Mollisol Carbon Emission Characteristics and Changes in Carbon Pool Fractions and Enzyme Activities Under Long-Term Fertilization: Postprint

Authors: He Mei, Wang Ligang, Zhu Ping, Qi Ruimin, Wang Yingchun

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Abstract

As a crucial soil resource underpinning China' s food security and ecological security, black soil' s carbon emission characteristics and carbon pool component changes have consistently been a research hotspot in ecology. Fertilization is a critical factor influencing organic carbon input and output in black soil, necessitating investigation at long-term scales. To clarify the characteristics and influencing mechanisms of soil carbon emissions under long-term different fertilization regimes, using the National Soil Fertility and Fertilizer Efficiency Monitoring Network Black Soil Monitoring Base at Gongzhuling (established in 1990) as the research platform, six treatments were selected: no fertilization (CK), mineral NPK fertilizer alone (NPK), combined mineral and low-rate organic manure (NPKM1), 1.5-fold mineral fertilizer combined with low-rate organic manure (1.5(NPKM1)), combined mineral and high-rate organic manure (NPKM2), and combined mineral fertilizer and straw (NPKS). The study investigated changes in soil carbon emissions (CO2-C) and soil carbon pool components including dissolved organic carbon (DOC), microbial biomass carbon (MBC), particulate organic carbon (POC), readily oxidizable carbon (ROC), and enzyme activities such as -glucosidase (BG), xylanase (BXYL), cellulase (CBH), and N-acetyl- -glucosaminidase (NAG) under long-term different fertilization. The results showed that, compared with CK, all fertilization treatments significantly increased black soil carbon emissions ($P < 0.05$). Specifically, the NPK treatment exhibited soil carbon emissions of approximately 2633.33 kg/hm2, which was 37.36% significantly higher than CK. Long-term combined mineral-organic fertilization (NPKM1, 1.5(NPKM1), NPKM2) significantly increased soil carbon emissions by 71.81%-88.51%, demonstrating the most pronounced effect. NPKS significantly increased soil carbon emissions by 56.32%, and no significant differences in carbon emissions were observed among the three long-term combined mineral-organic fertilization practices. Relative to the CK treatment, combined mineral-organic fertilization significantly increased DOC, MBC, POC, and ROC ($P < 0.05$), with these indicators being 16.07%-56.34%, 128.84%-185.77%, 284.15%-497.45%, and 841.03%-1145.94% higher than CK, respectively, with the 1.5(NPKM1) treatment showing the best performance. Simultaneously, combined mineral-organic fertilization increased NAG, BG, BXYL, and CBH activities by 313.22%-452.65%, 129.45%-250.74%, 159.08%-273.32%, and 72.21%-193.53% relative to CK, respectively, with the 1.5(NPKM1) treatment again demonstrating the best effect. Correlation analysis between soil carbon emissions and soil enzyme activities as well as soil active carbon pool components revealed that soil carbon emissions under long-term different fertilization practices were not only extremely significantly correlated with soil ROC, DOC, POC, and MBC contents ($P < 0.001$), but also extremely significantly correlated with soil BG, NAG, CBH, and BXYL enzyme activities ($P < 0.001$), indicating that fertilization can influence soil carbon emissions by altering the contents of various soil active carbon pool components and soil microbial activity.

Full Text

Carbon Emission Characteristics, Carbon Pool Components, and Enzyme Activity Under Long-Term Fertilization Conditions of Black Soil

HE Mei[1], WANG Ligang[1], ZHU Ping[2], QI Ruimin[1], WANG Yingchun[1]

[1]Institute of Agricultural Resources and Regional Planning, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[2]Institute of Agricultural Resources and Environment, Jilin Academy of Agricultural Sciences, Changchun 130033, China

Abstract

Black soil represents a critical soil resource underpinning China' s food and ecological security. Fertilization is a key factor influencing organic carbon input and output in black soils, yet its effects require investigation over extended time scales. This study examined carbon emission characteristics and carbon pool component changes at the Gongzhuling monitoring site of the National Soil Fertility and Fertilizer Efficiency Network, which was established in 1990. Six fertilization treatments were selected: no fertilizer (CK), chemical fertilizer only (NPK), chemical fertilizer combined with low organic manure (NPKM1), 1.5 times the inorganic and organic fertilizer amount of NPKM1 (1.5(NPKM1)), chemical fertilizer combined with high organic manure (NPKM2), and chemical fertilizer combined with straw (NPKS). The study investigated soil carbon emissions and changes in carbon pool components including dissolved organic carbon (DOC), microbial biomass carbon (MBC), particulate organic carbon (POC), readily oxidized organic carbon (ROC), and enzyme activities such as -glucosidase (BG), -xylosidase (BXYL), cellulase (CBH), and N-acetylglucosaminidase (NAG).

Results showed that all fertilization treatments significantly increased black soil carbon emissions compared to CK ($P < 0.05$). The NPK treatment emitted approximately 2633.33 kg CO -C/hm^2, 37.36% higher than CK. Long-term combined organic-inorganic fertilization (NPKM1, 1.5(NPKM1), NPKM2) increased soil carbon emissions by 71.81%-88.51%, with no significant differences among these three treatments. The NPKS treatment increased emissions by 56.32%. Relative to CK, NPKM1, 1.5(NPKM1), and NPKM2 treatments significantly increased DOC by 16.07%-56.34%, MBC by 128.84%-185.77%, POC by 284.15%-497.45%, and ROC by 841.03%-1145.94% ($P < 0.05$), with 1.5(NPKM1) showing the best effect. Combined organic-inorganic fertilization also increased NAG, BG, and BXYL activities by 313.22%-452.65%, 129.45%-250.74%, and 159.08%-273.32%, respectively, with 1.5(NPKM1) again performing best.

Correlation analysis revealed that soil carbon emissions were extremely significantly correlated with ROC, DOC, POC, and MBC contents ($P < 0.001$), as well as with BG, NAG, CBH, and BXYL enzyme activities ($P < 0.001$), indicating that fertilization affects soil carbon emissions by altering active carbon pool components and microbial activity.

Keywords: long-term fertilization; soil carbon emissions; soil active carbon pool components; soil enzyme activity

1. Study Area Description

The experiment was conducted at the Gongzhuling Long-Term Monitoring Base of the National Black Soil Fertility and Fertilizer Efficiency Network, located in Gongzhuling City, Jilin Province. This region represents a typical black soil area with a temperate semi-humid monsoon climate. The site has an annual average temperature of 4–5°C, with monthly averages of 19–25°C in July and -35°C in January. Annual precipitation ranges from 450–650 mm, with 70% occurring during the crop growing season. The frost-free period lasts 120–140 days, and the 10°C effective accumulated temperature is 2600–3000°C. The soil parent material is Quaternary loess-like sediment. The 0–20 cm soil layer has a bulk density of 1.19 g/cm^3, pH of 7.6, organic matter content of 13.2 g/kg, total nitrogen of 1.4 g/kg, available phosphorus of 11.79 mg/kg, and available potassium of 158.33 mg/kg. The cropping system is single-season rain-fed spring maize (*Zea mays* L.) with a planting density of 45,000 plants/hm^2. Since the experiment began in 1990, different maize varieties have been used: Jidan 304 (1991–1994), Jidan 209 (1995–1996), Simi 25 (1997–1999), Jidan 209 (2000–2003), and Xianyu 335 (2004–2012).

2. Experimental Design

This study analyzed six treatments from the long-term fertilization experiment: (1) CK (no fertilizer), (2) NPK (chemical fertilizer only), (3) NPKM1 (chemical fertilizer + low organic manure), (4) 1.5(NPKM1) (1.5 times the inorganic and organic fertilizer rate of NPKM1), (5) NPKM2 (chemical fertilizer + high organic manure), and (6) NPKS (chemical fertilizer + straw). The long-term experiment was initiated in 1990 with no replications for each treatment, which was common practice at the time. Each plot area was 400 m^2. To account for this limitation, each plot was divided into three sampling subplots during sample collection. Phosphorus and potassium fertilizers were applied as base fertilizer before sowing, with nitrogen applied as topdressing at the jointing stage. In organic-inorganic combination treatments, manure (pig manure before 1999, cattle manure thereafter) was applied after maize harvest, with one-third as base fertilizer and two-thirds topdressed. For the NPKS treatment, previous year' s straw was removed, air-dried, manually crushed, and applied to furrows during late June topdressing. All treatments left 15 cm stubble at harvest, which was returned to the field with root residues. Sowing occurred annually from April 25–30, with harvest around September 25–30.

Fertilizer application rates for each treatment

3. Methods

3.1 Soil Nutrient Measurement

After harvest, 0-20 cm soil samples were collected from each subplot using a five-point sampling method, then sieved and mixed. DOC was extracted with 0.5 mol/L K SO and measured using a TOC/TN auto-analyzer (Multi N/C 3100). POC was determined by the sodium hexametaphosphate dispersion method. ROC was measured using 333 mmol/L KMnO oxidation. MBC was determined by chloroform fumigation-extraction.

3.2 Soil Enzyme Activity Measurement

Four soil enzymes were measured using fluorometric microplate assays: -glucosidase (BG), -xylosidase (BXYL), cellulase (CBH), and N-acetylglucosaminidase (NAG). Fresh soil equivalent to 200 mL dry weight was suspended in 50 mmol/L sodium acetate buffer (pH 5.0) to create a soil slurry. Buffer, standards, and fluorogenic substrates were pipetted into 96-well microplates following the partitioned method. After incubation at 25°C for 4 hours, fluorescence was measured.

3.3 Soil Respiration Rate and CO -C Emission Estimation

Soil respiration was measured using a LI-8100 open-path soil CO flux system with an infrared gas analyzer (IRGA). To minimize disturbance effects, PVC collars (20 cm diameter, 10 cm height) were installed 3 cm into the soil one day before measurement. Measurements were taken between 9:00–11:00 AM during the maize growing season (see for dates), with three replicates per plot. The average value represented the plot' s respiration rate for that measurement day. Soil temperature was relatively stable during this period. Crop growth season rainfall and temperature data were obtained from the Siping Meteorological Station.

Cumulative CO -C emissions were calculated using linear interpolation between measurement dates:

$$CO\text{ -C } (kg/hm^2) = \Sigma(Rs \times 3600 \times 24 \times 12 \times 10 \quad \times N)$$

where Rs is soil respiration rate (mol m ^{2} s ^{1}), 12 is the molar mass of C (g/mol), 3600 converts seconds to hours, 24 converts hours to days, and N is the number of days between adjacent measurements.

[Figure 1: see original paper] Precipitation and average air temperature during crop growth season

4. Data Analysis

Data were processed using Microsoft Excel 2003 and statistically analyzed with SAS 9.1 software.

Results

4.1 Effects of Long-Term Different Fertilization on Soil Cumulative Carbon Emissions

All fertilization treatments significantly increased soil cumulative carbon emissions compared to the no-fertilizer control ($P < 0.05$). The NPK treatment emitted 2633.33 kg CO -C/hm^2, 37.36% higher than CK. Combined organic-inorganic fertilization treatments (NPKM1, 1.5(NPKM1), NPKM2) showed the greatest increases, raising emissions by 71.81%-88.51% with no significant differences among them. The NPKS treatment increased emissions by 56.32%.

[Figure 2: see original paper] Changes in soil cumulative carbon emission characteristics under different long-term fertilization treatments

4.2 Changes in Soil Active Organic Carbon Pool Components Under Long-Term Different Fertilization

Long-term combined organic-inorganic fertilization significantly increased soil DOC content by 16.08%-56.34% ($P < 0.05$), with 1.5(NPKM1) showing the highest DOC content at 515.56 mg/kg. Long-term NPK fertilization alone also increased DOC, while CK showed the lowest at 329.76 mg/kg.

All fertilization treatments significantly increased MBC content ($P < 0.05$). The 1.5(NPKM1) treatment achieved the highest MBC at 327.91 mg/kg, increasing by 128.84%-185.77% compared to other treatments. NPK alone increased MBC by 77.86%.

Combined organic-inorganic fertilization significantly increased POC content by 157.62%-169.63% for NPKM1, 387.95%-410.70% for 1.5(NPKM1), and 470.84%-497.46% for NPKM2 ($P < 0.05$). Similarly, ROC content increased dramatically by 659.90%-1145.94% across these treatments.

[Figure 3: see original paper] Changes in soil active organic carbon pool under long-term different fertilization treatments

4.3 Changes in Soil Enzyme Activities Under Long-Term Different Fertilization

Fertilization treatments substantially affected soil enzyme activities. The 1.5(NPKM1) treatment showed the highest activities for all four enzymes: BG activity reached 58.19 nmol g ^{1} h ^{1} (260.56% higher than CK), BXYL activity was 37.39 nmol g ^{1} h ^{1} (452.65% higher than CK), CBH activity was 62.81 nmol g ^{1} h ^{1} (273.32% higher than CK), and NAG activity was 257.36 nmol g ^{1} h ^{1} (250.74% higher than CK). The activity ranking was generally: 1.5(NPKM1) > NPKM2 > NPKS > NPKM1 > NPK > CK.

[Figure 4: see original paper] Soil enzyme activity under long-term different fertilization treatments

4.4 Correlation Analysis Between Soil Carbon Emissions and Active Organic Carbon/Enzyme Activities

Soil carbon emissions were extremely significantly correlated with ROC, DOC, POC, and MBC contents ($P < 0.001$), and with BG, NAG, CBH, and BXYL enzyme activities ($P < 0.001$). This indicates that soil carbon emissions are jointly regulated by active carbon pool components and microbial activity.

Correlation coefficients between soil CO -C emissions and soil active organic carbon components as well as enzyme activity

Discussion

5.1 Effects of Long-Term Different Fertilization on Soil Respiration Carbon Emissions

Field management practices strongly influence soil respiration rates, leading to significant differences in carbon emissions among treatments. Organic manure and straw application provide abundant substrates for microorganisms, while straw incorporation reduces soil bulk density and improves aeration, promoting aerobic respiration. Long-term fertilization also increases root residue input. The higher enzyme activities under combined organic-inorganic fertilization indicate faster material turnover and greater microbial activity, further stimulating soil respiration. Our results are slightly higher than those reported by Huang et al. in red soil under wheat-maize rotation, likely due to differences in soil type, cropping system, and climate. The NPK treatment significantly increased emissions compared to CK, possibly because root residue return in the CK treatment provided fresh carbon sources that replenished active carbon pools, while slower plant growth in CK reduced root respiration (40%-64% of total soil respiration), resulting in lower total emissions despite higher microbial activity per unit of soil.

5.2 Effects of Long-Term Different Fertilization on Active Carbon Pool Components

Soil active carbon components (DOC, MBC, POC, ROC) serve as energy sources for microorganisms and drive soil biochemical processes. Studies show that organic manure application increases MBC by 51.7%-85% and DOC by 56%-85% compared to no fertilization, while chemical fertilizer alone may decrease MBC. In our study, long-term combined fertilization increased MBC by 128.84%-185.77% and DOC by 16.08%-56.34%, likely due to high organic manure rates enriching microbial sources. The superior effect of combined fertilization on black soil active carbon pools may be attributed to direct carbon input, enhanced microbial diversity and activity, and stimulated plant residue decomposition, collectively increasing active carbon storage.

5.3 Effects of Long-Term Different Fertilization on Soil Enzyme Activities

All soil biochemical reactions involve enzymes, whose activities reflect microbial metabolic intensity and soil fertility. Long-term fertilization can enhance, inhibit, or maintain enzyme activities depending on soil type, climate, and management. In this study, chemical fertilizer significantly increased enzyme activities, possibly by optimizing soil C:N ratios. Combined organic-inorganic fertilization and straw return markedly increased enzyme activities (BG, BXYL, CBH, NAG) by 72.21%-452.65%, consistent with previous research showing that organic amendments introduce microorganisms and nutrients, stimulating biological activity. Straw decomposition enhances enzyme activity more effectively than burning, though our straw treatment showed lower enzyme activity than manure treatments, possibly due to differences in microbial community structure induced by different substrates.

5.4 Correlation Between Soil Respiration Carbon Emissions and Active Carbon Components/Enzyme Activities

Understanding these relationships helps explain soil carbon cycling mechanisms. Our results show extremely significant correlations between carbon emissions and all active carbon components (ROC, DOC, POC, MBC) and enzyme activities (BG, CBH, NAG, BXYL), consistent with some but not all previous studies. The strongest correlation occurred with ROC, followed by MBC, suggesting microbial substrate preference differences. The consistent correlations support that fertilization influences carbon emissions by modifying substrate availability and microbial activity. These relationships provide guidance for balanced fertilization management, though the mechanisms underlying crop growth effects on soil respiration require further investigation.

Conclusion

All fertilization treatments significantly increased black soil carbon emissions in Northeast China ($P < 0.05$). Long-term combined organic-inorganic fertilization showed the greatest effect, increasing emissions by 71.81%–88.51%, while straw return increased emissions by 56.32% and chemical fertilizer alone by 37.36%. Combined fertilization also most effectively increased active carbon pool components and enzyme activities. The extremely significant correlations between carbon emissions and both active carbon components and enzyme activities ($P < 0.001$) demonstrate that fertilization affects soil carbon emissions by altering substrate concentrations and microbial activity. These findings provide theoretical support for rational farmland management in Northeast China' s black soil region. While organic manure significantly enhances soil carbon sequestration potential, its associated increase in greenhouse gas emissions cannot be ignored. The relationship between crop growth and soil respiration substrates/microbial activity warrants systematic future research.

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