The Effect Mechanism of Fertilization Measures on the Three-dimensional Fluorescence Characteristics of Soil DOM Components

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Abstract

The composition of soil dissolved organic matter (DOM) can reflect the soil maturity and fertilizer supply capacity. Fertilization is an important management strategy to regulate soil DOM transformation by regulating fertility, crop growth, microbial biomass and enzyme activity. However, there are few studies on the effect mechanism of fertilization to soil microecology. Here, the data from a four-year fertilization experiment in Liaohe irrigation area of China was used to evaluate and quantify the biological and nutrient effect mechanism of fertilization on the three-dimensional fluorescence characteristics of DOM components by the three-dimensional fluorescence spectral area volume integral analysis method (EEM-FRI). The results showed that the combined application of organic and inorganic fertilizers had increased the proportion of soil humic acids and could affect the DOM contents in the soil depth. The single application of organic fertilizer had reduced the proportion of fulvic acids. The proportion of protein-like components was increased with the application of phosphate fertilizer. The single application of nitrogen fertilizer increased the proportion of microbial metabolites. The proportion of humic acids was decreased and the proportion of small molecule DOM was increased in the soil of planting spring maize and applying fertilizer. The planting of spring maize could increase the exogenous input and the terrigenous characteristics of soil DOM. The organic fertilizer could increase soil microbial source DOM. Fertilization measures had affected microbial biomass, enzyme activity and spring maize yield, regulated the degradation of DOM and affected the characteristics of DOM components. The yield of spring maize was negatively correlated with humic acids components and positively correlated with fulvic acids components. The characteristics of DOM components were affected by their sources, degradation of soil enzymes and crop growth. Fertilization measures had affected soil DOM components by supplying DOC, affecting microbial enzyme activities and regulating the growth of spring maize. Fertilization had mainly affected DOM components in soil layer of

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10-20 cm. Mixed fertilization had affected soil DOM in deep layer (20-40 cm). Combined application of organic and inorganic fertilizers had increased the proportion of soil DOM, application of inorganic fertilizer, especially nitrogen fertilizer alone significantly reduced of fulvic acids component in DOM, application of N, P, K was beneficial to increase the proportion of microbial metabolites in DOM. The proportion of fulvic acids components in soil DOM could be used to characterize soil fertility and microbial activity, and its proportion was a key factor to affect crop yield.

**Keywords:** Dissolved Organic Matter (DOM), soil, three-dimensional fluorescence spectra (EEM), fertilization measure

### Introduction

Soil dissolved organic matter (DOM) is an important part of organic matter in soil ecosystem and plays an important role in a series of soil biochemical processes [1-2]. It is generally found in the natural environment such as soil, sediment and various water bodies. It can change the migration, transformation and spatial distribution of metal elements in soil through ion exchange, adsorption and redox reactions [3]. Soil DOM mainly comes from plant residues, root exudates, exogenous inputs (organic fertilizer) and humus in soil organic matter [4]. Due to its high activity, strong migration ability, and positive response ability to soil disturbance, crop growth, soil microbial biomass, soil enzyme activity, human agricultural activities, etc., the characteristics of DOM components in the soil can use to indicate soil fertility [5] and respond to the changes of soil micro-ecological environment. In recent years, soil DOM has gradually become a hot area in environmental geochemistry research [6]. It is urgent to conduct research on the influencing mechanism of soil DOM components, so as to reveal the process of soil micro-ecological circulation. Fertilization is the main agricultural measure that affects soil physical and chemical environment and soil fertility. A large number of studies have been carried out on the effects of fertilization on crop growth and yield [7], soil microorganisms [8], and soil nutrients [9]. However, there are relatively few studies on the effects of different fertilization types on the structure of soil DOM components and soil micro-ecological cycle, especially the research on the northern farmland soils.

At present, in order to clarify the structure and properties of soil DOM from the micro-ecological and molecular level, and further understand the influence mechanism of DOM ecological environmental effect and transformation, there are many techniques and methods for DOM research at home and abroad. Compared to infrared spectroscopy, nuclear magnetic resonance, mass spectrometry, et al., the exciter-emission matrix fluorescence (EEM) technique is very sensitive, easy to use and does not disrupt the sample structure [10]. It is widely used for the study of DOM composition, source and biogeochemical cycling in soils, lakes and river sediments [11-13]. Fluorescence regional integration (FRI) can perform quantitative analysis on EEM, determine DOM configuration and heterogeneity [14], and distinguish subtle changes in EEM quantitatively to improve the ability of spectral analysis [15]. EEM spectra were divided into 5 regions [16]: Regional I (Ex/Em = 220-250/280-330 nm), for Tyrosine proteins. Regional II (Ex/Em = 220-250/330-380 nm), for Tryptophan proteins. Regional III (Ex/Em = 220-250/380-550 nm), for kind of fulvic acids. Regional IV (Ex/Em = 250-400/280-380 nm), for soluble microbial metabolites. Regional V (Ex/Em = 250-400/380-550 nm) for kind of humic acids. By calculating the specific area standard volume ($\phi_{T,n}$) and the percentage of each area standard volume ($P_{i,n} = 100\times\phi_{i,n}/\phi_{T,n}$, standard volume of the whole area), the content and relative content of fluorescent substances with specific structure in the corresponding area were determined. Using EEM to study the characteristics of soil DOM components can better reveal the impact mechanism of fertilization and other agricultural measures on soil microecology, and provide micro evidence for the impact of human activities such as fertilization on the mechanism of farmland nutrient transformation.

### Materials and Methods

#### Overview of the Study Area

The experiment was conducted at Kerqin District, Tongliao City, Inner Mongolia Autonomous Region, Northeast China (42°15′-45°41′N, 119°15′-123°43′E). The planting area of spring maize in this area is nearly 70×10^4 hm², it accounts for nearly 1/3 of the total in Inner Mongolia. The frost-free period of the test site was 100-150 days. The area receives an average annual precipitation of 350-450 mm, it is mainly concentrated in July-August. The test area is the Liaohe River irrigated area, the soil is mainly formed by siltation of the river, and the test area is sandy loam soil. Physical and chemical properties of soil at different levels were shown in Table 1.

#### Experimental Treatment

The experiment was randomly designed, and nine treatments were selected for this research: N (synthetic nitrogen), P (synthetic nitrogen), NP (synthetic N and
urea (N, 46%), calcium superphosphate (P)
respectively. The synthetic fertilizers were applied as
1:10 (m/V, g·mL
P2O5
and potassium chloride (K 2
manure (organic matter 14.7%, N 0.42%,
195 kg·hm-2
reference. The amount of N, P, K and mature applied were
manure), NPKM (synthetic NPK and manure), and CK
M (mature); NM (synthetic N and manure), PM
manure), NPM (synthetic N, P and
phosphorus); NPK (synthetic N, P and potassium);
M (mature); NM (synthetic N and manure), PM
manure), NPM (synthetic N, P and
manure), NPKM (synthetic NPK and manure), and CK
(no fertilizer). The soils of before sowing were taken as
accounted for 1/5, top dressing accounted for 4/5. Other
was Zhengdan-958, and the planting density was
7500 plants·hm -2. Field management was similar to
local traditional cultivation.

Soil Sampling

In 2010 (before sowing, May 10) and in 2013 (during
the corn ripening period, September 20), soil samples
were randomly collected in the field experiment plot
according to the „S” shaped sampling method and
5 points. Sampling depth was divided into three layers:
0-10 cm, 10-20 cm and 20-40 cm. Then, the soil samples
were thoroughly mixed, removed soil plant residues and
root systems and stored in plastic bags at 4ºC for later
analysis. The soil samples were divided to two, the fresh
samples were used to determine soil microbial biomass
and enzyme activity, and the air-dried soil samples
were crushed to determine DOM with 3D fluorescence
spectrum and soil physical and chemical indexes. Yield indicators were averaged over 4 years.

Soil Analysis

DOM fluorescence spectroscopic analysis of soil: took a certain amount of soil, extracted (20ºC, 200 r min⁻¹, 24 h) by 1 mol·L⁻¹ KCl in a ratio of
1:10 (m/V, g·mL⁻¹), centrifuged (5000 r min⁻¹, 15 min),
then filtered with 0.45 µm glass fiber membrane, DOM
solution was obtained and diluted to 10 times to reduce
the internal filter effect, the diluent were determined
DOM and DOC. The DOM three-dimensional
fluorescence spectrum was determined by HITACHI
F-7000 fluorescence spectrometer (F-7000, Hitachi
High-Technology Corp, Tokyo, Japan). The fluorescence
proton photometer was equipped with a 150 W Xenon
are lamp (Hitachi High-Technology Corp, Tokyo, Japan)
as the light source and two grating monochromators
engaged with a slit as the EEM wavelength selectors,
with quartz cuvettes of 1 cm, scanning speed was
set to 60,000 nm/min, each measurement period was
2 min in duration. The EEM parameters were as
follows, Bandpass: excitation wavelength, \( \lambda_{ex} = 5 \) nm,
emission wavelength, \( \lambda_{em} = 5 \) nm; Scanning speed:
2400 nm·min⁻¹. Three-dimensional fluorescence
spectrometric determination: excitation wavelength (Ex)
200-440nm, emission wavelength (Em) 250-600 nm,
MilliQ water were used as EEM blanks and
subtracted from each sample EEM. Emission and
excitation correction files generated by the fluoromax
manufacturer were applied to each MilliQ-subtracted
sample EEM [17]. DOM fluorescence spectra of soil
were quantitatively analyzed by regional integration
volume integration method. Dissolved organic carbon
(DOC) was measured by TOC-5000A (Shimadzu,
Japan). Microbial biomass carbon (MBC) and Microbial
biomass nitrogen (MBN) were measured with the
methods of Calbrix , MBC was determined by a multi
N/C 3100 analyzer (Analytik Jena AG, Jena, Germany).
MBN was analyzed by a FOSS, Kjeldahl nitrogen
analyzer (Model Kjeltc 2300, FOSS Analytical AB,
Hoganas, Sweden) [18]. A dual-beam ultraviolet/
visible spectrometer (TU-1900, Beijing Purkinje General
Instrument Co., Ltd, Beijing, China) automatically
controlled by computer was used to measure
quantitatively soil sucrose by 3,5-dinitrosalicylic
acid colorimetric method, soil phosphatase by the
colorimetric method of benzene disodium phosphate,
sodium urease by indigophenol colorimetry [19]. Soil
catalase was determined by potassium permanganate
titration [19]. Soil physical and chemical properties
were determined by conventional analysis [20].

Data Analysis

In the data statistics, the average and standard
development data are retained with two significant figures,
and the drawing is performed with Excel 2010 software.
Data correlation analysis, cluster analysis and path
analysis are carried out by SPSS 17.0 software, the
correlation coefficient data are retained with four

| Index | TOM (g·kg⁻¹) | TN (g·kg⁻¹) | TP (g·kg⁻¹) | TK (g·kg⁻¹) | pH    | Bulk density (g·cm⁻³) | Porosity |
|-------|--------------|-------------|-------------|-------------|-------|------------------------|----------|
| 0-10  | 12.56±1.45   | 1.22±0.04   | 0.15±0.05   | 33.47±2.87  | 7.86±0.17 | 1.32±0.13              | 43%      |
| 10-20 | 12.40±1.02   | 1.23±0.02   | 0.14±0.03   | 32.16±3.56  | 7.80±0.17 | 1.38±0.20              | 41%      |
| 20-40 | 12.36±1.89   | 1.10±0.04   | 0.13±0.02   | 31.48±3.21  | 7.81±0.15 | 1.40±0.15              | 40%      |

Table 1. The physical and chemical properties of 0-40 cm soil in test fields.

In the data statistics, the average and standard
development data are retained with two significant figures,
and the drawing is performed with Excel 2010 software.
Data correlation analysis, cluster analysis and path
analysis are carried out by SPSS 17.0 software, the
correlation coefficient data are retained with four
significant figures, and the path coefficient data are retained with three significant figures. The three-dimensional fluorescence is processed by MATLAB 2007 software.

**Results**

Changes of DOM Fluorescence Components in 0-10 cm Soil of Spring Maize Field under Different Fertilization Conditions

The 0-10 cm soil is the soil-crop interface, and the DOM component changes are sensitive to fertilization and crop growth. The EEM changes of 0-10 cm soil DOM were more complex (Fig. 1). The $P_{(I,n)}$ of DOM ranged from 0.86% to 6.45%, $P>NPK>NP>NPM>P>M>NM>NPKM>M>CK>BS$. All treatments were significantly ($P<0.05$) higher than those CK and before sowing. The $P_{(II,n)}$ of DOM ranged from 3.02% to 10.45%, $NP>P>NPK>NPM>PM>M>NPKM>N>NP>CK>BS$. All fertilization treatments were significantly ($P<0.05$) higher than CK and before sowing, and the applications of N, M and NM were significantly ($P<0.05$) lower than other fertilization treatments. The $P_{(III,n)}$ ranged from 18.74% to 25.81%, $NPKM>PM>M>NPK>NPM>NM>NP>CK>BS$. All treatments were significantly ($P<0.05$) higher than those CK and before sowing, and the applications of N, M and NM were significantly ($P<0.05$) lower than other fertilization treatments. The $P_{(IV,n)}$ ranged from 8.40% to 16.65%, $NP>N>NP>M>NPK>M>NPKM>CK>BS$. All the treatments were significantly ($P<0.05$) higher than those CK and before sowing, the applications of NP were significantly ($P<0.05$) lower than other fertilization treatments. The $P_{(V,n)}$ ranged from 44.61% to 68.98%, $BS>CK>NPKM>M>NM>NPK>PM>P>NPKM>NP$. All the treatments were significantly ($P<0.01$) lower than CK and before sowing.

![Fig. 1. EEM $P_{(in)}$ of DOM in 0-10 cm soil under different fertilization measures.](image)

**Dendrogram using Average Linkage (0-10cm)**

| CASE | 0 | 5 | 10 | 15 | 20 | 25 |
|------|---|---|----|----|----|----|
| Label | + | + | + | + | + | + |
| P    | + |   |   |   |   |   |
| NPM  | + |   |   |   |   |   |
| NP   | + |   |   |   |   |   |
| N    |   |   |   |   |   |   |
| NM   |   |   |   |   |   |   |
| M    |   |   |   |   |   |   |
| NP   |   |   |   |   |   |   |
| BS   |   |   |   |   |   |   |
| CK   |   |   |   |   |   |   |

*Region V* | *Region IV* | *Region III* | *Region II* | *Region I*
those CK and before sowing. Clustering analysis on the proportions of different components of DOM showed that it can be clearly divided into two categories, CK and various fertilization treatments. Various fertilization treatments can be divided into three categories: all the treatments applied with N fertilizer were one category, all the treatments applied with phosphate fertilizer were another category, and the treatments of NM, M, and NPKM were the other category. It revealed that the influence mechanism of nitrogen fertilizer, phosphorus fertilizer and organic fertilizer on DOM was different.

Changes of DOM Fluorescence Components in 10-20 cm Soil of Spring Maize Field under Different Fertilization Conditions

The 10-20 cm soil layer is the main distribution layer of spring maize root system. Fertilization and root system had great influence on soil microecology. The EEM of soil DOM could reflect the soil fertilizer supply capacity (Fig. 2). The $P_{(I,n)}$, $P_{(II,n)}$, $P_{(III,n)}$, $P_{(IV,n)}$ of 10-20 cm soil DOM were lower than 0-10 cm soil DOM, and the $P_{(V,n)}$ were higher than 0-10 cm. The $P_{(I,n)}$, $P_{(II,n)}$, $P_{(III,n)}$ and $P_{(IV,n)}$ of all the treatments were significantly ($P<0.05$) higher than those CK and before sowing. The $P_{(V,n)}$ of all the treatments ranged from 0.77% to 6.68%, M>PM>NPK>NPM>M>NPKM>P>NP>CK>BS, the treatment of M was significantly ($P<0.05$) higher than others treatments. The $P_{(I,n)}$ of all the treatments ranged from 2.75% to 11.08%, NPK>PM>M>N>NPM>P>NPKM>NM>NP>CK>BS, the treatment of NPK and PM were significantly ($P<0.05$) higher than other treatments. The $P_{(II,n)}$ of all the treatments ranged from 17.70% to 25.58%, NM>NPKM>PM>N>NPKM>P>M>NPK>N>CK>BS, the treatment of N was significantly ($P<0.05$) lower than other treatments. The $P_{(IV,n)}$ of all the treatments ranged from 0.77% to 6.68%, M>PM>NPK>NPM>M>NPKM>P>NP>CK>BS, the treatment of M was significantly ($P<0.05$) lower than others treatments. The $P_{(V,n)}$ of all the treatments ranged from 0.77% to 6.68%, M>PM>NPK>NPM>M>NPKM>P>NP>CK>BS, the treatment of M was significantly ($P<0.05$) lower than others treatments.

![Fig. 2. EEM $P_{(I,n)}$ of DOM in 10-20 cm soil under different fertilization measures.](image-url)
Changes of DOM Fluorescence Components in 20-40 cm Soil of Spring Maize Field under Different Fertilization Conditions

The 20-40 cm soil layer is the plough pan, which is less affected by crops and fertilization. The EEM of soil DOM was mainly affected by microorganisms (Fig. 3). The $P_{i,n}$ of 20-40 cm soil DOM were lower than 10–20 cm soil DOM. The $P_{V,n}$ were higher than 10–20 cm. The $P_{I,n}$, $P_{II,n}$, and $P_{V,n}$ of all the treatments were significantly ($P<0.05$) higher than those CK and before sowing, and the $P_{V,n}$ were significantly ($P<0.05$) lower than those CK and before sowing. The $P_{I,n}$ of all the treatments ranged from 0.91% to 5.18%, $M>PM>NP>NM>NPKM>N>P>NPKM>CK>BS$, the treatment of $P$ and $NPKM$ were significantly ($P<0.05$) lower than other treatments. The $P_{II,n}$ of all the treatments ranged from 3.07% to 9.79%, $PM>M>NPK>NM>NPM>N>P>NPKM>CK>BS$, and the treatment of $NP$ and $NPKM$ were significantly ($P<0.05$) lower than other treatments. The $P_{III,n}$ of all the treatments ranged from 18.72% to 28.20%, $NPKM>PM>NM>NPM>NPK>M>N>NP>P>CK>BS$, and the treatment of $NPKM$ was significantly ($P<0.05$) higher than other treatments. The $P_{IV,n}$ of all the treatments ranged from 8.70% to 14.33%, $NPK>M>NP>N>NP>M>NPM>N>CK>BS$, the treatment of $N$ and $NPK$ were significantly ($P<0.05$) higher than other treatments. The $P_{V,n}$ of all the treatments ranged from 42.96% to 70.27%, $BS>CK>NP>NPKM>PM>P>NPKM>N>NP>NPKM>NP>CK>BS$, the treatment of $P$ and $NPKM$ were significantly ($P<0.05$) higher than other treatments. The $P_{V,n}$ of all the treatments ranged from 8.50% to 17.37%, $N>NPK>M>PM>P>NPM>NK>NP>P>NPKM>NP>CK>BS$, and the treatment of $NP$ and $NPKM$ were significantly ($P<0.05$) higher than other treatments. The $P_{V,n}$ of all the treatments ranged from 0.91% to 5.18%, $M>PM>NP>NM>NPKM>N>NP>NPKM>CK>BS$, the treatment of $P$ and $NPKM$ were significantly ($P<0.05$) lower than other treatments.

The 20-40 cm soil layer is the plough pan, which is less affected by crops and fertilization. The EEM of soil DOM was mainly affected by microorganisms (Fig. 3). The $P_{i,n}$ of 20-40 cm soil DOM were lower than 10–20 cm soil DOM. The $P_{V,n}$ were higher than 10–20 cm. The $P_{I,n}$, $P_{II,n}$, $P_{III,n}$, and $P_{IV,n}$ of all the treatments were significantly ($P<0.05$) higher than those CK and before sowing, and the $P_{V,n}$ were significantly ($P<0.05$) lower than those CK and before sowing. The $P_{I,n}$ of all the treatments ranged from 0.91% to 5.18%, $M>PM>NP>NM>NPKM>NPK>N>P>NPKM>CK>BS$, the treatment of $P$ and $NPKM$ were significantly ($P<0.05$) lower than other treatments. The $P_{II,n}$ of all the treatments ranged from 3.07% to 9.79%, $PM>M>NPK>NM>NPM>N>P>NPKM>CK>BS$, and the treatment of $NP$ and $NPKM$ were significantly ($P<0.05$) lower than other treatments. The $P_{III,n}$ of all the treatments ranged from 18.72% to 28.20%, $NPKM>PM>NM>NPM>NPK>M>N>NP>P>CK>BS$, and the treatment of $NPKM$ was significantly ($P<0.05$) higher than other treatments. The $P_{IV,n}$ of all the treatments ranged from 8.70% to 14.33%, $NPK>M>NP>N>NP>M>NPM>N>CK>BS$, the treatment of $N$ and $NPK$ were significantly ($P<0.05$) higher than other treatments. The $P_{V,n}$ of all the treatments ranged from 42.96% to 70.27%, $BS>CK>NP>NPKM>PM>P>NPKM>N>NP>NPKM>NP>CK>BS$, and the treatment of $P$ and $NPKM$ were significantly ($P<0.05$) lower than other treatments.
BS>CK, and the treatment of NPKM was significantly (P<0.05) lower than other treatments. The P(V,n) of all the treatments ranged from 46.19% to 68.52%, BS>CK>NPKM>P>NP>N>NPM>NPK>M>PM, and the treatment of NPKM was significantly (P<0.05) higher than other treatments. Clustering analysis on the proportions of different components of DOM showed that it can be clearly divided into three categories: CK, NPKM fertilization treatments and other fertilization treatments.

Effects of Fertilization on Soil DOM Fluorescence Parameters in Spring Maize Farmland

Fluorescence parameter is an important indicator to reveal the source and degradation characteristics of soil DOM. It can reflect the origin and evolution of soil organic matter in a certain extent, and then reflect the fertility of soil. Fluorescence index (f_450/500) is the ratio of fluorescence intensity at 450 nm and 500 nm when the excitation wavelength is 370 nm. It can be used to distinguish the source of organic matter [21]. McKnight et al. [22] revealed that: the f_450/500>1.9 indicated that DOM was mainly derived from microbial metabolites in the soil, the f_450/500 <1.4 indicated that the DOM was mainly derived from external input. After planting for 4 years, the f_450/500 of spring maize field soil samples were lower than that before planting, the f_450/500 of fertilization treatments were all lower than CK (Fig. 4a). The f_450/500 was the highest in 10-20 cm soil layer and the lowest in 20-40 cm soil layer. In 0-10 cm, BS>CK>NM>P>PM>NPKM>NP>NPK>N>NP>M; in 10-20 cm, BS>CK>NM>P>PM>NPKM>N>NP>NPK>NPM; in 20-40 cm, BS>CK>NPKM>PM>NPK>N>NP>M. Therefore, planting spring maize and fertilization could increase DOM external input. The treatment of M increased DOM microbial source in 10-20 cm soil and decreased DOM microbial source in 0-10 cm and 20-40 cm soil. The treatment of NPK and NPKM increased DOM microbial source in -40 cm soil. The treatment of NPK and NPKM promoted DOM microbial sources in the 20-40 cm soil.

Biological index (BIX) is the fluorescence intensity ratio of the emission wavelength at 380 nm and 430 nm, when the excitation wavelength at 254 nm, which can be used to distinguish the source of organic matter. Huguet et al. [23] revealed that: with the increase of BIX, the autogeneic characteristics of DOM were enhanced and the terrigenous characteristics were decreased. With the deepening of soil layer, BIX showed a downward trend (Fig. 4b). In 0-10 cm, the BIX was M>CK>B S>NPKM>NP>P>NPM>NPK>N>NP>N. In 10–20 cm, the BIX was CK>N>BS>P>NPKC-NMP>NPKM-NPM >NP>NPM>N>PM. In 20–40 cm, the BIX was NPM-NM> CK>M>NPKM>NP>NPK>N>BS>P>PM. The f_450/500 and BIX revealed that the fertilization and planting of spring maize increased the terrigenous characteristics of 0-20 cm soil DOM, the treatment of N and P increased DOM biogenic characteristics in 0-20 cm soil layer, and the treatment of M increased DOM biogenic characteristics in 0-10 cm soil layer.

Effects of Fertilization on Soil Microorganism and Enzyme Activities in Spring Maize Field

Soil microorganism is an important source of DOM and a major factor affecting its composition. After planting for 4 years, the content of BMC of different layer were higher than that before sowing (Fig. 5a), fertilization treatments were all higher than CK. With the deepening of soil layer, BMC content decreased. The BMC content in 0-10 cm soil was highest in NPKM treatment, lowest in N treatment, and that of the application of organic fertilizer was higher than that of chemical fertilizer. The BMC content in 20-40 cm soil was the highest in NPM, the lowest in the treatment of N, and that of the application of organic fertilizer was higher than that of chemical fertilizer. After planting for 4 years, the content of BMN in 0-20 cm soil layer were higher than that before sowing (Fig. 5b), the BMN content in 0-10 cm soil layer of all fertilization treatments was higher than that of CK, the BMN content in 20-40 cm of all fertilization treatments was lower than that of CK and before.

Fig. 4. EEM parameter of DOM in 0-40 cm soil under different fertilization measures.
sowing. With the deepening of soil layer, BMN content decreased. In 0-20 cm, the BMN content was the highest in the N treatment, was the lowest in the M treatment. It revealed that the application of organic fertilizer had increased the content of BMC, and alone application of N had increased the content of BMN and had decreased the content BMC. Planting maize and fertilization had increased the content of BMC and the content of BMN in 0-10 cm soil layer.

Soil enzyme activity is an important factor affecting soil DOM components. Soil sucrase activities under different fertilization treatment were shown in Fig. 6a). In 0-10 cm soil, the sucrase activity of NPK treatment was the highest, and that of NPKM treatment was the lowest. The sucrase activity of organic fertilizer treatments was lower than that of chemical fertilizer treatments, CK, and before sowing. In 10-20 cm soil, the sucrase activity of fertilization treatments were
lower than that of CK and before sowing, the treatment of NPK was the highest, and the treatment of N was the lowest. In 20-40 cm soil, the sucrase activity of fertilization treatments were lower than that of CK, and higher than that of before sowing, the treatment of M was the highest, and the treatment of NPKM was the lowest. After planting for 4 years, soil urease activities under different fertilization treatments were shown in Fig. 6b). The urease activities of all soil layers were lower than that of before sowing. In 0-10 cm soil, the treatment of NPKM was the highest, and the treatment of M was the lowest. In 10-20 cm, the treatment of P was the highest, the treatment of PM was the lowest, and the treatment of organic fertilizer treatments was lower than that of chemical fertilizer treatments. In 20-40 cm, the treatment of fertilizer was higher than CK, the treatment of P was the highest, and the treatment of NPK was the lowest. Soil catalase activity under different fertilization treatments was shown in Fig. 6c). In 0-10 cm, the treatment of NPK was the highest, and the treatment of NPM was the lowest. In 10-20 cm, the fertilization treatment was higher than before sowing and CK, the treatment of NM was the highest, the treatment of NPM was the lowest. In 20-40 cm, the fertilization treatments were higher than CK, the treatment of M was the highest, and the treatment of NPKM was the lowest. Soil phosphatase activities under different fertilization treatments were shown in Fig. 6d). After planting for 4 years, the soil phosphatase activities of different layer were lower than that of before sowing. In 0-10 cm, the treatment of NP was the highest, and the treatment of N was the lowest. In 10-20 cm, the treatment of P was the highest, and the treatment of PM was the lowest. In 20-40 cm, the fertilization treatments were higher than CK, the treatment of M was the highest, and the treatment of NPKM was the lowest.

Discussion

Effects of Soil Microorganisms and Enzyme Activities on the Three-Dimensional Fluorescence Characteristics of Soil DOM

Soil microorganisms are the main participants of soil organic matter mineralization and carbon and nitrogen cycling, and are also the drivers of soil nutrient transformation and supply [24], which can affect the availability of soil nutrients, plant development and environmental quality. They play a key role in soil ecosystem functions, including pollutant biodegradation, biogeochemical cycling, and soil formation [25-26]. The content of microbial carbon and nitrogen is an important index to soil microbial activity [27]. The correlation analysis between soil microbial biomass and DOM fluorescence components was shown in Table 2. There was a significant positive correlation between MBC and Region I, Region II, Region III and Region IV, and there was a significant negative correlation between MBC and Region V and $f_{450/500}$. It revealed that with the increase of soil microbial biomass, the protein like, fulcruide-like acid and microbial metabolite component in soil DOM showed an upward trend, and the humic acids component in soil DOM showed a downward trend. The increase of microbial biomass promoted the microbial source of soil DOM, that was the macromolecule DOM were degraded to the small molecule DOM by soil microorganisms.

Based on Table 2, Sucrase activity had a significantly negative correlation with Region III, had a significantly positively correlation with BIX. Urease activity had a very significantly negative correlation with Region I, Region II, Region III and Region IV, and had a very significantly positive correlation with Region V and $f_{450/500}$. Catalase had a very significantly positive correlation with Region I, Region II, Region IV, had a significantly positive correlation with Region III, had a very significantly negative correlation with Region V, and had a significantly negative correlation with $f_{450/500}$. The degradation of DOM in soil was roughly divided into two steps. One was the macromolecular organics degraded into simple organics, in this step, the proportion of humic acids components soil DOM decreased, and the microbial source characteristics increased. The other step was the simple organic matter degraded into water and CO$_2$ to provide nutrients to the plants, in this step, the proportion of small molecule components of soil DOM (such as protein-like, fulcruide-like acid, microbial metabolites) decreased. Sucrase could hydrolyze sucrose in soil into glucose and fructose [28], and drive the mineralization of soil carbon which mainly hydrolyzed the fulvic acids component to mineralize carbon. Soil urease was the only enzyme that catalyzed urea hydrolysis, and its activity could reflect the level of soil nitrogen supply [29]. Soil urease could degrade small molecule DOM into inorganic substances to supply nitrogen to crops. Catalase activity could reflect the transformation of carbon and nitrogen in soil [8], its main purpose was to degrade large molecule DOM into small molecule DOM. Phosphatase had little effect on soil DOM.

Effects of Nutrient Salts Content on Three-Dimensional Fluorescence Characteristics of Soil DOM

The content of soil nutrient salts reflects soil fertility and has an impact on soil microorganisms and crop growth, so it can affect the DOM components. The correlation analysis between soil nutrient salts content and DOM components was shown in Table 3. TK promoted the proportion of DOM of protein-like, fulvic acids and microbial metabolite components, and decreased the proportion of DOM of humic acids components. The main reason was that TK promoted soil MBC content and inhibited soil urease activity. TP and AP promoted the ratio of DOM of protein-like,
fulvic acids and microbial metabolite components, and decreased the ratio of DOM of humic acids components. The main reason was that TP inhibits the sucrase activity, decreased the degradation of small molecule DOM, increased the activity of catalase, promoted the degradation of large molecule DOM.

AP increased microbial biomass, and then promoted the conversion of soil large molecular DOM to small molecule DOM. TOM increased the proportion of fulvic acids components and decreased the proportion of humic acids components, the main reason was that TOM increased microbial biomass, inhibited the sucrase activity, decreased the degradation of small molecule DOM, increased the catalase activity, and promoted the degradation of large molecule DOM. DOC promoted the DOM ratio of protein-like, fulvic acids and microbial metabolites, decreased the DOM ratio of humic acids, mainly because DOC increased the soil microbial biomass, inhibited the activities of sucrase and urease, promoted the degradation of large molecule DOM, and inhibited the degradation of small molecule DOM.

Effects of Spring Maize Yield on Three-Dimensional Fluorescence Characteristics of Soil DOM

DOM is a relatively active organic component in soil, which can be biodegraded into inorganic salts for biological use, and small molecules DOM can also be directly absorbed and utilized by crops [30]. The crop residues and root exudates are also the main sources of soil DOM. Therefore, the effects of crops on soil DOM are various. Figs 1-3 revealed that, after planting 4 years, the proportions of protein-like, fulvic acids and microbial metabolites in the soil DOM were significantly increased compared with before sowing.

| Table 2. Correlations of EEM components of DOM, enzymatic activity, microbial carbon and nitrogen in soil. |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Relevance         | MBC                  | MBN                | Sucrase           | Urease           | Catalase         | Phosphatase       |
| Region I       | 0.6606**              | 0.2548             | -0.0537          | -0.6130**       | 0.5357**        | -0.2642          |
| Region II      | 0.6452**              | 0.2432             | -0.0751          | -0.5569**       | 0.5126**        | -0.1859          |
| Region III     | 0.5618**              | -0.0096            | -0.3861*         | -0.5785**       | 0.4236*         | -0.3063          |
| Region IV      | 0.5123**              | 0.2829             | 0.0133           | -0.4870**       | 0.4380**        | -0.1172          |
| Region V       | -0.6530**             | -0.2096            | 0.1440           | 0.6145**        | -0.5244**       | 0.2370           |
| \( f_{450/500} \) | -0.5366**             | -0.1133            | 0.2445           | 0.5947**        | -0.3656*        | 0.2244           |
| \( BIX \)      | -0.0681               | 0.3017             | 0.3428*          | 0.1277          | -0.2717         | -0.0019          |

Note: \( n = 33 \), * is a significant correlation when \( P<0.05 \); ** is a highly significant correlation when \( P<0.01 \)

| Table 3. Correlations of EEM components of DOM and nutrient salts in soil. |
|-----------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Relevance         | TK              | AK              | TN              | AK              | TP              | AP              | TOM              | DOC             |
| Region I       | 0.4222*              | -0.0135           | 0.1303           | -0.0765         | 0.5446**        | 0.4255*         | 0.3220          | 0.5569**        |
| Region II      | 0.4356*              | -0.0637           | 0.0978           | -0.1706         | 0.5286**        | 0.4643**        | 0.3307          | 0.5546**        |
| Region III     | 0.4175*              | -0.3146           | -0.0926          | -0.1331         | 0.5530**        | 0.4638**        | 0.5367**        | 0.6916**        |
| Region IV      | 0.4533**             | 0.1768            | 0.1916           | -0.0673         | 0.4143*         | 0.3581*         | 0.1562          | 0.4975**        |
| Region V       | -0.4806**             | 0.0613            | -0.0874          | 0.1274          | -0.5622**       | -0.4747**       | -0.3729*        | -0.6390**       |
| \( f_{450/500} \) | -0.5060**             | 0.0805            | -0.0191          | 0.0932          | -0.4384**       | -0.5020**       | -0.3615*        | -0.6809**       |
| \( BIX \)      | 0.0257               | 0.1220            | 0.2854           | 0.2857          | -0.4928**       | -0.1356         | -0.3412         | -0.3240         |
| MBC             | 0.3418*              | -0.2985           | 0.1507           | -0.0718         | 0.3258          | 0.7712**        | 0.4395**        | 0.3949*         |
| MBN             | 0.1188               | -0.0877           | 0.3465*          | 0.2676          | -0.1096         | 0.4382**        | -0.0759         | 0.0319          |
| Sucrase         | 0.1911               | 0.3960*           | 0.2608           | 0.3828*         | -0.4410**       | 0.0359          | -0.5040**       | -0.4311*        |
| Urease          | -0.5395**             | 0.0665            | 0.1237           | -0.2188         | 0.0178          | -0.1076         | 0.0327          | -0.4699**       |
| Catalase        | 0.0902               | 0.0995            | 0.1470           | -0.4124*        | 0.6887**        | 0.0821          | 0.4153*         | 0.2077          |
| Phosphatase     | -0.2524              | 0.0784            | 0.1338           | -0.2041         | 0.1433          | 0.1238          | 0.0477          | -0.2277         |

Note: \( n = 33 \), * is a significant correlation when \( P<0.05 \); ** is a highly significant correlation when \( P<0.01 \)
the proportion of humic acids in soil DOM was significantly decreased. Therefore, the growth of spring maize could promote the soil macromolecule DOM to transform small molecule. The correlation analysis was shown in Table 4. Maize yield had a significantly positively relation with Region I and Region II, had a very significantly positively relation with Region III, and had a very significantly negative correlation with Region V. The growth of spring maize had different effects on different soil layers. In the 0-10 cm surface soil, the growth of spring maize could improve the soil physical and chemical properties to effect the change of soil DOM components, especially increased the soil oxidation environment, and then increased the microbial biomass (Table 4), thus promoted the transformation of DOM from large molecules to small molecules. In the 10-20 cm root distribution soil, the growth of spring maize had double function of root absorption and the root exudates supplement. Spring maize could use the small molecule DOM such as soluble microbial metabolites and proteins during the growth process. Therefore, the correlation between spring maize yield and DOM components was poor compared with that of surface soil (Table 4). The main influence on microorganism was MBC, and which has a low correlation with MBN. In the 20-40 cm plough bottom soil, the main effect of growth of spring maize was the degradation of DOM by roots, so there was a significantly negative correlation with the humic acids components of DOM, and had a significantly positive correlation with the fulvic acids components of DOM.

The Main Controlling Factors Affecting the Three-Dimensional Fluorescence Characteristics of Soil DOM

According to the results of correlation analysis, the path analysis was carried out between DOM components and the factors such as MBC, catalase, sucrase, TP, TK and crop yield that had good correlation with DOM fluorescence components in the soil. The results were shown in Table 5. Compared with other factors, DOC had a significant effect on the DOM components, had a positive effect on the Region I, Region II, Region IV, and had a negative effect on the Region V. TP had a dominant effect on Region III, spring maize yield has a secondary effect on Region I, and sucrase activity had a secondary effect on Region II, Region IV and Region V. DOC content could represent DOM content to a certain extent [31], and was the main source of DOM components, while the characteristics of DOM components were also affected by degradation of soil enzymes and crop growth.

The Influence Mechanism of Fertilization Measures on Three-Dimensional Fluorescence Characteristics of Soil DOM

Nowadays fertilization mainly includes the application of organic fertilizer and inorganic fertilizer. According to the measures of fertilizer application, fertilization can be divided into single fertilizer application and mixed fertilizer application. Manure can not only directly increase soil DOM, but also indirectly

| Relevance | Soil (0-10 cm) | Soil (10-20 cm) | Soil (20-40 cm) |
|-----------|---------------|-----------------|---------------|
| Relevance | Yield | Thousand grain weight | Yield | Thousand grain weight | Yield | Thousand grain weight |
| Region I  | 0.6724* | 0.0573 | 0.5344 | 0.3755 | 0.4388 | 0.3397 |
| Region II | 0.7160* | 0.2441 | 0.6286* | 0.2676 | 0.4776 | 0.2547 |
| Region III | 0.7522** | 0.5473 | 0.6740* | 0.4054 | 0.8293** | 0.5630 |
| Region IV | 0.5089 | 0.0624 | 0.5068 | 0.0786 | 0.3380 | 0.0808 |
| Region V  | -0.7509** | -0.2558 | -0.6980* | -0.3174 | -0.6514* | -0.3742 |
| \( f_{\text{ecos}} \) | -0.8150** | -0.5213 | -0.7664** | -0.2874 | -0.7116* | -0.2831 |
| BILX  | -0.3909 | -0.1270 | -0.4629 | -0.4804 | -0.0211 | 0.2511 |
| MBC   | 0.7349** | 0.6488* | 0.7899*** | 0.6296* | 0.4654 | 0.5549 |
| MBN   | 0.7948** | 0.5585 | 0.3648 | 0.3418 | -0.6924* | -0.5059 |
| Sucrase | -0.2412 | -0.1153 | -0.5660 | -0.2049 | -0.4855 | -0.0825 |
| Urease | 0.0434 | 0.3725 | 0.3138 | -0.0743 | 0.2226 | -0.4597 |
| Catalase | 0.4632 | 0.2788 | 0.6461* | 0.3650 | 0.4495 | 0.4940 |
| Phosphatase | 0.5643 | 0.5923 | 0.4303 | -0.0154 | 0.0367 | -0.6009 |

Note: \( n = 10, * \) is a significant correlation when \( P<0.05 \); \( ** \) is a highly significant correlation when \( P<0.01 \)}
affect the characteristics of DOM components by changing soil microbial and enzyme activities, and promote crop growth. In this experiment, the soil of 10-20 cm and 20-40 cm soil layer treated with manure could increase the Region I, Region II, Region III, Region IV, and decrease the Region V. Organic fertilizer could not only increase active organic carbon and humic substances [35]. The correlation analysis of Table 1 and Table 2 showed that, with the increase of the content of soil DOC and MBC, the Region I, Region II, Region III, Region IV maintained an upward trend, Region V showed a downward trend. The increasing organic fertilizer application increased the activity of catalase, decreased the activity of sucrase (Fig. 6), which revealed that the increasing application of organic fertilizer was beneficial to promote the transformation of large molecule DOM to small molecule DOM. Nitrogen, phosphorus and potassium were the main inorganic fertilizers. The experiment showed that single nitrogen could improve the Region IV of 0-20 cm soil. The N treatment in 0-10 cm soil could be grouped into one category, which had different effects on DOM components from other treatments (Fig. 1). Nitrogen application alone could significantly increase the BIX of DOM (Fig. 4) and decrease the MBC content (Fig. 5). Previous studies had shown that long-term single application of nitrogen fertilizer led to soil acidification and decreased the pH value, which seriously reduced the microbial community that promoted root function [36-37]. Continuous application of nitrogen fertilizer for a short time (4 years) could promote the release of metabolites and inhibit microbial activity. Phosphorus and potassium fertilizer was the main means to increase the contents of phosphorus and potassium in soil, especially the content of activated phosphorus and activated potassium. The phosphorus and potassium content in soil had a significantly positive correlation with Region I, Region II, Region III and Region IV, had a significantly positive correlation with Region V (Table 2). Single application of phosphorus fertilizer could significantly increase the proportion of protein-like DOM in 0–10 cm soil layer, mainly because phosphorus was the main element of biological genetic material, and could promote the synthesis of biological protein.

Fertilizer co-application is an important measure to improve soil fertility efficiency. The results showed that the co-application of N, P, K and organic fertilizer could increase significantly the proportion of humic acids and fulvic acids in DOM, and decrease the proportion of protein-like components in DOM. The treatment of NPKM separately clustered into one category in the 20-40 cm soil layer, which could increase the content of soil nutrients, especially the content of active components of soil nutrients and the content of MBC and MBN in soil (Fig. 5), decreased the activities of catalase and sucrase, increased the ability of soil fertility preservation. The co-application of N, P could significantly increase the proportion of humic acids components of DOM in 10-20 cm soil layer, decreased the proportion of protein-like components of DOM and microbial metabolites (Fig. 2). The main reason was that the treatment of NP decreased the catalase activity (Fig. 6) and inhibited the conversion of large molecular components to small molecular components of DOM. The co-application of N, P, K was the main application method of inorganic fertilizer, the treatment could increase the proportion of microbial metabolites and protein-like components of DOM in 10-40 cm soil layer, decreased the proportion of humic acids components, which reason is that the treatment of NPK increased the soil nutrient content and MBC content, promoted soil catalase and sucrase activities, as well as increased the yield and root activity of spring maize, and promoted the transformation of soil DOM. The co-application of inorganic-organic fertilizers could decrease the degradation of DOM, while the co-application of inorganic fertilizer could promote the degradation of DOM. At the same time, mixed fertilization treatment promoted the growth of spring maize and increased root biomass, thus enhanced the effect of fertilization on DOM in the deep (20-40 cm) soil layer.

Table 5. Path analyses of EEM components of DOM, enzymatic activity, microbial carbon, nutrient salts of soil (0-20 cm) and yield of spring corn.

| Path coefficient | MBC | TK | TP | DOC | Catalase | Sucrase | Yield | R² |
|------------------|-----|----|----|-----|----------|---------|-------|----|
| Region I         | 0.392 | 0.018 | 0.182 | 0.711 | 0.343 | 0.250 | -0.437 | 0.955 |
| Region II        | 0.384 | 0.038 | 0.293 | 0.698 | 0.208 | 0.438 | -0.221 | 0.841 |
| Region III       | 0.069 | 0.021 | 0.859 | -0.380 | -0.107 | -0.366 | 0.251 | 0.874 |
| Region IV        | 0.345 | 0.229 | -0.487 | 1.899 | 0.623 | 0.776 | -0.667 | 0.934 |
| Region V         | -0.302 | -0.051 | -0.212 | -0.786 | -0.322 | -0.355 | 0.263 | 0.832 |
Conclusions

The component of soil DOM is an important factor affecting the soil micro-ecological environment. The partial application of a fertilizer in farmland would promote the conversion of macromolecular DOM to small molecules, which is likely to cause soil carbon loss and reduce soil nutrient micro-immobilization. Therefore, the combined application of inorganic-organic fertilizers is beneficial to increase the carbon sequestration capacity in farmland soil and to reduce nutrient loss from soil microecology.

The components of DOM were significantly different between different fertilization treatments. Compared with before sowing, the proportion of humic acids components of DOM was significantly decreased and the proportion of other components was significantly increased after consecutive planting spring corn for four years. Compared with the control, the treatment of fertilization significantly decreased the proportion of humic acids components in DOM, and significantly increased the proportion of other components. The treatment of fertilization affected the components of DOM and the microecological environment by affecting soil microorganisms, enzyme activities and spring maize biomass. Sucrase mainly degraded small molecules of DOM such as fulvic acids and protein-like components. Catalase mainly degraded humic acids and other large molecules of DOM, and urease mainly degraded protein-like components of DOM. DOC was the main source of DOM components, and the characteristics of DOM components were affected by the degradation of soil enzymes and crop growth. Increasing application of organic fertilizer was beneficial to promote the conversion of large molecules to small molecules of DOM. The treatment of N fertilizer affected soil environment, changed soil microecology, and increase the proportion of microbial metabolites of soil DOM in a short period of time. P and K in soil had significant effects on DOM components. The increase of P and K content decreased the proportion of humic acids components of DOM, and increased the proportion of other components. The mixed treatment of inorganic-organic fertilizers was beneficial to increase the proportion of humic acids components of DOM. Fertilization mainly affected the components of DOM in the 0-20 cm soil layer, the co-application of fertilizers promoted the growth and yield of spring maize, and increased the influence on the DOM in the 20-40 cm soil layer.

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Conflict of Interest

The author declares no conflict of interest.

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Schedule

Schedule 1. Annual changes of yield and thousand grain weight of spring corn under different fertilization treatments.

| Year | Yield (kg.hm⁻²) | Thousand grain weight (g) |
|------|----------------|--------------------------|
|      | CK            | N            | P            | NP           | NPK          | M            | NM           | PM            | NPM           | NPKM          |
| 2011 | 9910.95       | 10869.30     | 9799.80      | 11100.75     | 11692.80     | 12068.10     | 10060.65     | 9645.75       | 9632.10       | 11885.10     |
| 2012 | 7878.45       | 11359.20     | 10970.10     | 11128.20     | 12820.35     | 10276.50     | 11295.90     | 10638.60      | 12367.50      | 13135.80     |
| 2013 | 7299.90       | 10752.75     | 11160.45     | 11170.50     | 12053.85     | 10156.80     | 11382.60     | 11061.30      | 11773.20      | 12267.45     |
| 2014 | 8894.70       | 11114.25     | 10384.95     | 11114.40     | 12256.50     | 11172.30     | 10678.20     | 10142.10      | 10999.80      | 12510.45     |

| Year | CK            | N            | P            | NP           | NPK          | M            | NM           | PM            | NPM           | NPKM          |
|------|----------------|--------------|--------------|--------------|--------------|--------------|--------------|---------------|---------------|---------------|
| 2011 | 325.45         | 315.95       | 282.35       | 325.80       | 327.10       | 333.25       | 298.55       | 273.20        | 304.95        | 329.90        |
| 2012 | 327.18         | 313.57       | 303.76       | 328.94       | 341.42       | 331.22       | 340.34       | 341.53        | 342.20        | 345.57        |
| 2013 | 282.57         | 320.17       | 310.36       | 335.54       | 348.02       | 337.82       | 346.94       | 348.13        | 348.80        | 352.17        |
| 2014 | 326.32         | 314.76       | 293.05       | 327.37       | 334.26       | 332.23       | 319.45       | 307.36        | 323.58        | 337.74        |
Schedule 2. Contents of nutrient salts, microbial carbon and nitrogen in the soil of spring corn fields under different fertilization treatments.

| Soil layer | Fertilization | TK (g·kg⁻¹) | AK (mg·kg⁻¹) | TN (g·kg⁻¹) | AN (mg·kg⁻¹) | TP (g·kg⁻¹) | AP (mg·kg⁻¹) | TOC (g·kg⁻¹) | DOC (mg·kg⁻¹) |
|------------|---------------|-------------|--------------|-------------|--------------|-------------|--------------|---------------|---------------|
| 0-10 cm    | BS            | 33.47±0.29  | 218.96±5.95  | 1.24±0.09   | 22.15±1.08   | 0.15±0.01   | 21.20±0.56   | 11.72±0.99   | 78.27±6.44    |
|            | CK            | 50.23±0.20  | 208.60±7.31  | 1.18±0.08   | 30.14±2.09   | 0.07±0.00   | 16.68±0.79   | 9.43±0.74    | 88.61±6.15    |
|            | N             | 43.64±0.23  | 212.51±4.33  | 1.35±0.08   | 78.81±2.16   | 0.13±0.03   | 21.62±1.04   | 10.85±0.71   | 238.58±2.60   |
|            | P             | 38.70±0.23  | 194.85±5.55  | 1.04±0.08   | 14.89±1.15   | 0.14±0.03   | 27.19±0.43   | 11.13±0.77   | 194.58±8.24   |
|            | NP            | 48.44±0.23  | 318.30±7.50  | 1.47±0.07   | 62.37±2.17   | 0.14±0.03   | 29.10±0.63   | 11.63±0.87   | 163.88±7.85   |
|            | NPK           | 50.08±0.24  | 269.95±7.25  | 1.25±0.08   | 22.19±1.14   | 0.14±0.04   | 27.48±0.86   | 12.89±0.86   | 172.68±1.44   |
|            | M             | 46.05±0.25  | 212.98±5.43  | 1.10±0.07   | 13.97±1.13   | 0.14±0.05   | 22.07±0.64   | 12.03±0.77   | 156.08±6.89   |
|            | NM            | 48.97±0.24  | 244.76±7.31  | 1.17±0.08   | 25.84±2.14   | 0.15±0.04   | 21.99±0.73   | 14.26±0.86   | 230.88±3.84   |
|            | NPM           | 41.80±0.24  | 97.15±5.31   | 0.93±0.07   | 30.93±2.15   | 0.14±0.04   | 28.81±0.38   | 13.20±0.76   | 291.98±5.67   |
|            | NPKM          | 45.26±0.26  | 102.17±7.50  | 1.10±0.13   | 34.06±1.12   | 0.15±0.02   | 34.47±0.37   | 13.72±0.87   | 182.38±3.81   |
| 10-20 cm   | BS            | 32.16±0.29  | 185.68±5.08  | 1.19±0.08   | 18.01±1.09   | 0.14±0.02   | 17.54±0.99   | 12.75±0.95   | 93.93±5.96    |
|            | CK            | 50.65±0.15  | 218.55±4.76  | 1.26±0.07   | 80.64±2.09   | 0.07±0.01   | 14.24±0.74   | 9.86±0.68    | 91.36±9.13    |
|            | N             | 47.47±0.17  | 227.73±4.13  | 1.25±0.07   | 16.67±1.17   | 0.14±0.02   | 23.14±0.60   | 11.58±0.70   | 244.58±9.09   |
|            | P             | 45.90±0.19  | 191.5±8.54   | 0.99±0.07   | 23.11±2.14   | 0.14±0.04   | 30.72±0.83   | 11.30±0.92   | 223.38±13.46  |
|            | NP            | 46.42±0.24  | 156.5±6.90   | 0.83±0.06   | 22.19±1.11   | 0.14±0.05   | 36.90±0.55   | 11.05±0.84   | 238.08±5.30   |
|            | NPK           | 47.26±0.17  | 165.6±7.78   | 0.94±0.07   | 14.89±1.16   | 0.13±0.03   | 35.2±0.89    | 12.8±0.88    | 182.38±9.48   |
|            | M             | 45.36±0.20  | 211.39±4.36  | 1.08±0.06   | 20.37±2.14   | 0.18±0.04   | 21.18±0.75   | 12.48±0.71   | 222.08±12.59  |
|            | NM            | 46.05±0.22  | 193.02±6.58  | 1.31±0.06   | 71.51±1.13   | 0.14±0.06   | 21.42±0.85   | 12.89±0.82   | 152.18±4.76   |
|            | PM            | 47.69±0.16  | 154.69±2.75  | 1.18±0.07   | 15.8±1.14    | 0.16±0.05   | 26.96±0.98   | 12.11±0.63   | 192.06±1.95   |
|            | NPM           | 34.08±0.14  | 109.63±3.97  | 1.2±0.06    | 22.15±2.14   | 0.14±0.05   | 32.0±0.94    | 13.76±0.69   | 188.08±8.26   |
|            | NPKM          | 48.21±0.23  | 88.23±6.02   | 1.22±0.06   | 22.10±1.12   | 0.15±0.06   | 26.38±0.52   | 14.51±0.80   | 263.88±9.33   |
The Effect Mechanism of Fertilization Measures

Schedule 2. Continued.

|     | BS       | CK        | N         | P         | NP        | NPK       | M         | NM        | PM        | NPM       | NPKM      |
|-----|----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| 20–40 cm | 31.48±0.19 | 39.65±0.19 | 46.88±0.24 | 46.30±0.23 | 47.04±0.24 | 50.19±0.24 | 46.02±0.24 | 42.63±0.24 | 44.10±0.23 | 48.62±0.23 | 40.78±0.29 |
|      | 177.98±4.35 | 193.78±3.33 | 186.09±7.37 | 224.38±7.46 | 229.03±4.34 | 258.94±3.91 | 231.98±4.25 | 233.94±5.19 | 86.84±4.54 | 213.28±5.76 | 138.45±5.19 |
|      | 1.08±0.09   | 0.61±0.06  | 1.06±0.08  | 0.71±0.07  | 1.00±0.06  | 0.95±0.06  | 1.18±0.07  | 1.29±0.06  | 0.99±0.07  | 0.97±0.13  | 1.01±0.13  |
|      | 14.14±1.09  | 64.20±2.09 | 11.23±1.14 | 19.41±1.13 | 36.80±2.14 | 23.11±1.14 | 18.49±1.14 | 37.72±1.14 | 17.58±1.13 | 26.76±2.13 | 25.75±1.09  |
|      | 0.13±0.01   | 0.07±0.00  | 0.13±0.04  | 0.15±0.04  | 0.15±0.04  | 0.15±0.05  | 0.15±0.05  | 0.15±0.05  | 0.17±0.016 | 0.155±0.049 | 0.153±0.008 |
|      | 10.01±0.47  | 12.62±0.58 | 12.56±0.64 | 19.91±0.55 | 21.53±0.84 | 14.70±0.43 | 18.29±0.37 | 12.62±0.53 | 20.41±0.53 | 26.38±0.40 | 27.89±0.63  |
|      | 12.63±0.91  | 9.45±0.62  | 11.09±0.86 | 12.69±0.87 | 12.38±0.71 | 12.72±0.69 | 13.13±0.71 | 12.32±0.75 | 12.52±0.72 | 14.20±0.66 | 14.94±0.63  |
|      | 71.69±4.81  | 123.75±10.28 | 258.88±10.16 | 207.48±4.08 | 209.48±5.99 | 113.68±5.48 | 230.08±10.95 | 285.38±7.11 | 178.81±3.84 | 169.58±4.76 | 314.18±3.29 |