Stoichiometric Characteristics of Carbon, Nitrogen, and Phosphorus in Soil-Organic Matter-Microorganisms of Farmland Under Long-Term Fertilization: Postprint

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Abstract

Investigating the effects of exogenous nutrient input on the stoichiometric characteristics of carbon, nitrogen, and phosphorus within soil systems is of great significance for deeply understanding the cycling of soil organic carbon (C) and nutrients and their interaction processes in farmland. Based on a 26-year long-term located fertilization experiment in farmland, we analyzed the contents and stoichiometric characteristics of carbon, nitrogen, and phosphorus in soil, organic fractions, and microbial biomass under different long-term fertilization conditions, and calculated their stoichiometric homeostasis index H according to the homeostasis model y=cx1/H. The results showed that: compared with the long-term fallow treatment (CK0), the 26-year chemical fertilizer combined with organic fertilizer treatments (MNPK and 1.5MNPK) under crop cultivation conditions significantly reduced microbial biomass nitrogen content, but significantly increased microbial biomass phosphorus content. Compared with the fallow treatment, even the long-term chemical phosphorus fertilizer treatments (NP, PK, NPK) showed significant decreases in soil organic phosphorus. Regarding the C:N ratio, the soil C:N ratio, organic matter C:N ratio, and microbial biomass C:N ratio under chemical fertilizer combined with organic material treatments (straw or organic fertilizer) were all significantly lower than those under chemical fertilizer treatments (N, NP, PK, and NPK). Regarding the C:P ratio, compared with the fallow treatment, 26 years of phosphorus fertilizer application (chemical phosphorus or organic phosphorus) significantly reduced the soil C:P ratio and microbial biomass C:P ratio, while CK and unbalanced chemical fertilizer treatments (N, NP, and PK) significantly reduced the soil organic matter C:P ratio. Regarding the soil N:P ratio, the N:P ratio in the fallow treatment was significantly higher than in other treatments, and the soil organic matter N:P ratio in the fallow treatment was significantly higher than in CK and chemical fertilizer treatments, indicating that crop cultivation under no-fertilization or chemical fertilizer conditions exacerbated nitrogen consumption in soil organic matter. The homeostasis indices H of microbial biomass C:N, C:P, and N:P ratios were 0.24, 0.75, and 0.64, respectively, which do not exhibit homeostatic characteristics. Microbial biomass C:N, C:P, and N:P ratios showed significant positive correlations with soil C:N, C:P, and N:P ratios, respectively, but had no significant correlations with the stoichiometric ratios of carbon, nitrogen, and phosphorus in soil organic matter. This indicates that changes in soil carbon, nitrogen, and phosphorus elements can directly lead to changes in the stoichiometric ratios of microbial biomass carbon, nitrogen, and phosphorus, but the stoichiometric ratios of microbial biomass carbon, nitrogen, and phosphorus have no significant effect on the stoichiometric ratios of carbon, nitrogen, and phosphorus in soil organic matter, which may be more influenced by nutrient management practices such as cropping and fertilization.

Full Text

Preamble

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Carbon, Nitrogen, and Phosphorus Stoichiometry Characteristics of Bulk Soil, Organic Matter, and Soil Microbial Biomass Under Long-Term Fertilization in Cropland

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Abstract

Investigating the impact of exogenous nutrient inputs on the carbon (C), nitrogen (N), and phosphorus (P) stoichiometric ratios in agricultural soil systems is crucial for understanding nutrient cycling and interaction processes in agroecosystems. Using a 26-year long-term fertilization experiment as a platform, we analyzed the contents and stoichiometric characteristics of C, N, and P in bulk soil, soil organic matter, and microbial biomass under various fertilization treatments. The homeostasis index (H) was calculated according to the model y = c x^(1/H), where y represents consumer stoichiometry, x represents resource stoichiometry, c is a constant, and H is the regulation coefficient.

Results showed that, compared with the fallow treatment (CK0), long-term combined application of chemical fertilizer and manure (MNPK and 1.5MNPK) with cropping significantly decreased soil microbial biomass nitrogen but significantly increased microbial biomass phosphorus ($P < 0.05$). Even with long-term P fertilizer application (chemical or organic P), soil organic phosphorus decreased significantly. The C:N ratios in bulk soil, soil organic matter, and microbial biomass under organic amendment treatments (straw or manure) were significantly lower than those under chemical fertilizer treatments (N, NP, PK, NPK). Compared with CK0, P application (chemical P fertilizer or organic amendments) significantly reduced the C:P ratio in bulk soil and microbial biomass, but no fertilization (CK) and unbalanced fertilization treatments (N, NP, and PK) significantly decreased the C:P ratio of soil organic matter. The N:P ratio under CK0 was the highest among all treatments, and the N:P ratio of soil organic matter under CK0 was higher than that under CK and chemical fertilizer treatments, indicating N depletion in soil organic matter under cropping and chemical fertilization conditions.

The homeostatic regulation coefficients (H) for microbial biomass C:N, C:P, and N:P ratios were 0.24, 0.75, and 0.64, respectively, indicating no stoichiometric homeostasis. Microbial biomass C:N, C:P, and N:P ratios showed significant positive correlations with the corresponding ratios in bulk soil, but no significant relationships existed between soil organic matter and microbial biomass stoichiometric ratios. Our results indicate that changes in soil C, N, and P can directly affect microbial biomass stoichiometry, while soil organic matter stoichiometry is more likely influenced by crop cultivation and nutrient management practices rather than microbial biomass stoichiometry.

Keywords: stoichiometry; long-term fertilization; black soil; soil organic matter; microbial biomass; homeostasis

Introduction

Soil organic matter, including soil organic carbon (SOC), soil organic nitrogen (SON), and soil organic phosphorus (SOP), is a critical indicator of soil fertility. It participates in the decomposition of organic matter and humus formation, regulates soil energy balance and various enzyme activities, and drives nitrogen and phosphorus transformation processes. Soil microorganisms are the primary drivers of soil organic matter and nutrient cycling. The cycling of C, N, and P represents fundamental biogeochemical processes that are also essential for assessing soil fertility. In the context of intensifying global change, these processes involve extremely complex interactions.

Increasingly, researchers are applying ecological stoichiometry to investigate and evaluate the cycling characteristics and interactions of C, N, and P in soil, organic matter, and microorganisms. Ecological stoichiometry is the science studying the balance of energy and multiple chemical elements (primarily C, N, and P) in ecosystems and their effects on ecological interactions. Global analyses indicate that C:N:P ratios in surface soils (0–10 cm) are relatively stable, with average values ranging from 60:5:1 to 186:13:1, while soil humus C:N:P ratios average 134:9:1. Studies have shown that microbial biomass C:N:P ratios in global soils range from 42:6:1 to 60:7:1, with significant positive correlations between soil and microbial biomass C:N:P ratios.

Homeostasis refers to an organism' s ability to maintain constant internal chemical composition despite varying nutrient environments and is a prerequisite for ecological stoichiometry. Research on global soil and microbial biomass C:N:P data revealed that compared to natural ecosystems like forests, cropland soils exhibit the weakest microbial biomass homeostasis. Different fertilization conditions create varying nutrient input statuses, which can cause different degrees of nutrient limitation to soil microorganisms, thereby affecting soil ecosystem structure and function. To understand how nutrient inputs influence C:N:P stoichiometry in cropland soils, this study utilized a long-term fertilization experiment to analyze the stoichiometric characteristics of C, N, and P in soil, organic matter, and microbial biomass under different fertilization regimes, revealing key internal relationships to provide scientific guidance for nutrient management and sustainable soil utilization.

1. Site Description

Soil samples were collected from a long-term fertilization experimental site at the Jilin Academy of Agricultural Sciences, located in a temperate semi-humid region (124°48 33.9 E, 43°30 23 N). The area has an average annual temperature of 4–5°C, annual precipitation of 450–600 mm, annual sunshine hours of 2500–2700 h, and a frost-free period of 125–140 days. The soil is a medium-layer black soil (Mollisol) developed from Quaternary loess-like sediments. The long-term experiment was established in 1990 with continuous annual corn cropping. Initial topsoil properties included: SOC 22.8 g/kg, SON 1.40 g/kg, total phosphorus 1.39 g/kg, total potassium (K O) 22.1 g/kg, available phosphorus 27 mg/kg, and available potassium (K O) 190 mg/kg, with pH 6.3.

This study selected nine treatments: (1) Fallow (CK0); (2) No fertilizer (CK); (3) Nitrogen only (N); (4) Nitrogen and phosphorus (NP); (5) Phosphorus and potassium (PK); (6) Nitrogen, phosphorus, and potassium (NPK); (7) Low NPK plus manure (MNPK); (8) 1.5× manure rate (1.5MNPK); (9) NPK plus straw (SNPK). Fertilizer application rates are shown in .

2. Soil Sampling and Analysis

Soil samples were collected after corn harvest in 2016. Each plot was sampled with a soil auger at 0–20 cm depth, with multiple cores composited into one sample per plot. Samples were divided: one portion air-dried and ground to 0.25 mm and 0.15 mm for determination of soil and organic matter C, N, P; another portion stored at 4°C for microbial biomass analysis.

Soil organic carbon (SOC) was determined by potassium dichromate oxidation, total nitrogen (TN) by Kjeldahl digestion, and total phosphorus (TP) by NaOH fusion-molybdenum antimony colorimetry. Soil organic phosphorus (SOP) was measured on 0.15 mm soil: samples were ultrasonicated in 0.1 mol/L HCl (5:1 ratio) for 10 minutes to remove inorganic N, centrifuged (8000 r/min for 5 minutes), washed 2–3 times, dried, and analyzed by ignition-0.2 N H SO extraction. Soil organic nitrogen was determined on 0.15 mm soil after HCl treatment.

Soil microbial biomass carbon (SMBC) and nitrogen (SMBN) were measured by chloroform fumigation-extraction: 40 mL of 0.5 mol/L K SO was added to 20 g fresh soil (4:1 extract ratio), fumigated for 24 hours, then extracted for 30 minutes and filtered. Carbon and nitrogen in extracts were analyzed using a Multi N/C 3100 analyzer. Microbial biomass phosphorus (SMBP) was determined by chloroform fumigation-0.5 mol/L NaHCO extraction with recovery correction. Soil water content was determined gravimetrically.

3. Data Analysis

Stoichiometric ratios were calculated as mass ratios. The homeostasis index (H) was calculated using Sterner' s model: $y = c\ x\hat{}(1/H)$, where y represents consumer stoichiometry, x represents resource stoichiometry, and H is the homeostasis index. When $H = 1$, organisms show no homeostasis and composition varies directly with the environment ("You are what you eat"). When $H >$ 1 but finite, organisms exhibit partial homeostasis with composition changing proportionally with resources ("Constant Proportional"). When H is infinite, organisms maintain absolute homeostasis with constant composition regardless of environment ("Strict Homeostasis").

Data were analyzed using SPSS Statistics 19.0. One-way ANOVA was used to test treatment differences ($P < 0.05$). Pearson correlation analysis was performed, and figures were prepared using SigmaPlot 10.0.

2. Results and Analysis

2.1 Soil C, N, P Contents and Microbial Biomass Characteristics After Long-Term Fertilization

After 26 years, soil organic carbon content under manure treatments (MNPK and 1.5MNPK) was significantly higher than under fallow (CK0), no fertilizer (CK), and chemical fertilizer treatments (N, NP, PK, NPK), but significantly lower than under fallow ($P < 0.05$). The NPK treatment had the lowest organic carbon content (20.89 g/kg), indicating that crop cultivation and harvest accelerated organic carbon depletion. Total nitrogen showed similar patterns, with manure treatments having significantly higher TN than chemical fertilizer treatments but lower than fallow. Due to phosphorus not participating in atmospheric cycling, total phosphorus increased with P input. Fallow and no-P treatments had the lowest TP (0.17–0.19 g/kg). Manure treatments had significantly higher organic phosphorus (0.87-0.97 g/kg) than other treatments, while fallow organic P (0.56-0.64 g/kg) was significantly higher than chemical fertilizer treatments (0.22 g/kg).

For microbial biomass, long-term organic amendment treatments (straw or manure) and fallow had significantly higher SMBC (428.28-620.26 mg/kg) and SMBN (68.08-93.80 mg/kg) than cropped treatments. Combined organic-inorganic fertilization significantly reduced SMBN compared to fallow (93.80 mg/kg), even with organic N inputs, indicating long-term cropping significantly decreased microbial N. Manure treatments (MNPK, 1.5MNPK) had significantly higher SMBP than fallow and other treatments, while fallow SMBP (29.69 mg/kg) was significantly higher than no-P treatments.

2.2 Stoichiometric Ratios of Soil, Organic Matter, and Microbial Biomass

Soil C:N ratios under long-term organic amendment treatments (10.56-10.89) were significantly lower than under chemical fertilizer treatments (11.42-12.39) and showed no significant difference from fallow. Microbial biomass C:N ratios under no fertilizer and medium manure treatments (10.72-11.27) were significantly lower than chemical fertilizer treatments (11.40-12.41) but higher than organic amendment treatments (6.29-6.84) [Figure 2: see original paper].

Soil C:P ratios decreased with increasing P input. Fallow soil C:P ratio (32.95) was significantly higher than other treatments and lower than no-P treatments. Chemical P and manure treatments significantly reduced organic matter C:P ratios (17.09-18.26) compared to fallow, with high manure treatment (1.5MNPK) having the lowest ratio (9.05) [Figure 1: see original paper].

Microbial biomass C:P ratios under chemical P and manure treatments (20.89-21.59) were significantly lower than fallow (78.53-84.29), while N-only treatment significantly increased the ratio (94.05). Microbial biomass N:P ratios showed no significant differences among treatments (0.74-1.22) except straw treatment, which significantly increased the ratio [Figure 2: see original paper].

2.3 Homeostasis Characteristics of Soil Microbial Biomass

Differences in nutrient input under long-term fertilization affected microbial nutrient supply, altering microbial biomass C:N:P stoichiometry. The homeostasis indices (H) for microbial biomass C:N, C:P, and N:P ratios were 0.24, 0.75, and 0.64, respectively, indicating no stoichiometric homeostasis. As soil C:N, C:P, and N:P ratios increased by one unit, microbial biomass ratios increased by 3.11, 1.05, and 0.64 units, respectively, showing partial proportional changes with nutrient environment [Figure 4: see original paper].

2.4 Relationships Among Stoichiometric Ratios

For individual elements, significant positive correlations existed between SMBC, SMBN, and SMBP contents. For stoichiometric ratios, microbial biomass C:N, C:P, and N:P ratios showed significant positive correlations with corresponding ratios in bulk soil but no significant relationships with soil organic matter ratios. Soil organic matter stoichiometry showed no significant correlation with microbial biomass stoichiometry [TABLE:2, TABLE:3].

3. Discussion

Long-term chemical fertilizer treatments resulted in significantly higher soil C:N ratios than fallow and organic amendment treatments because fertilizer application increased crop yield, accelerating soil N depletion without external organic N supplementation. Organic amendments provided substantial organic N sources that met microbial N demands and promoted N accumulation, thereby reducing C:N ratios.

The large variation in soil C:P ratios and their decrease with P input reflects P' s unique characteristics: it does not cycle atmospherically, is easily immobilized in soil, and is not a structural component of humic acids. Low P fertilizer utilization efficiency leads to P accumulation with long-term application. No fertilizer and unbalanced chemical fertilizer treatments had significantly lower organic matter C:P ratios than fallow due to accelerated organic carbon depletion from continuous cropping.

Chemical P and manure treatments significantly reduced microbial biomass C:P ratios compared to fallow, likely because adequate P supports higher microbial growth rates. According to growth rate theory, high growth rates require increased allocation to P-rich ribosomes, reducing biomass C:P ratios. When P meets microbial demand, activity increases, enhancing carbon utilization efficiency. However, long-term N fertilization increases crop yield and biomass, reducing dissolved organic carbon availability for microbes, thereby decreasing microbial biomass C:P ratios.

The lack of homeostasis in microbial biomass stoichiometry ($H < 1$) indicates that microbial communities cannot maintain stable internal composition under changing nutrient environments. Long-term fertilization alters substrate stoichiometry, and microbial communities adjust their nutrient uptake and assimilation ratios physiologically. The significant positive correlation between soil and microbial biomass stoichiometry demonstrates that soil nutrient status directly influences microbial composition.

The absence of significant relationships between microbial biomass and organic matter stoichiometry may be because organic matter comprises complex compounds formed from decomposed plant/animal residues, with crop management and climate being dominant controlling factors rather than microbial processes alone. Over 90% of soil nitrogen exists as organic compounds, and high N losses through volatilization and leaching in croplands create large variation in N:P ratios.

4. Conclusion

Nutrient input and crop uptake significantly affected the C:N:P stoichiometry of soil, organic matter, and microbial biomass in agroecosystems. Compared to natural recovery systems, long-term fertilization and cropping significantly reduced microbial biomass nitrogen but increased microbial biomass phosphorus. Chemical fertilization accelerated soil nitrogen depletion, significantly increasing soil and organic matter C:N ratios while decreasing C:P ratios. The homeostasis indices for microbial biomass C:N, C:P, and N:P were all below 1, indicating no homeostasis. Changes in soil C:N:P directly altered microbial biomass stoichiometry, while organic matter stoichiometry was more influenced by crop management and nutrient input practices. Combined application of straw and manure played an important role in maintaining stoichiometric balance of C, N, and P in soil organic matter.

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Note: Figure translations are in progress. See original paper for figures.