Investigation of the spatial heterogeneity of soil microbial biomass carbon and nitrogen under long-term fertilizations in fluvo-aquic soil

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Abstract

Soils are heterogeneous and microbial spatial distribution can clearly indicate the spatial characteristics of the soil carbon and nitrogen cycle. However, it is not clear how long-term fertilization affects the spatial distribution of microbial biomass in fluvo-aquic soil. We collected fluvo-aquic soil samples (topsoil 0–7.5 cm and sub-topsoil 7.5–20 cm) using a spatially-explicit design within three 40.5 m² plots in each of four fertilization treatments. Fertilization treatments were: cropping without fertilizer inputs (CK); chemical nitrogen, phosphorus, and potassium fertilizer (NPK); chemical fertilizer with straw return (NPKS); and chemical fertilizer with animal manure (NPKM). Variables included soil microbial biomass carbon (MBC) and nitrogen (MBN), and MBC/MBN. For both soil layers, we hypothesized that: microbial biomass was lowest in CK but with the largest spatial heterogeneity; and microbial biomass was highest in NPKM and NPKS but with the lowest spatial heterogeneity. Results showed that: (1) Fertilization significantly increased MBC and MBN more in topsoil than sub-topsoil but had no MBC/MBN changes. (2) The coefficient of variation (CV) and Cochran’s C showed that variation was largest in CK in topsoil and NPK in sub-topsoil and that variation of topsoil was generally lower than in sub-topsoil. The sample size of the three variables was largest in CK in topsoil but had little variation among the other treatments. (3) The trend-surface model showed that within-plot heterogeneity varied substantially with fertilization (NPKM = NPK > NPKS > CK), but Moran’s I and the interpolation map showed that spatial variability with fertilization followed the order NPK > NPKS > CK = NPKM at a fine scale in topsoil. In sub-topsoil, the trend-surface model showed that within-plot heterogeneity followed the order NPKM = CK > NPK > NPKS and that the fine-scale pattern was NPKM > NPK = NPKS > CK. MBC had the highest spatial heterogeneity among the three variables in both soil layers. Our results indicate that the application of organic fertilizer (straw or manure) reduced the variation of MBC and MBN but increased the spatial variability of MBC and MBN. The spatial variation of the three variables was MBC > MBN > MBC/MBN regardless of whether variation was considered at the plot-scale or the fine-scale in both layers.
Introduction

With the increasing application of chemical fertilizers in recent decades, fertilizer efficiency has gradually decreased. This phenomenon is especially common in the North China Plain, which is mainly dominated by fluvo-aquic soil [1]. Some studies have shown that fertilization, especially straw return and organic manure, can effectively improve the fertility of fluvo-aquic soil [2, 3]. Fertilization has major effects on soil properties, including improving soil physical structure, increasing soil available nutrients, and increasing soil microbial activity [4], and can modify the spatial distribution of soil properties [5–7].

Soil microorganisms are key factors in the degradation of soil organic matter and nutrient cycling [8]. Soil microbial indicators are very sensitive to the effects of human activities on soil, especially in agricultural activities [9, 10]. With the development of precision agriculture, the spatial distribution of soil microorganisms frequently affects efficient use of nutrients [11, 12] and the spatial heterogeneity of microbes strongly influences the infiltration, migration, and adsorption of soil nutrients [13, 14]. Fluvo-aquic soil is derived from alluvial soil and thus has greater spatial heterogeneity than aeolian soil [15]. Spatial variation in soil can be detected, estimated, and mapped using geostatistical analysis [16], which can help identify changes in spatial trends and characteristics of space in the soil.

Compared with physical and chemical properties, soil microbes are more susceptible and more sensitive to the influence of external sources of nutrients [17]. Soil microorganisms exhibit high spatial and temporal variation, even in homogenously managed agroecosystems [18, 19]. At present, studies of spatial heterogeneity of soil microorganisms are mainly conducted in forests [20, 21], grasslands [22, 23], or large scale farmlands [24]. However, even at the small scale level, there is large spatial variability of microorganisms in the soil [25, 26]. Spatial variability of soil properties are controlled by inherent variations in soil characteristics and are affected by exogenous factors such as cultivation of crops or grazing of large herbivores [27, 28]. Soil microbial heterogeneity can lead to spatial dependence in soil microbial communities at multiple scales, ranging from the rhizosphere to scales of more than 100 m [29, 30].

Previous work has found that variation in microbial biomass can be partially explained by soil type and drainage class [31, 32], soil texture [33, 34], organic matter content, and pH [35]. The conventional plow depth is 20 cm [36, 37], but roots reach from 0–30 cm [38, 39], and microbes are usually present in the topsoil (0–7.5 cm) [40, 41]. This spatial pattern leads to variation between the strongest microbial activity and the depth of nutrient uptake by plant roots, so it is necessary to study differences in the spatial distribution of microbes in the topsoil and sub-topsoil layers. The spatial distribution and structure of soil properties can provide us with a better understanding of the potential factors (e.g., grazing, cultivation, conservation, fertilization, and plant density) that influence the other soil properties [29].

For long-term fertilization of farmland, the availability of external materials, especially carbon and nitrogen sources, has become an important guideline of microbial distribution. Röver, et al. [6] focused on changes in spatial heterogeneity of a microbial community due to different fertilization treatments in a small farmland in Germany. They found that banding fertilizer caused microbial biomass carbon (MBC) to reach more than 15%, a moderate degree of variation, forming "hotspots" in some areas. Heinze, et al. [7] showed that organic fertilizer improved the stability of soil pH compared with a single application of chemical fertilizer, however, soil heterogeneity lead to spatial heterogeneity of soil pH, which ultimately masked the effect of fertilization on soil microbial biomass. Wang, et al. [42] found that variation of soil MBC had a strong spatial autocorrelation with a dependence distance of 3.17 m, which was the smallest among all variables (e.g. soil respiration, soil moisture) in 16×14 m plots.
The spatial heterogeneity of soil MBC and microbial biomass nitrogen (MBN) in different fertilization treatments can be used to determine the spatial characteristics of long-term soil evolution. Different fertilization treatments in long-term studies result in uneven distributions of nutrients in the soil, which may further lead to spatial variation of microbial biomass distribution. Dissolved organic carbon and dissolved organic nitrogen vary greatly among treatments, which is the main factor limiting microorganisms\[43\].

Our objective was to examine the effects of long-term fertilization on soil MBC, MBN and MBC/MBN dynamics and spatial heterogeneity in both topsoil and sub-topsoil. In view of previous studies, our hypothesis is that spatial heterogeneity in both layers will be in the following order: CK>NPK>NPKM>NPKS.

**Materials and methods**

**Site description**

Soil samples were collected on July 11, 2016 (during the maize season) from the national soil fertility and fertilizer efficiency long-term monitoring station, located in Yuanyang County, Henan Province, latitude 35˚00’00”N, longitude 113˚41’00”E. This site has a mean annual temperature of 14˚C; annual precipitation and annual evaporation of 645 mm and 1450 mm, respectively; groundwater depth of 50–80 cm during the rainy season and 150–200 cm during the dry season; and a frost free period of 224 d. In 1991, after two years of uniform planting (1989–1990), the initial soil physicochemical properties were: clay mineral types, hydromica 1.24 g/kg, total porosity 43%; soil texture, silt loam, clay content (< 2 microns) 13.4%, silt content (2–50 microns) 60.7%, and sand content (50–2000 microns), 26.5%; soil organic carbon 6.7 g/kg; total nitrogen 0.67 g/kg; and soil C/N ratio, 10.

**Experimental design**

There were four fertilization treatments in this study. Fertilization treatments included chemical fertilizer nitrogen (N), phosphorus (P), and potassium (K) (NPK); NPK with straw return (NPKS, 70% N from straw before 2004, 50% N from straw after 2004); animal manure and chemical fertilizer NPK (NPKM); and no fertilizer (CK). The cropping system was wheat-maize rotation in one year. Cow Manure and maize straw were only used in the wheat season. In the wheat season, the fertilization amounts were N-165 kg • hm\(^{-2}\), P\(_2\)O\(_5\)-82.5 kg • hm\(^{-2}\), K\(_2\)O- 82.5 kg • hm\(^{-2}\), with a ratio of N: P\(_2\)O\(_5\): K\(_2\)O = 1: 0.5: 0.5; topdressing occurred at the wheat turning green stage. In the maize season, fertilization amounts were N 188 kg • hm\(^{-2}\), P\(_2\)O\(_5\) 94 kg • hm\(^{-2}\), K\(_2\)O 94 kg • hm\(^{-2}\); with a ratio of N: P\(_2\)O\(_5\): K\(_2\)O = 1: 0.5: 0.5, topdressing was conducted during the jointing period period. Nitrogen fertilizer was urea, phosphorus fertilizer was calcium superphosphate, potassium fertilizer was potassium chloride.

The topdressing direction went from north to south; the row spacing was 40 cm in the wheat season and 60 cm in the maize season; and the plow depth was 20 cm.

Four fertilization treatments with a total of twelve plots were studied, each fertilization treatment had three replicates. Fertilization plots were rectangular: the length (south-north) was 9 m and the width (east-west) was 4.5 m. We set the coordinate origin at the northeast corner of every plot; the Y-axis was south and the X-axis was west. We divided every plot into eight squares, with side lengths of 2.25 m. At the center of each square we set a circle with a radius of 1.125 m, and then randomly selected three points within one circle and recorded the coordinate point. These were our sampling points for topsoil and sub-topsoil; the topsoil was 0–7.5 cm underground and the sub-topsoil layer from 7.5–20 cm. In total, 24 samples were collected per plot, 72 samples per fertilization treatment, 288 samples in one layer, and 576 samples in total. Details of the sampling are shown in Fig 1.
Laboratory analysis

Plots were divided into 8 parts using a measuring rope, and then we positioned cards at sampling locations in the grid. Samples were taken from both soil levels (0–7.5 cm and 7.5–20 cm) in each location using a soil drill. Fresh soil samples were stored in the refrigerator at -20˚C. After selecting the debris and sieving using a 2 mm screen, soil MBC and MBN were determined using the chloroform fumigation extraction method. The principle of the method and detailed operation steps are in Vance, et al. [44] and Lin, et al. [45]. Carbon and nitrogen were measured in leachate with a C/N analyzer (multi N/C 3100, Analytik Jena AG, Germany). The formulas used were from Vance et al. (1987a), the calculation methods of MBC and MBN are the same.

Microbial biomass carbon:

\[
E_C(\mu g/g) = C_{\text{Fumigation}} - C_{\text{non-Fumigation}}
\]

\[
B_C(\mu g/g) = E_C / 0.45
\]

Microbial biomass nitrogen:

\[
E_N(\mu g/g) = N_{\text{Fumigation}} - N_{\text{non-Fumigation}}
\]

\[
B_N(\mu g/g) = E_N / 0.45
\]
Where Ec is extracting organic carbon, and En is extracting nitrogen, they represent the difference between fumigated and non-fumigated soils. CFumigation represents the content of organic carbon extracted from fumigated soil, NFumigation represents the content of nitrogen extracted from fumigated soil. Cnon-Fumigation represents the content of organic carbon extracted from non-fumigated soils, Nnon-Fumigation represents the content of nitrogen extracted from non-fumigated soils. Bc represents microbial biomass carbon, BN represents microbial biomass nitrogen. In the calculation of microbial biomass carbon and nitrogen, the conversion coefficient is 0.45.

Data analysis

**Conventional statistical methods for different fertilization treatments.** Means, variances, and frequency were estimated for each soil property in each plot. The distributions of values from three plots in the same treatment were plotted on a common scale for comparison purposes. Cochran’s C test was used to test the assumption of variance homogeneity. The test statistic is a ratio that relates the largest empirical variance of a particular treatment to the sum of the variances of the remaining treatments. The theoretical distribution with the corresponding critical values can be specified [46–48]. The purpose of frequency distribution analysis is to illustrate the overall distribution of soil attribute values in different intervals. Thus, the distribution of MBC and MBN under different fertilization treatments was also analyzed.

**Geostatistics methods.** We performed spatial statistics using the trend-surface model, Moran’s I, and an interpolation map.

**Trend-surface model**

The trend-surface model is usually used to analyze the spatial rules of soil properties at the plot scale, and then indicates changes in properties on an X, Y coordinate system. The two trend-surface model is more suitable for small scale spatial data analysis. The two trend-surface model is as follows:

\[
\text{Soil variable} = \beta_0 + \beta_1 x + \beta_2 y + \beta_3 xy + \beta_4 x^2 + \beta_5 y^2
\]

This model sums up the spatial distribution of attributes in order to filter out random interference [49]. According to the complexity of the elements to be evaluated, the model can be divided into three parts: the one trend-surface model, the two trend-surface model, and the three trend-surface model. The study area was small, so the two trend-surface model was used [50]:

The coefficients of the trend-surface model (\( \beta_0 - \beta_5 \)) can be obtained by regression: \( \beta_0 \) is the intercept; \( \beta_1 \) and \( \beta_2 \) are the effect of soil property changes in the X and Y axes; \( \beta_3 \) is the changes in the attribute values on the diagonal; and \( \beta_4 \) and \( \beta_5 \) represent the abnormality along the X and Y axes of the parameters to determine the degree of influence of each attribute in each direction of the coordinates. The regression coefficient of the trend-surface model was calculated in SPSS (version 20.0, IBM) at the p<0.05 level.

**Moran’s I**

Moran’s I is the degree of spatial autocorrelation within a plot. Spatial autocorrelation refers to how data are related in space, and whether the relationship of such data is concentrated, dispersed or has no correlation. Spatial autocorrelation analysis is widely used in the field of geographical statistics and the most important indices are Moran’s I and Geary’s C. Moran’s I, which uses the local spatial autocorrelation distance as the maximum variation from different treatments, was the selected. Because the correlation coefficients are between -1 and 1, they are
related to the relationship between attribute values within a plot, which is not relevant when the correlation coefficient is 0. Local spatial autocorrelation allows for testing of spatial variability at the small scale [51].

Spatial autocorrelation of Moran’s I can be expressed as:

\[
I = \frac{n}{S_0} \sum_{i=1}^{n} \sum_{j=1}^{n} W_{ij} z_i z_j / \sum_{i=1}^{n} z_i^2
\]

where \(z_i\) is the deviation of \(I\) and its average value \((x_i - \bar{X})\), \(W_{ij}\) is the spatial weight between \(i\) and \(j\); \(n\) represents the total number of elements; and \(S_0\) is the aggregation of all spatial weights.

Interpolation map

Interpolation maps are made by converting known discrete measured data to a continuous data surface and then interpolating into the overall plot. The advantages and disadvantages of various interpolation methods are then compared. The Inverse Distance Weighted (IDW) interpolation method does not need the semi variance as a function parameter and the interpolation is inversely proportional to the distance. Therefore, IDW is suitable in small plots of few samples and has high precision [52]; ArcGIS 10.2.1 was used to generate the interpolation map.

Results

Descriptive statistics

Mean and coefficient of variation. For topsoil, concentrations of MBC and MBN were in the following order: CK < NPK < NPKS = NPKM; there were no significant differences in MBC/MBN among the fertilization treatments (\(P<0.05\)). For sub-topsoil, MBC was the highest in NPKM and the lowest in CK. For MBN, there were no significant differences among NPK, NPKS, and NPKM, but it was the lowest in CK. MBC/MBN was highest in CK and lowest in NPK (\(P<0.05\)).

The concentrations of MBC and MBN in topsoil were generally higher than in sub-topsoil (\(P<0.05\)) (Fig 2). The MBC concentration was 52.77–67.24% of topsoil in sub-topsoil; MBC concentration decreased fastest in NPKS and slowest in NPK. MBN concentration was 51.71–70.95% of topsoil in sub-topsoil (\(P<0.05\)). MBC/MBN was only significantly different in NPKM between topsoil and sub-topsoil, where MBC/MBN was 10% lower in topsoil than sub-topsoil (\(P<0.05\)).

The coefficient of variation (CV) was typically lower in topsoil than sub-topsoil in all four fertilization treatments (Table 1), except for MBC and MBN of CK. In topsoil, CVs of MBC,
MBN, and MBC/MBN were the highest in CK; CV of MBC was the lowest in NPKM; and MBN and MBC/MBN were the lowest in NPKS. In sub-topsoil, the CVs of MBC, MBN, and MBC/MBN were the highest in NPKS, NPK, and CK, respectively, while the CVs of MBC and MBN were lowest in NPKM, and MBC/MBN was the lowest in NPKS.

**Frequency.** To introduce the effects of fertilization history on soil heterogeneity, individual samples from the three plots of each fertilization treatment were pooled (n = 72), illustrating the frequency distributions of soil properties (Fig 3).

The frequency of MBC in topsoil was the highest in 200–300 mg/kg for CK, between 300–500 mg/kg for NPKS and NPKM, and between 200–400 mg/kg for NPK. MBC in sub-topsoil was the highest in 100–200 mg/kg for CK, 200–300 mg/kg for NPKM and NPKS, and 100–300 mg/kg for NPK.

The frequency of MBN in topsoil was highest in 20–60 mg/kg for CK, and between 40–80 mg/kg for NPK, NPKS, and NPKM. MBN in sub-topsoil was the highest in 0–40 mg/kg in CK, 20–60 mg/kg for NPK, and NPKS and NPKM had the highest frequency between 20–60 mg/kg.

For MBC/MBN, these four treatments were concentrated at 4–12 in topsoil and sub-topsoil.

In Fig 3, we can also see that MBC and MBN are more concentrated in sub-topsoil than topsoil, and the distribution range of MBC was relatively narrow due to its low concentration in CK. The distribution of MBC in NPKM was more concentrated than in NPKS. The highest concentration of MBC in sub-topsoil still occurred in NPKM, and the lowest concentration was in CK.

### Table 1. Coefficients of variance (CV) of MBC, MBN, and MBC/MBN in topsoil and sub-topsoil in four fertilization treatments (CK, NPK, NPKS, and NPKM).

| Layer       | CK MBC% | CK MBN% | CK MBC/MBN% | NPK MBC% | NPK MBN% | NPK MBC/MBN% | NPKS MBC% | NPKS MBN% | NPKS MBC/MBN% | NPKM MBC% | NPKM MBN% | NPKM MBC/MBN% |
|-------------|---------|---------|-------------|---------|---------|-------------|---------|---------|-------------|---------|---------|-------------|
| Topsoil     | 44.65   | 55.18   | 36.11       | 28.94   | 35.71   | 27.36       | 27.56   | 28.76   | 19.68       | 26.85   | 30.97   | 26.14       |
| Bottomsoil  | 36.30   | 49.43   | 46.03       | 39.84   | 49.99   | 33.44       | 39.93   | 43.66   | 30.06       | 29.74   | 40.77   | 32.72       |

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[Fig 3. Frequency distribution of MBC, MBN and MBC/MBN in the four fertilization treatments.](https://doi.org/10.1371/journal.pone.0209635.g003)
Within-plot variances. Coefficients of variation (CVs) of soil properties within-plot are summarized in Fig 4. The CVs of topsoil were mainly in the range of 18–36%, slightly higher in CK and lower in NPKS. CVs were higher in sub-topsoil than topsoil, especially for NPK where three plots were abnormally higher. Overall, MBC/MBN had the lowest CV.

Cochran’s C was used to test the within-plot variance among different treatments or different plots (Table 2). However, there were no significant differences in the variance of MBC in topsoil. The differences in variance of MBN and MBC/MBN were significant. Four of the highest six values of the three variables occurred in CK in both layers, and two of the three maximum values of each variable occurred in CK in topsoil. There were significant differences in variance of the three variables among 12 plots in sub-topsoil. However, we could not confirm which fertilization had the largest variance because the largest variances of the three variables were distributed among different fertilization treatments. For MBN, none of the largest three CV occurred in CK in sub-topsoil, showing higher variability of CK in four fertilization treatments. MBC and MBN were different in sub-topsoil than in topsoil, where they were relatively larger in NPK than the others. MBC/MBN still had higher variation in CK compared with the others.

The Cochran’s C test was also used to compare pairs of treatments conforming to our hypothesis as follows: CK vs. NPKS, CK vs. NPK, CK vs. NPKM, NPKS vs. NPKM, NPK vs. NPKM, and NPK vs. NPKS. The results showed that the three variables of topsoil had higher variation in CK, while MBN had the highest variation in NPKS in sub-topsoil, and MBN had the highest variation in NPK in sub-topsoil, followed by NPKM, NPKS, and CK.

Sample size requirements based on observed within-plot variances. Based on the estimated standard deviation, sample size requirements are different for different fertilization treatments in 40.5 m² plot [53, 54].

In topsoil, the number of samples required for each fertilizer treatment was significantly different (Fig 5). For MBC, MBN, and MBC/MBN, the sampling size of CK was the largest. When the relative expectation error was 5%, the number of samples required for CK treatment reached 136–424 and the minimum number of samples of NPKS was 88–90. For MBN, with a relative error of 5%, the sample size reached 248–601 in CK but was 128–132 in NPKS. The sample size of MBC/MBN was lowest relative to MBC and MBN, the maximum sample size of CK3 was 260 and sample size was lowest (48–66) in NPKS when the relative error was 5%.

The sample size was greater in sub-topsoil than topsoil. For MBC, the sample size of NPK reached the maximum (207–561) when relative expected error was 5%, and the sample size was 43–267 at the same relative expected error in NPKM. For MBN, the largest sample size was in NPK at 300–650 with a relative expected error of 5%. The sample size was lowest (between 207–561) in NPKM. MBC/MBN sample sizes did not have large differences; sample size was maximum (382–1016) in NPKS and minimum (81–301) in NPKM.

Sample size requirements depended on long term fertilization (Fig 5), with more samples required in CK than in NPKS and NPKM in topsoil; NPK and NPKS required the most samples, and NPKM required the fewest, in sub-topsoil.

Spatial statistical analysis

Trend-surface model. The trend-surface model revealed a number of significant patterns in the spatial variability of soil properties among different long-term fertilization treatments.

There was a total of 30 linear or nonlinear relationships in topsoil (Table 3), including ten in NPKM, ten in NPK, nine in NPKS, and one in CK. MBC had a total of 12, MBN had ten, and MBC/MBN had eight. These results indicated that the highest spatial heterogeneity occurred within plots in NPK and CK had the lowest spatial heterogeneity. MBC had greater spatial variability compared with MBN and MBC/MBN.
Fig 4. CVs of soil MBC, MBN, and MBC/MBN in each plot of four fertilization treatments.

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Sub-topsoil had greater spatial heterogeneity than topsoil. There was a total of 34 linear and nonlinear relationships in sub-topsoil, CK accounted for nine, NPK for eight, NPKS for eight, NPKM for nine, MBC for 12, MBN for 11, and MBC/MBN for 11. NPKM was the maximum both in topsoil and sub-topsoil and as MBC, MBC/MBN was the minimum both topsoil and sub-topsoil.

Moran’s I. We used local Moran’s I analysis to detect fine-scale spatial structure within each plot after removing coarse-scale trends across the 40.5m² plots. All of the Moran’s I of MBC in all fertilization treatments are showed in Fig 6. Results are summarized in Table 4.

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Moran’s I. We used local Moran’s I analysis to detect fine-scale spatial structure within each plot after removing coarse-scale trends across the 40.5m² plots. All of the Moran’s I of MBC in all fertilization treatments are showed in Fig 6. Results are summarized in Table 4.
Sub-topsoil: only four of nine plots in CK achieved spatial autocorrelation and there were five, five, and seven in NPK, NPKS, and NPKM, respectively. This suggests stronger spatial autocorrelation in NPKM, especially for MBC, which showed spatial autocorrelation in all three plots, with spatial autocorrelation distances between 4.5–7.5 m. MBN had two of three plots with spatial autocorrelation in CK, NPKS, and NPKM. However, only one plot of three had spatial autocorrelation in NPK. MBC/MBN was similar to MBN: each fertilization treatment had two of three plots with spatial autocorrelation in NPK, NPKS, and NPKM, while CK only had one of three.

Interpolation map. We used two-dimensional interpolation maps to visualize MBC, MBN, and MBC/MBN by inverse distance weighting (IDW) to better estimate soil in the whole plot. This analysis was done to compare the spatial distributions of the soil properties among the plots (Fig 7).

The interpolation maps had darker colors in CK compared to NPK, NPKS, and NPKM in both soil layers and concentrations of MBC and MBN were higher in topsoil than sub-topsoil. The color was more uniform and with few hotspots in CK while there were more hotspots in NPKS and NPKM. The largest hotspots appeared in CK1 in topsoil, with the largest hotspots of MBC and MBN at the bottom right corner of this plot. The largest hotspot of sub-topsoil occurred in NPKM3.

We did not find a significant impact on soil microbial biomass based on the direction of topdressing according our IDW maps, but hotspots were always at the edge of the plots.

Discussion

Effects of fertilization on MBC, MBN, and MBC/MBN and their variation
MBC and MBN concentration were the highest in NPKM and lowest in CK in both layers, following the order: NPKM = NPKS > NPK > CK. This result was the same as Liu, et al. [55], and MBC and MBN concentration were higher in topsoil than sub-topsoil. There was no
Table 3. Significant regression coefficients of trend-surface analysis, and coefficients of determination ($r^2$) for soil MBC, MBN, and MBC/MBN in the 12 plots.

| Plot   | Soil properties | $\beta_0$ | $\beta_x$ | $\beta_y$ | $\beta_{xy}$ | $\beta_{x^2}$ | $\beta_{y^2}$ | $r^2$ | Plot   | Soil properties | $\beta_0$ | $\beta_x$ | $\beta_y$ | $\beta_{xy}$ | $\beta_{x^2}$ | $\beta_{y^2}$ | $r^2$ |
|--------|----------------|----------|-----------|-----------|--------------|---------------|--------------|-------|--------|----------------|----------|-----------|-----------|--------------|---------------|--------------|-------|
| CK3    | MBC            | -        | -         | -         | -            | 0.03          | -            | 0.261 | CK1    | MBN            | -        | 26.135    | -         | -            | -5.75         | -            | 0.316 |
| NPK1   | MBC            | 566.11   | -248.102  | -         | -            | 64.227        | -            | 0.381 | MBC/MBN| MBN/MBC       | 9.527    | -         | -         | -            | 0.07          | -            | 0.326 |
|        | MBN            | 76.632   | -         | -         | -            | -             | 0.109        | -      | NPK2   | MBC            | 405.074  | -         | -         | -            | 0.065         | -            | 0.195 |
|        | MBC/MBN        | 7.355    | -         | -         | -            | -             | 0.224        | -      | NPK3   | MBC            | 299.871  | -         | -         | -            | 0.055         | -            | 0.296 |
| NPK2   | MBC            | 495.72   | -         | -         | -            | 0.055         | -            | 0.055 | NPK3   | MBC            | 87.5     | -         | -         | -            | 0.065         | -            | 0.111 |
|        | MBN            | 67.12    | -         | -         | -            | -             | 0.225        | -      | NPK4   | MBC            | 4.042    | -         | -         | -            | -             | 0.037        | 0.291 |
|        | MBN/MBN        | 4.042    | -         | -         | -            | -             | 0.037        | -      | NPKS1  | MBC            | 633.096  | -         | -         | -            | 0.013         | NPK3 MBN    | 1.338 |
| NPKS1  | MBC            | 89.357   | -         | -         | -            | 0.049         | -            | 0.117 | NPKS2  | MBC/MBN       | 7.308    | -         | -         | -            | 0.017         | NPKS2 MBN   | 0.37  |
|        | MBN            | 73.202   | -         | -         | -            | -             | 0.075        | -      | NPKS2  | MBC/MBN       | 5.987    | -         | -         | -            | -             | NPKS2 MBN   | 0.37  |
| NPKS2  | MBC            | 485.72   | -         | -         | -            | 0.264         | -            | 0.117 | NPKS1  | MBC/MBN       | 7.106    | -         | -         | -            | 0.071         | NPKS1 MBN   | 0.37  |
|        | MBN            | 84.78    | -         | -         | -            | 0.253         | -            | 0.253 | NPKS3  | MBC/MBN       | 81.832   | -         | -         | -            | 0.335         | NPKS3 MBN   | 0.421 |
| NPKS3  | MBC            | 575.037  | -         | -         | -            | 0.422         | -            | 0.422 | NPKS3  | MBC/MBN       | 81.832   | -         | -         | 3.964        | -             | NPKS3 MBN   | 0.421 |
|        | MBN            | 64.78    | -         | -         | -            | 0.274         | -            | 0.274 | NPKM1  | MBC/MBN       | 8.386    | -         | -         | -            | -             | NPKM1 MBN   | 0.189 |
| NPKM1  | MBC            | 531.015  | -         | -         | 18.416       | -             | 0.421        | -      | NPKM2  | MBC/MBN       | 6.331    | -         | -         | -            | 0.078         | NPKM2 MBN   | 0.365 |
|        | MBN            | 81.832   | -         | -         | 3.964        | -             | 0.335        | -      | NPKM2  | MBC/MBN       | 84.78    | -         | -         | -            | 0.133         | NPKM2 MBN   | 0.267 |
| NPKM2  | MBC            | 486.767  | -         | -         | -            | 0.173         | -            | 0.173 | NPKM3  | MBC/MBN       | 8.846    | -         | -         | -            | 0.138         | NPKM3 MBN   | 0.401 |
|        | MBN            | -        | -         | 20.286    | -            | -2.066        | -            | 0.415 | NPKM3  | MBC/MBN       | 8.846    | -         | -         | -            | 0.138         | NPKM3 MBN   | 0.42  |

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There was a significant difference of MBC/MBN among fertilization treatments or between layers (except it was higher in sub-topsoil than topsoil in NPKM). As we know, a large amount of microbial input comes from manure [56, 57] and sufficient dissolved organic carbon and nitrogen caused a high microbial biomass in NPKM. There was also a large amount of microbial biomass in NPKS due to straw providing a sufficient source of carbon and nitrogen for microorganisms. Furthermore, the weakly alkaline soil can neutralize the acids produced by microbes when they decompose the straw [58, 59], producing a soil environment suitable for microorganism growth and propagation and leading to higher MBC and MBN [60, 61]. NPK lacks

![Fig 6. Moran’s I correlograms for soil MBC at 12 plots of four fertilization treatments. Filled circles denote Moran’s I values that exhibited significant positive or negative autocorrelation. Obs: observations; LCL: low confidence limit; UCL: Upper confidence limit.](https://doi.org/10.1371/journal.pone.0209635.g006)

### Table 4. Summary of significant Moran’s I values for MBC, MBN, MBC/MBN in 12 plots.

The unit of the distance for spatial dependence is meters.

| Layer     | Soil variables | CK1 | CK2 | CK3 | NPK1 | NPK2 | NPK3 | NPKS1 | NPKS2 | NPKS3 | NPKM1 | NPKM2 | NPKM3 | CK, NPK, NPKS, NPKM* | Hypothesis |
|-----------|----------------|-----|-----|-----|------|------|------|-------|-------|-------|-------|-------|-------|----------------|------------|
| Topsoil   | MBC            | 5   | 4   | 3.5 | 3.5  | 5-5.5| 4.5  | 1-3   | -5.5  | 6.5   | 2  | 3 | 1 | | U |
|           | MBN            | -7.5| -1  | 3.5, 5.5| -2 | 5 | 4.5 | 1 | -5.5 | 6.5 | 1, 2, 2, 1 | R |
|           | MBC/MBN        | 3.5-7.5| 6.5-5.5| 1 | -4 | -6 | -5.6 | 6.5 | 1, 2, 1, 3 | R |
| Sub-topsoil| MBC            | 1.5 | 1-3,5| -4 | -6.5 | -6.5 | -7 | -5.5 | -4.5 | 1.5, 2, 2 | U |
|           | MBN            | -5.5| 5.5  | 1   | 1.5-6.5 | 1.5-6 1.5-4 | 4  | 2, 1, 2 | R |
|           | MBC/MBN        | 1.5-6 | 6 | 1.5-5.5 | -1 | -6-6.5 | -7 | 1.5-4 | 1, 2, 2 | R |

Note: for each variable, the first row indicates the significant negative autocorrelation at the listed lag distance and the second row indicates the positive autocorrelation at the listed lag distance.

* Denotes the total number of plots with significant autocorrelation detected by Moran’s I.

* S, R represent the variables that supported or rejected the original hypothesis, respectively; U denotes unclear evidence as to supporting or rejecting the original hypothesis.
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Legend

- 50 – 100
- 100 – 200
- 200 – 300
- 300 – 400
- 400 – 500
- 500 – 600
- 600 – 700
- 700 – 800
- 800 – 900
necessary carbon sources and leads to insufficient energy to supply more microorganisms, and thus has lower microbial biomass. CK had the lowest microbial biomass due to its low carbon and nutrient input. Even in the same tillage layer, topsoil was closer to the ground, with high porosity and good air permeability, which provided better aeration conditions for microbial activity [62, 63]. With increasing depth, the permeability is reduced, which decreases the microbial biomass [64]. The difference of MBC/MBN between the soil layers was not significant except with NPKM. This indicated that the microbial population structure did not change obviously up to a depth of 20 cm. MBC/MBN of topsoil was lower than sub-topsoil in NPKM, indicating that the proportion of fungi increased from topsoil to sub-topsoil, which is likely to be caused by the difference in aeration conditions and available organic carbon in the soil, and manure caused this difference [65–67].

Long-term fertilization treatments showed the highest variation in CK, while NPKS and NPKM had the lowest variation in general, and following the order: CK > NPK > NPKM > NPKS in topsoil and NPK > CK > NPKS > NPKM in sub-topsoil. Variation in sub-topsoil was higher than topsoil except for MBC and MBN in CK. The differences of MBC and MBN among different fertilization treatments matched our hypothesis, probably because of smaller bulk density, macro-water-stable aggregates (>2 mm), high porosity, and because soil moisture and oxygen content were more even in NPKM and NPKS [68–71]. On the other hand, low porosity and low oxygen content were not conducive to microbial survival in CK. A small portion of the soil had high porosity and oxygen content due to root penetration [68, 72] and root stubble, leading microbes to gather near the roots and resulting in an uneven distribution of microbes in the soil. Lateral root systems were developed in NPKM [73, 74], root stubble residues in soil were more balanced, and the overall spatial variability decreased.

The addition of exogenous organic carbon can also make the decomposition rate of soil original organic carbon react sharply in a short time and form priming effect[60]. Several studies have shown that the addition of exogenous organic carbon can promote the growth of microorganisms, thus promoting the decomposition of soil original organic carbon and producing positive priming effect. Microorganisms are in a nutrient-limited state when soil nutrient content is relatively low. The addition of cow manure stimulates the demand of microorganisms for nutrients, thus accelerating the decomposition of soil organic carbon[61]. De Nobili et al.[75] and Falchini et al. [76] also believed that soil with relative deficiency of nutrients was more affected by the priming effect than soil with relatively abundant nutrients. This is also one of the factors causing the high variability of CK and NPK treatments and sub-topsoil.

**Effects of fertilization on the spatial variation of MBC, MBN, and MBC/MBN**

Trend-surface models usually indicate spatial variability at the plot scale. In this study, the trend-surface model showed maximum spatial variability in NPKM in both layers and the minimum spatial variability in CK in topsoil. MBC had higher spatial variation than MBN at the plot scale; the minimum was found in MBC/MBN. This means that carbon or nitrogen sources might not be uniform at the plot scale. Manure caused the uneven distribution of dissolved organic carbon and nitrogen in NPKM because manure is difficult to decompose due to being a macromolecule and having high lignin content [77, 78], leading to higher spatial variability at the plot scale. Another reason may be that manure was usually scattered.
artificially in the plot, which strongly affected the spatial distribution in the plot. The trend-surface spatial properties of CK were almost the opposite in topsoil (smallest) and sub-topsoil (highest), which indicated that as the sole source of soil organic carbon and nitrogen, the distribution of root stubble in the two layers was completely different. The stubble was mainly in sub-topsoil and was relatively less common in topsoil in CK. Spatial variation of MBC/MBN was the lowest, indicating that the microbial communities had no large differences in all soils at the plot scale.

Moran’s I spatial autocorrelation distance represents spatial variability at the fine-scale. There were large differences in spatial heterogeneity between the two soil layers at the fine scale in this study. The spatial variability of NPK was highest, followed by NPKS, CK, and NPKM in topsoil; the spatial variation in sub-topsoil followed the order: NPKM > NPKS = NPK > CK. The only carbon input in NPK was wheat stubble, which is difficult to decompose due to its high lignin content [79], but microbes tended to gather near the root stubble, resulting in larger spatial variability. Straw was scattered and plowed into the soil in the plot and while the distribution was more uniform in NPKS than NPK, microbial decomposition of straw is uneven: microbial biomass was low in places where straw had higher lignin content or in straw-uncovered place, and other places had higher microbial concentration, so the spatial autocorrelation was high at the fine-scale [80, 81]. In sub-topsoil, the strongest spatial autocorrelation occurred in NPKM, possibly due to higher permeability from more dissolved organic carbon and nitrogen [82], resulting in the highest dissolved organic matter content in sub-topsoil. But this phenomenon was uneven, which is beneficial to the survival and aggregation of microorganisms in certain parts of the sub-topsoil [83, 84]. Straw provides a normal source of energy for microbial survival in NPKS [85–87], but it is more difficult for microbes to survive and the decomposition rate of straw is slower in sub-topsoil [40], soil microbes and other soil organisms also release a variety of organic matter in the soil [88, 89], supplying soil microbial nutrient sources and increasing spatial heterogeneity simultaneously at the fine scale. The root stubble depth also differs among fertilization treatments in sub-topsoil [90, 91]. In this study NPKM and NPKS both had high density roots in both layers, but NPK had the same root density in topsoil [92] and the root density was much lower in sub-topsoil. Therefore, the spatial heterogeneity was much lower in NPK. The root density was higher in sub-topsoil than topsoil in CK due to its poor nutrient soil, the root penetrated deeper can it get enough nutrition and moisture[93], which led to very low spatial heterogeneity at the fine-scale in CK in topsoil.

There were different spatial heterogeneity patterns among the treatments at the fine-scale and the plot-scale, so we made a two-dimensional map with the IDW interpolation method to express the spatial distribution of variables in plots. The largest hotspot was in CK1 (high MBC and MBN). This hotspot was caused by residual stubble because we found carbonized maize stubble when picking roots from the soil. In such samples, MBC and MBN were higher, indicating the emergence of microbial aggregation in this position; microbial decomposition was more intense and caused this hotspot. Another obvious hotspot was in NPKS1, but we did not find any abnormal phenomena when we were selecting and sieving. This hotspot might have resulted from rich carbon and nitrogen sources in this sample that had been consumed by microbial decomposition. A notable phenomenon in IDW maps was that the most hotspots were commonly found on the edge of the plot, indicating an obvious edge effect. Thus, sampling should be in the vicinity of the central area to avoid the error caused by the edge effect.

Conclusions

Fertilization significantly increases the concentration of MBC and MBN, especially with input of organic materials, and it is increased more in topsoil than sub-topsoil. However, there are
few differences of MBC/MBN between topsoil and sub-topsoil and among fertilization treatments.

Variation of MBC, MBN, and MBC/MBN caused by fertilization were in the following order MBC > MBN > MBC/MBN. Differences in soil properties among fertilization treatments were in the order CK > NPK > NPKM > NPKS (topsoil) and NPK > CK > NPKS > NPKM (sub-topsoil).

Spatial heterogeneity differed between topsoil and sub-topsoil at the plot-scale. The spatial variability at the plot-scale was NPKM = NPK > CK (topsoil) and NPKM = CK > NPK = NPKS (sub-topsoil). Spatial heterogeneity also differed at the fine-scale between topsoil and sub-topsoil: the order was NPK > NPKS > CK in topsoil and NPKM > NPK = NPKS > CK in sub-topsoil. Spatial variation of variables followed the order: MBC > MBN > MBC/MBN (topsoil) and MBC = MBN = MBC/MBN (sub-topsoil).

The “hotspots” of MBC and MBN in the plots were mainly on the plot edges. Sampling should be in the central area of the plot to better represent the situation in plots.

Author Contributions

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References

1. Chen G., Cao H., Liang J., Ma W., Guo L., Zhang S., et al., Factors Affecting Nitrogen Use Efficiency and Grain Yield of Summer Maize on Smallholder Farms in the North China Plain. Sustainability. 2018; 10: 363.

2. Wang L., Dong M., Zhang L., and Du X., Effects of organic manures with different carbon-to-nitrogen ratios on soil microbial biomass of organic agriculture. Chinese Journal of Eco-Agriculture. 2013; 21: 1073–1077.

3. Chen Q., “Effect of Matching Use of Straw and Fertilizer on Soil Oxidoreductase Activity,” Master, Northwest Agriculture and Forestry University, Yangling, China, 1, 2009.

4. Zhang J., Zhang B., and Qin Y., Spatial heterogeneity of soil nutrients in Liaocheng, Shandong Province. Science & Technology Review. 2009; 27: 49–52.

5. Carron M., Auriac Q., Snoeck D., Villenave C., Blanchart E., Ribeyre F., et al., Spatial heterogeneity of soil quality around mature oil palms receiving mineral fertilization. European Journal of Soil Biology. 2015; 66: 24–31.

6. Röver M., Kaiser E., Röver M., and Kaiser E., Spatial heterogeneity within the plough layer: Low and moderate variability of soil properties. Soil Biology & Biochemistry. 1998; 31: 175–187.

7. Heinze S., Raupp J., and Joergensen R., Effects of fertilizer and spatial heterogeneity in soil pH on microbial biomass indices in a long-term field trial of organic agriculture. Plant and Soil. 2009; 328: 203–215.

8. Shi W., Agricultural and Ecological Significance of Soil Enzymes: Soil Carbon Sequestration and Nutrient Cycling, Springer Berlin Heidelberg; 2010.
9. Sparling G., Pankhurst C., Doube B., and Gupta V., Soil microbial biomass, activity and nutrient cycling an indicator of soil health, Centre for Agriculture and Biosciences International; 1997.
10. Doran J. and Zeiss M., Soil health and sustainability: managing the biotic component of soil quality. Applied Soil Ecology. 2000; 15: 3–11.
11. Bonkowski M., Griffiths B., and Scrimgeour C., Substrate heterogeneity and microfauna in soil organic ‘hotspots’ as determinants of nitrogen capture and growth of ryegrass. Applied Soil Ecology. 2000; 14: 37–53.
12. Schulz S., Brankatsch R., Dünig A., and gelKnabner I., The role of microorganisms and plants at different stages of ecosystem development for soil formation. Biogeosciences. 2013; 10: 3983–3996.
13. Shi X., Wu J., Wu J., Jiang B., and Xu H., Effects of the heterogeneity of multiple correlated random parameters on solute transport. Advances in Water Science. 2012; 23: 509–515.
14. Shi X., Wu J., Yuan Y., and Jiang B., Effect of the anisotropy in porous media on the spatial variability of the hydraulic conductivity. Advances in Water Science. 2005; 16: 679–684.
15. Iqbal J., Thomasson J., Jenkins J., Owens P., and Whisler F., Spatial variability analysis of soil physical properties of alluvial soils. Soil Science Society of America Journal. 2005; 69: 1338–1350.
16. Rossi R., Mulla D., Journel A., and Franz E., Geostatistical tools for modeling and interpreting ecological spatial dependence. Ecological Monographs. 1992; 62: 277–314.
17. Doran J., Sarrantonio M., and Liebig M., Soil health and sustainability. Advances in Agronomy. 1996; 56: 1–54.
18. Dupuis E. and Whalen J., Soil properties related to the spatial pattern of microbial biomass and respiration in agroecosystems. Canadian Journal of Soil Science. 2007; 87: 479–484.
19. Parkin T., Spatial variability of microbial processes in soil—a review. Journal of Environmental Quality. 1993; 22: 409–417.
20. Sales M., Souza C., Kyriakidis P., Roberts D., and Vidal E., Improving spatial distribution estimation of forest biomass with geostatistics: A case study for Rondônia, Brazil. Ecological Modelling, 2007; 205: 221–230.
21. Baldrian P., Merhautová V., Petránková M., Caižham T., and Šnajdr J., Distribution of microbial biomass and activity of extracellular enzymes in a hardwood forest soil reflect soil moisture content. Applied Soil Ecology. 2010; 46: 177–182.
22. Berner D., Marhan S., Keil D., Poll C., Schützenmeister A., Piepho H.-P., et al., Land-use intensity modifies spatial distribution and function of soil microorganisms in grasslands. Pedobiologia. 2011; 54: 341–351.
23. Boedinghaus R., Nunan N., Berner D., Marhan S., and Kandel E., Do general spatial relationships for microbial biomass and soil enzyme activities exist in temperate grassland soils? Soil Biology & Biochemistry. 2015; 88: 430–440.
24. Fražiolo L., Piccolo M., Feigl B., and Cerri C., Inorganic nitrogen, microbial biomass and microbial activity of a sandy brazilian cerrado soil under different land uses. Agriculture, Ecosystems & Environment. 2010; 135: 161–167.
25. Martirosyan V., Ehrlich R., Freund Y., Barness G., and Steinberger Y., Spatial heterogeneity of a microbial community in a sandy soil ecosystem. Pedobiologia. 2013; 56: 195–203.
26. Morris S., Spatial distribution of fungal and bacterial biomass in southern Ohio hardwood forest soils: fine scale variability and macroscale patterns. Soil Biology & Biochemistry. 1999; 31: 1375–1386.
27. Burke I., Lauenroth W., Riggle R., and Brannen P., Spatial variability of soil properties in the shortgrass steppe: The relative importance of topography, grazing, microsite, and plant species in controlling spatial patterns. Ecosystems. 1999; 2: 422–438.
28. Yanai J., Sawamoto T., Oe T., Kusa K., Yamakawa K., Sakamoto K., et al., Spatial variability of nitrous oxide emissions and their soil-related determining factors in an agricultural field. Journal of Environmental Quality. 2003; 32: 1965–1977. PMID: 14674518
29. Ramette A. and Tiedje J., Multiscale responses of microbial life to spatial distance and environmental heterogeneity in a patchy ecosystem. Proceedings of the National Academy of Sciences of the United States of America. 2007; 104: 2761–6. https://doi.org/10.1073/pnas.0610671104 PMID: 17296935
30. AgKin T. and Kızılkaya R., Spatial distribution patterns of soil microbial biomass carbon within the pasture. Agriculturea Conspicuus Scientificus. 2007; 72: 75–79.
31. Rogers B. and lii R., Temporal analysis of the soil microbial community along a toposequence in Pine-land soils. Soil Biology & Biochemistry. 2001; 33: 1389–1401.
32. Girvan M., Bullimore J., Pretty J., Osborn A., and Ball A., Soil type is the primary determinant of the composition of the total and active bacterial communities in arable soils. Applied & Environmental Microbiology. 2003; 69: 1800–9.
33. Schutter M., Sandeno J., and Dick R., Seasonal, soil type, and alternative management influences on microbial communities of vegetable cropping systems. Biology and Fertility of Soils. 2001; 34: 397–410.

34. Mvan G., Mercx R., and Vlassak K., Spatial distribution of microbial biomass in microaggregates of a silty-loam soil and the relation with the resistance of microorganisms to soil drying. Soil Biology & Biochemistry. 1996; 28: 503–510.

35. Wang Y., Zhou L., Wu J., Buttery C., Tang C., and Xu J., "Soil microbial biomass and pH as affected by the addition of plant residues," in The International Symposium of Molecular Environmental Soil Science at the Interfaces in the Earth, 2010, 320–322.

36. Qiu H., Studies on the potential ecological risk and homology correlation of heavy metal in the surface soil. 2010; 2: 194–201.

37. Prasad J., Rao C., Srinivas K., Jyothi C., Venkateswarlu B., Ramachandrapa B., et al., Effect of ten years of reduced tillage and recycling of organic matter on crop yields, soil organic carbon and its fractions in alfisols of semi arid tropics of southern India. Soil & Tillage Research. 2016; 156: 131–139.

38. Jokubauskaitė I., Slepetiūnė A., and Karcauskienė D., Influence of different fertilization on the dissolved organic carbon, nitrogen and phosphorus accumulation in acid and limed soils. Eurasian Journal of Soil Science. 2015; 4: 137–143.

39. Vance E., Brookes P., and Jenkinson D., Microbial biomass measurements in forest soils: The use of the chloroform fumigation-incubation method in strongly acid soils. Soil Biology & Biochemistry. 1987; 19: 697–702.

40. Underwood A., Experiments in ecology: Their logical design & interpretation using analysis of variance. Cambridge Univ Press; 2012.

41. Kennedy A. and Smith K., Soil microbial diversity and the sustainability of agricultural soils. Springer Netherlands; 1999.

42. Haynes R. and Naidu R., Influence of lime, fertilizer and manure applications on soil organic matter content and soil physical conditions: a review. Nutrient Cycling in Agroecosystems. 1998; 51: 123–137.
58. Fan W., Wu J., Li J., and Hu J., Comparative effects of different maize straw returning modes on soil humus composition and humic acid structural characteristics in Northeast China. Chemistry & Ecology. 2018; 34: 1–16.

59. Chen Y., Senesi N., and Schnitzer M., Information Provided on Humic Substances by E4/E6 Ratios. Soil Science Society of America Journal. 1977; 41: 352–358.

60. Kuzyakov Y., Friedel J. K., and Stahr K., Review of mechanisms and quantification of priming effects. Soil Biology & Biochemistry. 2000; 32: 1485–1498.

61. Yuan s., Wang s., and Zhang w., Effect of external organic carbon and temperature on SOC decomposition. Chinese Journal of Soil Science. 2015; 46: 916–922.

62. Li C., Yan K., Tang L., Jia Z., and Li Y, Change in deep soil microbial communities due to long-term fertilization. Soil Biology & Biochemistry. 2014; 75: 264–272.

63. Cao C., Jiang S., Ying Z., Zhang F., and Han X., Spatial variability of soil nutrients and microbiological properties after the establishment of leguminous shrub Caragana microphylla Lam. plantation on sand dune in the Horqin Sandy Land of Northeast China. Ecological Engineering. 2011; 37: 1467–1475.

64. Blume E., Bischoff M., Reichert J., Moorman T., Konopka A., and Turco R. F., Surface and subsurface microbial biomass, community structure and metabolic activity as a function of soil depth and season. Applied Soil Ecology. 2002; 20: 171–181.

65. Baldrian P., Ectomycorrhizal Fungi and Their Enzymes in Soils: Is There Enough Evidence for Their Role as Facultative Soil Saprotrophs? Oecologia. 2009; 161: 657–660. https://doi.org/10.1007/s00442-009-1433-7 PMID: 19685081

66. Lim S., Baah-Achamfour M., Choi W., Arshad M., Fatemi F., Banerjee S., et al., Soil organic carbon stocks in three Canadian agroforestry systems: From surface organic to deeper mineral soils. Forest Ecology & Management. 2018; 417: 103–109.

67. Mohammed I., Longterm effects of treatments on soil physical properties, Lap Lambert Academic Publishing; 2011.

68. Liu Y., Gao M., Wu W., Tanveer S., Wen X., and Liao Y., The effects of conservation tillage practices on the soil water-holding capacity of a non-irrigated apple orchard in the Loess Plateau, China. Soil & Tillage Research. 2013; 130: 7–12.

69. Abiven S., Heim A., and Schmidt M. W. I., Lignin content and chemical characteristics in maize and wheat vary between plant organs and growth stages: consequences for assessing lignin dynamics in soil. Plant & Soil. 2011; 343: 369–378.

70. Irshad M., Eneji A., Hussain Z., and Ashraf M., Chemical characterization of fresh and composted live-stock manures. Journal of Soil Science & Plant Nutrition. 2013; 13: 115–121.

71. Singh S., Ghoshal N., and Singh K., Variations in soil microbial biomass and crop roots due to differing resource quality inputs in a tropical dryland agroecosystem. Soil Biology & Biochemistry. 2007; 39: 76–86.
82. Zhao M., Zhou J., and Chen Z., Concentration and characteristics of soluble organic nitrogen (SON) and carbon (SOC) in different types of organic manures. Acta Ecologica Sinica. 2007; 67: 916–920.
83. Lin Y., Yang X., Zhang F., Gu Q., Sun B., and Ma L., Effect of long-term fertilization on cropland soil fauna community in loess soil, shaanxi, China. Scientia Agricultura Sinica. 2005; 38: 1213–1218.
84. Xiang C., Zhang P., Pan G, Qiu D., and Chu Q., Changes in diversity, protein content, and amino acid composition of earthworms from a paddy soil under different long-term fertilizations in the Tai Lake Region, China. Acta Ecologica Sinica. 2006; 26: 1667–1673.
85. Broder M. and Wagner G., Microbial colonization and decomposition of corn, wheat, and soybean residue. Soil Science Society of America Journal. 1988; 52: 112–117.
86. Bai Z., Liang C., Bodé S., Huygens D., and Boeckx P., Phospholipid 13C stable isotopic probing during decomposition of wheat residues. Applied Soil Ecology. 2016; 98: 65–74.
87. Zhang S., Huang J., Luo Z., Dong S., Wang Y., Zhu Q., et al., Effect of adding different amounts of wheat straw and phosphorus on soil microorganism community. Chinese Journal of Applied Ecology. 2014; 25: 797–802. PMID: 24984499
88. Dong W., Li X., and Song Y., Role of soil fauna on soil organic matter formation. Soils. 2016; 48: 211–218.
89. Barot, Jiménez S., Sousa J., Filser J., Deckym J., Kutuzović G., et al., “Modeling the impact of soil fauna on soil organic matter dynamics,” presented at the 5th International Ecosummit. Ecological sustainability–Engineering change, Montpellier, Francuska, 2016.
90. Xiong S., Wang X., Li C., Ma X., Du S., and Zhang Y., Responses of the spatial-temporal distribution of winter wheat(Triticum aestivum) roots and yield to different ratios of nitrogen sources. Chinese Journal of Plant Ecology. 2011; 35: 759–768.
91. Chen J., Xu J., and Kuang S., Mathematics model of area and spatial density distribution in maize root system. Anhui Agricultural Science Bulletin. 2007; 23: 717–720.
92. Shahzad T., Rashid M., Maire V., Barot S., Perveen N., Alvarez G., et al., Root penetration in deep soil layers stimulates mineralization of millennia-old organic carbon. Soil Biology & Biochemistry. 2018; 124: 150–160.
93. Kuchenbuch R. and Barber S., Yearly variation of root distribution with depth in relation to nutrient uptake and corn yield1. Communications in Soil Science & Plant Analysis. 1987; 18: 255–263.