Effects of Different Fertilization Treatments on Soil Microbial Characteristics in the Loess Hilly Region (Postprint)

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Abstract

This study aimed to investigate the effects and mechanisms of fertilization on soil microorganisms in nutrient-poor loess hilly regions. The experiment was conducted using long-term located fertilization plots at the Ansai Station, with treatments including CK (control), N (nitrogen fertilizer), P (phosphorus fertilizer), M (organic fertilizer), NP (nitrogen fertilizer + phosphorus fertilizer), MN (organic fertilizer + nitrogen fertilizer), MP (organic fertilizer + phosphorus fertilizer), and MNP (organic fertilizer + nitrogen fertilizer + phosphorus fertilizer), to study the impacts of long-term fertilization on soil microbial community structure and respiration. Soil microbial activity and PLFA content in the 0-20 cm tillage layer were higher than those in the 20-40 cm soil layer, with the tillage layer exhibiting 63.61%-116.78% higher basal respiration, 53.45%-137.64% higher induced respiration, and 16.16%-43.67% higher total PLFA content compared to the 20-40 cm layer. Single application of N and P enhanced soil respiration intensity, with basal respiration intensity in the 0-20 cm layer increasing by 34.11% and 48.89%, respectively, and induced respiration intensity increasing by 40.83% and 63.59%, respectively; in the 20-40 cm layer, basal respiration increased by 40.83% and 63.59%, respectively, while induced respiration increased by 14.70% and 20.49%, respectively. Single application of N significantly altered G- microbial communities, with PLFA content in the 0-20 cm and 20-40 cm layers increasing significantly by 63.19% and 53.07%, respectively; single application of P also significantly affected soil microbial community structure, whereas the effect of NP on microbial community structure was not significant. Combined application of organic and inorganic fertilizers significantly increased soil respiration and microbial PLFA content. Three-way ANOVA revealed that the single nitrogen fertilizer factor had no significant effect on soil microbial properties; the single phosphorus fertilizer factor had significant effects on microbial respiration intensity and some phospholipid fatty acid contents, with the phosphorus fertilizer factor accounting for 11.4%-54.0%

of the variation in these microbial properties in the tillage layer. RDA analysis indicated that soil available phosphorus was the primary factor influencing microbial properties in the loess hilly region. Long-term combined application of nitrogen, phosphorus, and organic fertilizers helps improve soil microbial properties, thereby enhancing the stability and health of farmland ecosystems.

Full Text

Effects of Long-Term Fertilization on Soil Microbial Properties in the Loess Hilly-Gully Region, China

Preamble

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Title: Effects of Long-Term Fertilization on Soil Microbial Properties in the Loess Hilly-Gully Region, China

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Abstract

This study investigated the effects and mechanisms of fertilization on soil microbial properties in the nutrient-poor Loess Hilly Region. Using long-term fertilization plots at the Ansai Field Research Station, we examined soil microbial community structure and respiration under eight treatments: CK (control), N (nitrogen fertilizer), P (phosphorus fertilizer), M (manure), NP (nitrogen + phosphorus), MN (manure + nitrogen), MP (manure + phosphorus), and MNP (manure + nitrogen + phosphorus). Results showed that microbial activities and phospholipid fatty acid (PLFA) contents in the surface soil layer (0–20 cm) were significantly higher than in the subsoil layer (20–40 cm). Specifically, basal respiration, substrate-induced respiration, and total PLFA contents in the surface layer were 63.61%–116.78%, 53.45%–53.45%, and 16.16%–43.67% higher than in the subsoil, respectively.

Compared to CK, single applications of N and P fertilizers increased surface soil

basal respiration by 34.11% and 48.89%, respectively, while substrate-induced respiration intensities increased by 40.83% and 63.59%. In the subsoil, basal respiration increased by 40.83% and 63.59%, and substrate-induced respiration increased by 14.70% and 20.49%, respectively. Nitrogen fertilizer significantly affected the microbial community, increasing PLFA contents by 63.19% in the 0–20 cm layer and 53.07% in the 20–40 cm layer. Phosphorus fertilizer also significantly altered microbial community structure, though the NP combination showed no significant effect. The mixture of organic and inorganic fertilizers significantly enhanced soil respiration and microbial community structure.

Three-way variance analysis revealed that single nitrogen fertilizer had no significant effect on most soil microbial properties, while phosphorus fertilizer significantly affected all indicators except actinomycetes. In the 0–20 cm layer, phosphorus fertilizer' s influence ratio on these microbial characteristics ranged from 11.4% to 54.0%. Redundancy analysis (RDA) indicated that available phosphorus played a major role in driving changes in microbial characteristics. Long-term combined application of manure, nitrogen, and phosphorus fertilizers improved soil microbial properties, thereby contributing to the stability and health of farmland ecosystems.

Keywords: long-term fertilization; soil respiration; phospholipid fatty acid

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1. Study Area Overview

This study was conducted at the Ansai Soil and Water Conservation Comprehensive Experimental Station (109°19 23 E, 36°51 30 N) on the central Loess Plateau. The site has a warm temperate semi-arid climate with an average annual temperature of 8.8°C and annual precipitation of 535 mm, with 60% concentrated in July–September. The frost-free period is 160 days. The topography consists of typical loess ridge-hill gullies with severe soil erosion. The zonal soil is dark loessial soil, though the experimental area features loessal soil (Cumulic Haplustoll) due to natural conditions and human activity. The soil parent material is widely exposed, and the nutrient-poor soil is managed under rain-fed agriculture with a one-year, one-crop system focused on autumn crops.

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2. Experimental Design

The experiment was established in 2001 at the Duntan Flatland Long-Term Nutrient Experiment Field. Eight fertilization treatments were arranged in a randomized block design with three replications per treatment, each plot measuring 6 m × 10 m. The treatments included: CK (control), N (nitrogen fertilizer), P (phosphorus fertilizer), M (manure), NP (nitrogen + phosphorus), MN (manure + nitrogen), MP (manure + phosphorus), and MNP (manure + nitrogen + phosphorus). Nitrogen fertilizer was applied as urea at 97.5 kg N/ha, with

75% applied as base fertilizer and 25% topdressed between the corn trumpet stage and tasseling stage. Phosphorus fertilizer was applied as superphosphate at 75.0 kg P/ha as base fertilizer. Manure (winter sheep manure) was applied at 7500 kg/ha as base fertilizer. The crop rotation was continuous corn (*Zea mays* L.).

3. Sample Collection and Testing Analysis

Soil samples were collected from 0–20 cm and 20–40 cm layers using an S-shaped sampling method after harvest. Each sample was divided into two portions after removing roots and litter. One portion was air-dried and sieved through 0.25 mm and 0.149 mm meshes for determination of soil organic carbon, total nitrogen, total phosphorus, available phosphorus, and alkali-hydrolyzable nitrogen using conventional methods. The other fresh portion was sieved through 2 mm and stored at 4°C for basal respiration (BR) and substrate-induced respiration (SIR) measurements, or at -20°C for PLFA analysis.

Soil organic carbon was determined by potassium dichromate oxidation, total nitrogen by Kjeldahl digestion, total phosphorus by H SO -HClO digestion with molybdenum-antimony colorimetry, available phosphorus by NaHCO extraction with molybdenum-antimony colorimetry, and alkali-hydrolyzable nitrogen by alkaline diffusion. BR and SIR were measured using an infrared gas analyzer following Hueso et al. [19]. PLFAs were extracted using a modified Bligh-Dyer method [20], separated on silica columns with chloroform, methanol, and citrate buffer, and analyzed by gas chromatography (GC7890A, Agilent Technologies) using the Sherlock MIS 4.5 system. Individual PLFAs were quantified using the internal standard C19:0 and expressed as nmol/g dry soil.

Specific PLFAs were used as biomarkers: Gram-positive bacteria (G) were represented by i13:0, a13:0, i14:0, a14:0, i15:1 w9c, i15:0, a15:0, i16:0, i17:1 w9c, i17:0, a17:0, a19:0, and i22:0; Gram-negative bacteria (G) by 12:1 w4c, 15:1 w8c, 17:1 w8c, 18:1 w9c, 18:1 w7c, 19:0 cyclo w7c, 20:1 w9c, and 22:1 w9c; actinomycetes by 16:0 10-methyl, 17:0 10-methyl, 17:1 w7c 10-methyl, and 18:0 10-methyl; and fungi by 18:2 w6c.

4. Data Processing

SPSS 20.0 software was used for one-way ANOVA to analyze soil respiration and microbial community structure across fertilization treatments. Three-way ANOVA was employed to examine the effects and interactions of nitrogen, phosphorus, and manure factors on each indicator. Redundancy analysis (RDA) was performed to assess the explanatory power and influence of environmental factors on microbial community structure variation.

1. Effects of Fertilization on Soil Respiration

Basal respiration and substrate-induced respiration showed consistent trends across treatments. In the 0–20 cm layer, all fertilization treatments except CK significantly increased soil respiration intensity by 31.18%–53.76% (Figure 1 [Figure 1: see original paper]). In the 20–40 cm layer, only the MN, MP, and MNP treatments significantly enhanced basal respiration, while other treatments showed no significant differences. Substrate-induced respiration was significantly increased by all treatments in the surface layer (40.12%–139.38%), but only MN, MP, and MNP treatments had significant effects in the subsoil layer.

2. Effects of Fertilization on Soil Microbial Community Structure Diversity

Fertilization significantly affected soil microbial community composition, though effects varied among treatments and microbial groups. In the 0–20 cm layer, all fertilization measures significantly increased total PLFA content compared to CK, with the greatest increase under MNP treatment. G PLFA, G PLFA, and bacterial PLFAs were significantly increased by most treatments, while fungal PLFAs were only significantly affected in individual treatments (PM, NPM). Actinomycete PLFAs decreased significantly under P and NP treatments. In the 20–40 cm layer, fertilization effects were weaker, with only G PLFA and G PLFA showing significant increases under PM and NPM treatments.

3. Interactive Effects of Fertilization Treatments on Soil Microbial Properties

Three-way ANOVA revealed differential effects of nitrogen, phosphorus, and manure factors on microbial indicators. In the 0–20 cm layer, nitrogen fertilizer had no significant effect on most properties except actinomycetes and total PLFAs. Phosphorus fertilizer significantly affected all indicators except fungi and showed significant interactions with manure for BR, SIR, G PLFA, G PLFA, and total PLFA. Manure significantly altered all indicators except fungi and interacted significantly with phosphorus for most properties. No significant three-way interactions were observed. In the 20–40 cm layer, results were similar to the surface layer, though nitrogen and phosphorus showed significant interactions for actinomycetes and total PLFA.

4. Driving Factors of Microbial Community Structure Changes

Principal component analysis (PCA) of microbial communities under different fertilization treatments showed distinct clustering. For the 0–20 cm layer, PC1 explained 24.3% of data variation and PC2 explained 19.37%, effectively separating treatments. For the 20–40 cm layer, PC1 explained 36.79% and PC2 explained 19.63% of variation, with some overlap between treatment clusters.

RDA was performed using soil microbial properties and environmental factors (soil organic carbon, total nitrogen, total phosphorus, available phosphorus, alkali-hydrolyzable nitrogen). In the 0–20 cm layer, the first axis explained 58.46% of variation and the second axis 14.96%. Available phosphorus had extremely significant effects on microbial characteristics, while total phosphorus and alkali-hydrolyzable nitrogen had significant effects. In the 20–40 cm layer, the first axis explained 61.6% of variation and the second axis 17.16%. Available phosphorus remained the dominant driver, while total phosphorus and alkali-hydrolyzable nitrogen showed significant but negative effects on microbial properties.

Discussion

This study found that fertilization effects were more pronounced in the 0–20 cm layer than in the 20–40 cm layer, though trends were consistent across depths. The surface layer, as the primary tillage horizon with abundant root distribution, receives direct fertilizer application and downward leaching of nutrients, creating favorable conditions for microbial growth. The weaker effects in the subsoil likely result from limited nutrient migration.

Single nitrogen application showed limited effects on microbial properties, consistent with some previous studies [23, 26]. Long-term inorganic nitrogen application may accelerate decomposition of native soil organic carbon, reducing total accumulation and creating unfavorable C/N ratios for microbial buildup [27]. However, nitrogen addition can increase nitrogen availability and reduce C/N ratio, potentially favoring bacteria adapted to low C/N environments [22].

Phosphorus fertilizer significantly affected microbial community structure, particularly increasing total PLFA, bacterial, and actinomycete contents. This aligns with findings from tropical forests [29] and grasslands [28] where phosphorus addition enhanced microbial biomass. In the phosphorus-deficient soils of the Loess Hilly Region, phosphorus application likely alleviates a primary growth limitation for microorganisms. The RDA results confirm that available phosphorus is the main driver of microbial community changes, particularly in the surface layer.

The non-significant effect of combined NP fertilization on microbial community structure agrees with some previous reports [30], possibly due to nutrient imbalances or antagonistic effects. In contrast, combined organic-inorganic fertilization significantly enhanced microbial activity and altered community structure, consistent with numerous studies [13, 16, 31]. Organic manure provides not only nutrients but also active microbial inoculum and readily available carbon sources, improving soil structure and creating favorable habitats for microbial proliferation [33, 34]. The synergistic effects of organic and inorganic fertilizers on root biomass and exudates further stimulate microbial growth [36].

Conclusion

In the nutrient-poor soils of the Loess Hilly Region, long-term fertilization significantly affects soil microbial properties, with effects decreasing with soil depth. Phosphorus fertilizer, particularly in its available form, emerges as the primary driver of microbial community changes, reflecting its status as a key limiting factor in this region. Combined organic-inorganic fertilization most effectively enhances soil respiration, microbial biomass, and community structure diversity. These findings demonstrate that establishing scientific fertilization regimes combining organic and inorganic amendments can improve soil fertility and biological properties, thereby enhancing ecosystem stability, health, and crop productivity in the Loess Hilly Region.

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