectively, compared with CK. PPO activity was lowest (0.46 mol/(g · h)) in N12P24 treatment (Fig. 3d). POD activity in N24P12 treatment was significantly lower (38.95%) than CK, while differences in other treatments were not significant (Fig. 3e).

3.4.1 Correlation analysis

RDA based on relationships between soil environmental factors and microbial PLFA content with different N and P additions (Fig. 4a [Figure 4: see original paper]) showed that soil physicochemical properties could explain 68.68% of changes in microbial community structure. SMC, TP, and TN were the most important factors, explaining 33.30%, 16.00%, and 6.20% of variation, respectively. Bacteria, fungi, Actinobacteria, GP bacteria, anaerobes, and F:B ratio were positively correlated with SOM, SMC, TP, AP, and TN, and negatively correlated with pH. RDA using five enzyme activities as response variables and soil environmental factors as explanatory variables (Fig. 4b) revealed that the two axes together explained 29.02% of variation in soil enzyme activity, with TN being the most explanatory environmental factor (14.20%). NAG was positively correlated with SOM, SMC, TN, and NO_3^--N, and negatively correlated with AP, TP, and pH. BG and AKP were positively correlated with TP, AP, and pH, and negatively correlated with TN and NO_3^--N.

3.4.2 Principal component analysis (PCA)

PCA of soil microbial PLFA showed (Fig. 5a [Figure 5: see original paper]) that soil microbial community structure differed among treatments, with PC1 and PC2 axes explaining 70.1% and 13.9% of differences, respectively. Points corresponding to N and P additions and CK treatment projected in the negative direction of PC1, indicating similar microbial community structure among these three treatments. Points corresponding to four combined N and P treatments projected in the positive direction of PC1, showing positive correlation and indicating that combined N and P additions had greater impact on microbial community structure. PCA of soil enzyme activity (Fig. 5b) revealed that P12, P24, and N24P24 treatments were close to CK, indicating these three treatments did not significantly affect overall soil enzyme activity, while N fertilizer, N12P12, and N24P12 treatments had greater effects.

3.5.1 Environmental factors driving changes in microbial community structure

RDA demonstrated that soil physicochemical properties (SMC, TP, and TN) dominated changes in microbial community structure (Fig. 4a). Therefore, we further quantified the effect of N and P additions on microbial community structure by altering soil nutrient content using a structural equation model (SEM; Fig. 6 [Figure 6: see original paper]). The model indicated that soil TN and TP contents together explained 54.0% of total variation in microbial community structure. Additionally, soil respiration was directly affected by N and P additions and microbial community changes, which together explained 84.0% of total variation in soil respiration.

3.5.2 Links between ecological processes

SEM examining effects of N and P additions on microbially mediated ecological processes showed (Fig. 7 [Figure 7: see original paper]) that N and P additions along with microbial biomass directly contributed to soil respiration rate, with these three factors together explaining 91.0% of total variation in soil respiration. N and P additions together explained 75.0% of accumulated soil N mineralization, whereas N addition indirectly affected microbial biomass and soil enzyme activity by inhibiting N mineralization.

4.1 Changes in microbial community structure

Microbial biomass and community structure play critical roles in soil fertility and stability. We found that N and P fertilizer application did not significantly affect total PLFA content (Table 2; Fig. 5a). Meta-analysis revealed a lack of total PLFA response to N addition (Zhu et al., 2016). Positive effects on microbial growth from increased litter input and N supply may be offset by negative effects of soil acidification and metal toxicity (Treseder, 2008; Tian and Niu, 2015), resulting in unchanged total PLFA.

Soil pH is a key factor altering microbial community structure, as long-term N application leads to soil acidification that negatively affects bacterial and fungal contents (Dai et al., 2018; Zhang et al., 2018; Wang et al., 2020). In this study, low N fertilizer application had no effect on soil pH but significantly reduced bacterial and fungal contents, whereas high N fertilizer significantly reduced soil pH but did not change bacterial and fungal contents (Tables 1 and 2). The impact of N-induced soil acidification depends on fertilizer application intensity, soil buffering capacity, land use history (Ma et al., 2023), and potential soil-specific responses of bacterial and fungal communities to fertilizer application (Liu et al., 2018). Consequently, unlike previous studies, alterations in microbial community structure observed here may be associated with environmental factors other than pH. RDA (Fig. 4b) and SEM (Fig. 6) showed that under nutrient addition conditions, soil TP and TN contents jointly dominated changes in microbial community structure, indicating a close relationship between soil nutrient pools and microorganisms, consistent with our first hypothesis.

Compared with N addition alone, total PLFA, bacterial, and fungal contents significantly increased when combined with P addition (Table 2; Fig. 5a), indicating a significant positive interaction effect of N and P, consistent with previous studies (Guo et al., 2017). Total PLFA, bacteria, fungi, actinomycetes, and GN bacteria were significantly higher in soils with long-term combined N and P application than in soils with N fertilizer alone. Increased levels of bacteria, fungi, and anaerobic bacteria may be due to P input increasing soil AP content and alleviating strict P limitation in the regional ecosystem, thus indirectly affecting microbial activity (Wang et al., 2022), while increased nutrients available to microorganisms after N and P fertilization directly promoted microbial growth and reproduction (Wang et al., 2017). We conclude that the soil microbial community in our study area was co-limited by N and P. When only P or N was added, limitations of other nutrients remained despite increased availability of the added element. However, when co-applied, the restrictive effects of both P and N were simultaneously alleviated, thereby stimulating microbial growth.

4.2 Changes in soil biotic and abiotic parameters

Our study showed that long-term N addition decreased soil TP and AP contents (Table 1), attributable to accelerated P demand in crops (Fisk et al., 2014). With crop rotation, soil P remains in deficit, decreasing its activation capacity (Wang et al., 2018). Moreover, N significantly reduced soil MBC and MBN contents (Fig. 1) and mineralized N accumulation (Fig. 2c) due to N-induced loss of associated basic cations and C limitation, inhibiting potential N saturation of β-glucosidase activity and reducing microbial C acquisition and biomass. When soil available N is sufficiently abundant, microorganisms no longer mineralize organic matter to obtain available N resources (Wang et al., 2020). P availability affects N mineralization by mediating microbial activity

(Bicharanloo et al., 2022), and our study revealed that P addition significantly increased N mineralization accumulation. The balance between mineralization and immobilization accelerates soil N mineralization (Wang et al., 2022).

Consistent with our second hypothesis, there was no significant difference in soil N mineralization among four combined N and P treatments, but all were lower than CK and higher than N treatments (Fig. 2c), probably because soil inorganic N content was saturated, resulting in greater N inhibition than P promotion.

N addition does not always negatively impact soil ecological functions. We found that N fertilizer treatment increased PPO and POD activities (Fig. 3d and e), explained by the "resource allocation theory" (Allison and Vitousek, 2005). This theory predicts that elevated N availability increases microbial demand for C, inducing production of C-acquiring enzymes (Huang et al., 2018). Changes in soil physicochemical properties drove changes in enzyme activities under N and P additions, such as pH and total available N and P contents (Fig. 4b). These findings suggest that soil nutrients stimulate enzyme synthesis to break down organic compounds, and soil microbial community (bacterial, fungal, and actinomycete PLFA content) increases in response to improved soil physical structure and chemical properties, promoting microbial enzyme synthesis capacity (Cui et al., 2019).

Ecological effects of N and P additions are not always opposing, primarily manifesting as synergistic effects on soil respiration (Fig. 2a). N enrichment stimulates plant growth by increasing substrate input into soil and promoting soil respiration (Chen et al., 2017). Soil AP significantly increases respiration by reducing microbial nutrient limitation and stimulating root growth (Lu et al., 2022), whereas P addition directly increases soil P availability. Significantly, combined N and P treatment altered the magnitude of individual N or P effects on soil respiration, confirming our third hypothesis that nutrient interaction enhances positive effects on soil respiration. The influence of combined N and P treatment on respiration response is partially contingent upon P. P addition mitigates inhibitory effects of litter-fall accumulation on soil respiration while concurrently enhancing leaf N content and promoting N use efficiency through improved soil N availability (Liu et al., 2021). These dual strategies amplify positive effects of N addition on soil respiration.

4.3 Relationships between soil ecological processes

Soil is an important natural reservoir of C and N, and soil ecological function is a key process in material cycling and energy exchange in terrestrial ecosystems. Forest soil N mineralization is known to be influenced by several factors, individually or interactively, including microbial activity, extracellular enzymes, and soil C:N ratio (Gao et al., 2015). However, our study reveals a previously overlooked aspect: mineralized N also plays a regulatory role in soil microbial and enzymatic activities. Our findings indicate that N mineralization significantly affects soil microbial activity (Fig. 6), including microbial biomass, respiration, and enzyme activity. Unlike forest ecosystems, soil heterotrophs in artificially cultivated environments face limited nutrient acquisition opportunities due to crop competition and diminished C metabolic efficiency (Kaye and Hart, 1997). Consequently, greater amounts of SOM must decompose to meet organism nutrient demands. N mineralization particularly influences soil C:N ratio, consequently impacting microbial nutrient uptake capabilities. Soil MBN content reflects microbial effects on N mineralization and fixation. Any factors affecting these processes change MBN content, thereby altering microbial biomass and enzyme synthesis for N consumption. Therefore, N mineralization level affects overall soil enzyme activity. Yan et al. (2021) discovered that excessive N input negatively affected soil microbial communities and biomass in agricultural fields, forming undegradable compounds that reduce microbial mineralization activity and inhibit BG activity. This explains negative effects of N addition on soil microbe-mediated ecological processes in our study (Figs. 1, 2c, and 3a) and demonstrates that the study area is susceptible to P limitation.

Microbial biomass, enzyme activity, and soil respiration varied with long-term nutrient additions. Previous studies found that N and P additions did not affect microbial community structure and elemental cycling (Jing et al., 2020; Ma et al., 2020). This difference may be due to multiple factors. Soil microorganisms may face distinct resource limitations based on ecosystem type. Forest ecosystems typically exhibit high N levels, while C and P are often limiting (Li et al., 2015). Furthermore, nutrient addition duration is also important for soil ecological function changes. Compared with short-term nutrient addition (Ma et al., 2020), the 36-year duration in this study represents a key innovation. However, soil microbial community structure and function may vary by season and soil depth, requiring sampling at different time points and depths to fully reveal microbial responses to nutrient addition in the semi-arid loess area.

5 Conclusions

Long-term N and P additions significantly increased total PLFA, bacterial and fungal PLFA contents, altered microbial community structure, and increased soil respiration rate and microbial biomass in loess area agricultural soils. N addition negatively affected soil microbial biomass, BG activity, and mineralized N accumulation, while P addition mitigated or modified these negative effects. Soil microbial community structure and ecological processes are driven by different environmental mechanisms: soil nutrient pool content (TN and TP) dominates changes in microbial community structure, thereby affecting soil respiration, while changes in soil enzyme activity are mediated by available nutrients (NO_3^--N and AP) and N mineralization. In conclusion, P addition and combined N and P addition positively impacted soil microbial characteristics, ecological functions, and soil productivity. Our study provides a scientific basis enabling local farmers to choose appropriate fertilizer application programs, thereby positively affecting sustainable environmental development on the Loess

Plateau.

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References

Allison S D, Vitousek P M. 2005. Responses of extracellular enzymes to simple and complex nutrient inputs. *Soil Biology & Biochemistry*, 37(5): 937-944.

Baath E, Anderson T H. 2003. Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology & Biochemistry*, 35(7): 955-963.

Bao S D. 2000. *Soil and Agricultural Chemistry Analysis* (3rd ed.). Beijing: China Agriculture Press, 20-97. (in Chinese)

Bicharanloo B, Shirvan M B, Keitel C, et al. 2022. Nitrogen and phosphorus availability have stronger effects on gross and net nitrogen mineralisation than wheat rhizodeposition. *Geoderma*, 405: 115440, doi: 10.1016/j.geoderma.2021.115440.

Bossio D A, Scow K M, Gunapala N, et al. 1998. Determinants of soil microbial communities: Effects of agricultural management, season, and soil type on phospholipid fatty acid profiles. *Microbial Ecology*, 36(1): 1-12.

Bunemann E K, Bossio D A, Smithson P C, et al. 2004. Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. *Soil Biology & Biochemistry*, 36(6): 889-901.

Cai X W, Zhang D, Wang Y Q, et al. 2022. Shift in soil microbial communities along similar to 160 years of natural vegetation restoration on the Loess Plateau of China. *Applied Soil Ecology*, 173: 104394, doi: 10.1016/j.apsoil.2022.104394.

Chen J, Luo Y, Li J, et al. 2017. Costimulation of soil glycosidase activity and soil respiration by nitrogen addition. *Global Change Biology*, 23(3): 1328-1337.

Chen X, Hao B H, Jing X, et al. 2019. Minor responses of soil microbial biomass, community structure and enzyme activities to nitrogen and phosphorus addition in three grassland ecosystems. *Plant and Soil*, 444(1-2): 21-37.

Cruz A F, Hamel C, Hanson K, et al. 2009. Thirty-seven years of soil nitrogen and phosphorus fertility management shapes the structure and function of the soil microbial community in a brown Chernozem. *Plant and Soil*, 315(1-2): 173-184.

Cui Y X, Fang L C, Guo X B, et al. 2019. Responses of soil bacterial communities, enzyme activities, and nutrients to agricultural-to-natural ecosystem conversion in the Loess Plateau, China. *Journal of Soils and Sediments*, 19(3): 1427-1440.

Dai Z M, Su W Q, Chen H H, et al. 2018. Long-term nitrogen fertilization decreases bacterial diversity and favors the growth of Actinobacteria and Proteobacteria in agro-ecosystems across the globe. *Global Change Biology*, 24(8): 3452-3461.

Fanin N, Kardol P, Farrell M, et al. 2019. The ratio of Gram-positive to Gram-negative bacterial PLFA markers as an indicator of carbon availability in organic soils. *Soil Biology & Biochemistry*, 128: 111-114.

Fisk M C, Ratliff T J, Goswami S, et al. 2014. Synergistic soil response to nitrogen plus phosphorus fertilization in hardwood forests. *Biogeochemistry*, 118(1-3): 195-204.

Galloway J N, Townsend A R, Erisman J W, et al. 2008. Transformation of the nitrogen cycle: Recent trends, questions, and potential solutions. *Science*, 320(5878): 889-892.

Gao W L, Yang H, Kou L, et al. 2015. Effects of nitrogen deposition and fertilization on N transformations in forest soils: A review. *Journal of Soils and Sediments*, 15(4): 863-879.

Grayston S J, Campbell C D, Bardgett R D, et al. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA and community DNA techniques. *Applied Soil Ecology*, 25(1): 63-84.

Guo Y, Sun B H, Wang Y, et al. 2017. PLFA fingerprint characteristics of an anthropogenic loess soil under long-term different fertilizations. *Scientia Agricultura Sinica*, 50(1): 94-103. (in Chinese)

Han J Q, Dong Y Y, Zhang M. 2021. Chemical fertilizer reduction with organic fertilizer effectively improve soil fertility and microbial community from newly cultivated land in the Loess Plateau of China. *Applied Soil Ecology*, 165: 103966, doi: 10.1016/j.apsoil.2021.103966.

He D, Xiang X J, He J S, et al. 2016. Composition of the soil fungal community is more sensitive to phosphorus than nitrogen addition in the alpine meadow on the Qinghai-Tibetan Plateau. *Biology and Fertility of Soils*, 52(8): 1059–1072.

He W, Ye W H, Sun M J, et al. 2022. Soil phosphorus availability and stoichiometry determine microbial activity and functional diversity of fluvo-aquic soils under long-term fertilization regimes. *Journal of Soils and Sediments*, 22(4): 1214–1227.

He W Y, Zhang M M, Jin G Z, et al. 2021. Effects of nitrogen deposition on nitrogen-mineralizing enzyme activity and soil microbial community structure in a Korean pine plantation. *Microbial Ecology*, 81(2): 410–424.

Huang J S, Chen W J, Qi K B, et al. 2018. Distinct effects of N and P addition on soil enzyme activities and C distribution in aggregates in a subalpine spruce plantation. *Biogeochemistry*, 141(2): 199–212.

Jing X, Chen X, Fang J Y, et al. 2020. Soil microbial carbon and nutrient constraints are driven more by climate and soil physicochemical properties than by nutrient addition in forest ecosystems. *Soil Biology & Biochemistry*, 141: 107657, doi: 10.1016/j.soilbio.2019.107657.

Kaye J P, Hart S C. 1997. Competition for nitrogen between plants and soil microorganisms. *Trends in Ecology & Evolution*, 12(4): 139–143.

Kracmarova M, Kratochvilova H, Uhlik O, et al. 2020. Response of soil microbes and soil enzymatic activity to 20 years of fertilization. *Agronomy-Basel*, 10(10): 1542, doi: 10.3390/agronomy10101542.

Krauss M, Berner A, Perrochet F, et al. 2020. Enhanced soil quality with reduced tillage and solid manures in organic farming–a synthesis of 15 years. *Scientific Reports*, 10(1): 4403, doi: 10.1038/s41598-020-61320-8.

Li J, Li Z A, Wang F M, et al. 2015. Effects of nitrogen and phosphorus addition on soil microbial community in a secondary tropical forest of China. *Biology and Fertility of Soils*, 51(2): 207–215.

Liu J S, Zhang X, Wang H, et al. 2018. Long-term nitrogen fertilization impacts soil fungal and bacterial community structures in a dryland soil of Loess Plateau in China. *Journal of Soils and Sediments*, 18(4): 1632–1640.

Liu Y Z, Zhao C C, Guo J W, et al. 2021. Short-term phosphorus addition augments the effects of nitrogen addition on soil respiration in a typical steppe. *Science of the Total Environment*, 761: 143211, doi: 10.1016/j.scitotenv.2020.143211.

Lu X Y, Lu W, Sun H Y, et al. 2022. Responses of soil respiration to phosphorus addition in global grasslands: A meta-analysis. *Journal of Cleaner Production*, 349: 131413, doi: 10.1016/j.jclepro.2022.131413.

Ma S H, Chen G P, Tian D, et al. 2020. Effects of seven-year nitrogen and phosphorus additions on soil microbial community structures and residues in a tropical forest in Hainan Island, China. *Geoderma*, 361: 114034, doi: 10.1016/j.geoderma.2019.114034.

Ma X M, Zhou Z, Chen J, et al. 2023. Long-term nitrogen and phosphorus fertilization reveals that phosphorus limitation shapes the microbial community composition and functions in tropical montane forest soil. *Science of the Total Environment*, 854: 158709, doi: 10.1016/j.scitotenv.2022.158709.

Nannipieri P, Trasar-Cepeda C, Dick R P. 2018. Soil enzyme activity: A brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biology and Fertility of Soils*, 54(1): 11–19.

Niu G X, Hasi M, Wang R Z, et al. 2021. Soil microbial community responses to long-term nitrogen addition at different soil depths in a typical steppe. *Applied Soil Ecology*, 167: 104054, doi: 10.1016/j.apsoil.2021.104054.

Palansooriya K N, Wong J T F, Hashimoto Y, et al. 2019. Response of microbial communities to biochar-amended soils: A critical review. *Biochar*, 1(1): 3–22.

Saiya-Cork K R, Sinsabaugh R L, Zak D R. 2002. The effects of long term nitrogen deposition on extracellular enzyme activity in an *Acer saccharum* forest soil. *Soil Biology & Biochemistry*, 34(9): 1309–1315.

Schleuss P, Widdig M, Heintz-Buschart A, et al. 2019. Stoichiometric controls of soil carbon and nitrogen cycling after long-term nitrogen and phosphorus addition in a mesic grassland in South Africa. *Soil Biology & Biochemistry*, 135: 294–303.

Shi Y C, Lalande R, Ziadi N, et al. 2012. An assessment of the soil microbial status after 17 years of tillage and mineral P fertilization management. *Applied Soil Ecology*, 62: 14–23.

Shi Y C, Lalande R, Hamel C, et al. 2013. Seasonal variation of microbial biomass, activity, and community structure in soil under different tillage and phosphorus management practices. *Biology and Fertility of Soils*, 49(7): 803–818.

Siegenthaler M B, Ramoneda J, Frossard E, et al. 2022. Microbial community responses to phosphorus and nitrogen inputs in the organic soil horizons of two contrasting temperate beech forests. *Applied Soil Ecology*, 172: 104357, doi: 10.1016/j.apsoil.2021.104357.

Tian D S, Niu S L. 2015. A global analysis of soil acidification caused by nitrogen addition. *Environmental Research Letters*, 10(2): 024019, doi: 10.1088/1748-9326/10/2/024019.

Treseder K K. 2008. Nitrogen additions and microbial biomass: A meta-analysis of ecosystem studies. *Ecology Letters*, 11(10): 1111–1120.

Wang H Y, Nie Y, Butterly C R, et al. 2017. Fertilization alters microbial community composition and functional patterns by changing the chemical nature of soil organic carbon: A field study in a Halosol. *Geoderma*, 292(15): 17–24.

Wang R, Balkanski Y, Boucher O, et al. 2015. Significant contribution of combustion-related emissions to the atmospheric phosphorus budget. *Nature*

Geoscience, 8(1): 48-54.

Wang R Z, Bicharanloo B, Hou E Q, et al. 2022. Phosphorus supply increases nitrogen transformation rates and retention in soil: A global meta-analysis. *Earth's Future*, 10(3): e2021EF002479, doi: 10.1029/2021EF002479.

Wang S Y, Fan T L, Ding N P, et al. 2018. Change characteristics of soil phosphorus and phosphorus fertilizer efficiency in Black Loessial soil of dryland in the Loess Plateau under long-term fertilization. *Chinese Journal of Eco-Agriculture*, 26(7): 1038-1047. (in Chinese)

Wang Z R, Yang S, Wang R Z, et al. 2020. Compositional and functional responses of soil microbial communities to long-term nitrogen and phosphorus addition in a calcareous grassland. *Pedobiologia*, 78: 150612, doi: 10.1016/j.pedobi.2019.150612.

Widdig M, Heintz-Buschart A, Schleuss P M, et al. 2020. Effects of nitrogen and phosphorus addition on microbial community composition and element cycling in a grassland soil. *Soil Biology & Biochemistry*, 151: 108041, doi: 10.1016/j.soilbio.2020.108041.

Wu S H, Huang B H, Gao J, et al. 2019. The effects of afforestation on soil bacterial communities in temperate grassland are modulated by soil chemical properties. *Peerj*, 7: e6147, doi: 10.7717/peerj.6147.

Yan W M, Zhong Y Q W, Liu J, et al. 2021. Response of soil respiration to nitrogen fertilization: Evidence from a 6-year field study of croplands. *Geoderma*, 384: 114829, doi: 10.1016/j.geoderma.2020.114829.

Zelles L. 1997. Phospholipid fatty acid profiles in selected members of soil microbial communities. *Chemosphere*, 35(1-2): 275-294.

Zeng Q C, Liu D, An S S. 2021. Decoupled diversity patterns in microbial geographic distributions on the arid area (the Loess Plateau). *CATENA*, 196: 104922, doi: 10.1016/j.catena.2020.104922.

Zhang C, Song Z L, Zhuang D H, et al. 2019. Urea fertilization decreases soil bacterial diversity, but improves microbial biomass, respiration, and N-cycling potential in a semiarid grassland. *Biology and Fertility of Soils*, 55(3): 229-242.

Zhang J Y, Ai Z M, Liu H F, et al. 2022. Short-term N addition in a *Pinus tabuliformis* plantation: Microbial community composition and interactions show different linkages with ecological stoichiometry. *Applied Soil Ecology*, 174: 104422, doi: 10.1016/j.apsoil.2022.104422.

Zhang T, Chen H Y, Ruan H H. 2018. Global negative effects of nitrogen deposition on soil microbes. *Isme Journal*, 12(7): 1817-1825.

Zhu J X, Wang Q F, He N P, et al. 2016. Imbalanced atmospheric nitrogen and phosphorus depositions in China: Implications for nutrient limitation. *Journal of Geophysical Research-Biogeosciences*, 121(6): 1605-1616.