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Soil Carbon and Nitrogen Transformations under Soybean as Influenced by Organic Farming

X.M. Tian, H. Fan, F.H. Zhang, K.Y. Wang,* J.A. Ippolito, J.H. Li, Z.W. Jiao, Y.B. Li, Y.Y. Li, J.X. Su, W.T. Li, and M.J. An

ABSTRACT

Organic farmers use natural inputs and ecological principles to produce crops in ways that protect soil, the environment, and human health. The objectives of this study were to determine (i) how organic farming with composted manure application affects soil properties under soybean and (ii) how soil C and N transformations in this system are related to soil microbial community structure, soil nutrient and heavy metal content, and soybean yield and quality. The results showed that three years of organic farming promoted soil aggregation and significantly increased aggregate associated C and N concentrations by 11.7 to 24.1% and 9.4 to 17.0%, respectively. Microbial biomass and species diversity (i.e., Shannon, Simpson, and Pielou indexes) were significantly greater in the organic farming than in the conventional farming. Organic farming also increased soil respiration by 56%, nitrification by 51%, and dentrification by 75%. Soybean yield was 13.7% in the organic farming than in conventional farming. Organic farming increased soil Cu and Ni; however, their concentrations were still less than the allowable limits for organic production. Redundancy analysis indicated that the increases in soil nutrient content, heavy metal content, and soybean yield in the organic farming were closely linked with C and N concentrations in the <0.25-mm size fraction. In summary, organic farming with composted manure application improved soil properties and altered the structure and function of the microbial community. The <0.25-mm aggregate fraction had a major influence on microbially mediated C and N transformations and soybean yield.

Core Ideas

- Organic farming significantly increased or improved many soil properties, including the mean weight diameter of aggregates, soil organic matter, available N, soil microbial diversity, and soil C and N transformation rates. Soybean yield was also 13.7 % greater in the organic farming treatment than in conventional farming treatment.
- Organic farming increased soil Cu and Ni; however, their concentrations were still less than the allowable limits for organic production.
- Organic farming with composted manure application improved soil properties and altered the structure and function of the microbial community. The <0.25-mm aggregate fraction had a major influence on microbially mediated C and N transformations and soybean yield.

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Soil supports, provides for, and regulates ecosystem services that are vital to sustainable agricultural production. Organic farming (OF) systems, which use natural inputs and emphasize ecological principles for crop production, can potentially deliver more soil ecosystem services than conventional farming (CF) systems. Previous research has shown that OF has many advantages over CF in promoting the formation of soil structure (Pulleman et al., 2003), enhancing soil biodiversity (Tu et al., 2006), protecting the environment (International Federation of Organic Agriculture Movements, 1998), improving soil quality (Patel et al., 2015), and ensuring food quality and safety (Giles, 2004).

As a key nutrient in terrestrial ecosystems, N has an important influence not only on crop growth and yield but also on many other ecological processes (Elser et al., 2007; Vitousek et al., 2010). Organic matter mineralization is one of the key processes affecting C and N cycling in agroecosystems (Janzen, 2004). Microorganisms are the mediators of soil N transformations, including biological N fixation, nitrification, and denitrification (Holst et al., 2007; Müller et al., 2007). These processes are affected by many biotic and non-biotic factors (e.g., soil temperature, moisture, organic matter content, and microbial community structure) (Liu et al., 2010; Zaman and Chang, 2004). Fertilization also has a crucial effect on soil N transformations (Zhang et al., 2012). Inorganic and organic fertilizers improve the productivity of soil ecosystems by increasing soil nutrient content and by stimulating organic matter decomposition (Köchy and Wilson, 2001; Changhui et al., 2014).

Chemical fertilizers have been linked to serious environmental problems (Cordell et al., 2009 Ju et al., 2006; Guo et al., 2010). In contrast, several studies have shown that organic fertilizers can increase yield and quality with less environmental risk than chemical fertilizers (Cai and Qin, 2006; Drinkwater et al., 1998; Nosengo, 2003). The objectives of this study were (i) to determine how OF with composted manure application

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Abbreviations: BaPS, barometric process separation; CF, conventional farming; OF, organic farming; SMC, soil microbial biomass; SMN, soil biomass nitrogen.

influences soil C and N transformation processes under soybean and (ii) to increase understanding about how these transformation processes are related to soil nutrient content, heavy metal content, microbial functional diversity, and crop yield and quality. The information obtained from this study could lead to the development of better management practices and more widespread adoption of OF systems.

MATERIALS AND METHODS

Description of the Experimental Site

The field experiment was performed at Biesituoke Township, Xinyuan County, Yili Autonomous Prefecture, Xinjiang Uygur Autonomous Region, China (43°27′ N lat., 83°17′ E long., 900 m above sea level). The area is located on the plain of the Kunnes River, a tributary of the Ili River. The study area has a continental climate. The mean annual temperature is 8.1 °C, and the mean annual precipitation is 480 mm. The region is suitable for planting soybeans, corn, sugar beet, flax, and wheat. The soil texture at the experiment site is silt loam (22% sand, 57% silt, and 21% clay). The soil was analyzed when the experiment began in 2009 using the methods listed in Table 1. The results were as follows: organic matter, 35.5 g kg^{-1}; alkali-hydrolyzable N, 88.3 mg kg^{-1}; available P, 2.73 mg kg^{-1}; and available K, 262 mg kg^{-1}. Alkali-hydrolyzable N is an indicator of the N supplying capacity of the soil.

Experimental Design

The experiment included two treatments: CF and OF. Randomized complete block design was used with three replications. The plots (40 m × 20 m) had been monocropped with soybean and fertilized with chemical fertilizer in the three years prior to the start of the experiment. In the CF treatment, the plots were fertilized with 135 kg ha^{-1} ammonium phosphate and 45 kg ha^{-1} potassium sulfate at planting and with 150 kg ha^{-1} urea during the growing season. In the OF treatment, the plots were fertilized at planting with 3000 kg ha^{-1} of composted animal (cattle, sheep, and horse) manure which had the following properties: organic matter, 45%; total N, 2.98%; total P, 1.51%; and total K, 0.65%. The crop management practices in the OF treatment were according to the standards for organic products GB/T19630 (Certification and Accreditation of People's Republic of China, 2005).

Soil Sampling and Analysis

Soil Sampling

Five soil samples were collected randomly from the 0 to 20 cm depth of each plot on September 28, 2011 (i.e., three years after the OF treatments began). The soybeans (experimental type 94–9B) were planted on 24 April, and the plots were irrigated on 12 June and on 23 August. The five soil subsamples samples were combined to form one composite sample per plot and then immediately taken to the laboratory. Half of each sample was stored at 4 °C and used to determine soil microbiological properties. The other half of each sample was air-dried and used to determine the soil physicochemical properties.

Isolation of Soil Aggregates

The soil samples were separated into four aggregate size fractions using a wet sieving method. Briefly, nested sieves (2, 0.25, and 0.053 mm) were suspended in a drum containing deionized water. The water level in the drum was adjusted so that the soil sample on the 2-mm sieve was just beneath the water surface when the sieves were at their lowest point. One hundred grams of air-dried soil were placed on the 2-mm sieve and soaked for 5 min. The sieves were moved up and down for 2 min (40 times per min, 5-cm-long stroke). The aggregates on the top of each sieve were transferred to aluminum boxes, dried at 60 °C for 48 h, and weighed.

Samples of the 0.25- to 2-mm fraction were dispersed ultrasonically to obtain microaggregates that were occluded within the macroaggregates. Briefly, 0.25- to 2-mm aggregates were put in a beaker containing 500 mL of deionized water and then sonicated at 21.5 Hz and 300 mA for 30 min in an ultrasonic cleaning tank (KQ-100VDE, KunShan Ultrasonic Instruments Co., Ltd., Kunshan, China). After sonification, the samples were wet sieved as described above to separate the following size classes: 0.25 to 2 mm, 0.053 to 0.25 mm, and <0.053 mm. The aggregate fractions were dried separately at 60 °C for 48 h, weighed, and then stored. Because its mass was very small, the 0.25 to 2 mm fraction was combined with the 0.053 to 0.25 mm fraction.

Aggregate stability was expressed as mean weight diameter (MWD) (Kemper and Rosenau, 1986):

$$\mathrm{MWD} = \sum_{i=1}^{4} \bar{X}_i W_i \qquad [1]$$

where X_i is mean diameter of size fraction i and W_i is the proportion (%) of the total sample mass in size fraction i.

Soil Chemical Analysis

The chemical properties of the whole soil and aggregate size fractions were determined according to the methods listed in Table 1. The concentrations storage of soil organic C (TOC_i, kg m^{-2}) and total N (TN_i, kg m^{-2}) in each fraction were calculated as follows (Rodriguez et al., 2001):

$$\mathrm{TOC}_i = \frac{\mathrm{SOC}_i \times \beta \times H}{100} \qquad [2]$$

$$\mathrm{TN}_i = \frac{\mathrm{SN}_i \times \beta \times H}{100} \qquad [3]$$

where SOC_i and SN_i are, respectively, the concentrations (g kg^{-1}) of soil organic C and total N in size fraction i; β is bulk density (g cm^{-3}); and H is soil depth (mm).

Respiration, Nitrification, and Denitrification

The rates of soil respiration, nitrification, and denitrification were determined using the Barametric Process Separation (BaPS) method (Ingwersen et al., 1999). Briefly, intact cores were collected on 28 August and then put into the reaction chambers of the BaPS system (UMS, Munich, Germany). The containers were sealed and put into a water bath at 20 °C, which was approximately the soil temperature at the time of sampling. The air tightness of the seals was checked by extracting 10 mL of gas and checking that the pressure change in the confined space was less than 0.2 hPa within 10 min. The

Table 1. Soil analysis methods.

Item	Method	Reference
Soil organic C	Dichromate oxidation method	Kalembasa and Jenkinson, 1973
Soil total N	Kjeldahl method	Jackson, 1973
Bulk density	Cutting-ring method	Carter, 1993
Soil total P and available P	Mo-Sb colorimetry	Cao et al., 2012
Alkaline hydrolyzable N (AN)	Alkali solution diffusion	Page et al., 1982
Heavy metals (Cu, Ni, Pb, As, Cd, Zn, Cr, Hg)	ICP and AFS	Sumner and Miller, 1996
Trace elements (Ca, Mg, Mn, Se, Fe, Mo)	ICP and AFS	Sumner and Miller, 1996
Ammonium nitrogen (NH_4^+–N)	Indophenol blue method	Keeney, 1982
Nitrate nitrogen (NO_3^-–N)	UV spectrophotometer	Keeney, 1982

samples were incubated for 12 h. The rates of respiration, nitrification, and denitrification were calculated using software that was supplied with the BaPS system and based on the theoretical work of Ingwersen et al. (1999).

Soil Microbial Analyses

Soil microbial biomass C (SMC) and soil microbial biomass N (SMN) were determined in fresh soil samples using a chloroform fumigation extraction method (Anderson and Domsch, 1978). The C source utilization patterns of the soil microbial communities were determined using the Average Well Color Development (AWCD) method (Bartelt-Ryser et al., 2005). Briefly, fresh soil samples were diluted 1:1000 with sterile 0.9% NaCl solution. After adjusting to pH 7.0, the suspensions were inoculated into BIOLOG Ecoplates (BIOLOG, Hayward, CA) containing 31 carbon sources. The plates were inoculated at 28 °C in the dark. Substrate utilization was measured every 12 h at 590 nm for 7 d. The data were collected with Microlog Release 4.20 software (ML 3402, Microlog). The readings for individual substrates were corrected, and each absorbance value was corrected by subtracting the absorbance value of the control well (0.9% NaCl solution). The diversity indexes were calculated using the absorbance values at 168 h according to the following equations:

$$\text{Shannon index} = -\sum P_i \log P_i \quad [4]$$

$$\text{Simpson index} = 1 - \sum P_i^2 \quad [5]$$

$$\text{Pielou index} = \text{Shannon index}/\text{LnS} \quad [6]$$

where P_i is the ratio of the activity on each substrate (ODi) to the sum of activities on all substrates (OD) and S is the total number of C sources (i.e., 31). The microbial quotient, which indicates how effectively microorganisms are using the soil organic matter, was calculated by dividing SMC by SOC.

Soybean Yield and Quality

Soybean production was measured in three 1 m × 1 m subplots within each plot. The following variables were measured: plant number, pod number per plant, 100-grain weight, and yield. Soybean grain samples were taken to the laboratory. After removing impurities, the grain samples were analyzed with an Infratec 1241 Grain Analyzer (Foss NIR Systems INC., Hoganas, Sweden) to determine their moisture, protein, and oil content (Quaranta et al., 2010).

Table 2. Selected soil chemical properties in the conventional farming (CF) and organic farming (OF) treatments.

Treatment	SOC†	TN	TK	TP	AN	AP
	g kg^{-1}				mg kg^{-1}	
CF	29.8b‡	1.79b	22.7a	2.36a	82.7b	13.9a
OF	33.8a	2.00a	21.7a	1.81b	95.3a	13.2a
	AK	Ca	Mg	Mn	Fe	Se
	mg kg^{-1}	g kg^{-1}	mg kg^{-1}			
CF	184a	8.62b	553b	916b	28.6a	14.9a
OF	182a	15.3	1935a	987a	30.0a	10.0b

† SOC, total soil organic carbon; TN, total soil nitrogen; TK, total soil potassium; AN, alkali-hydrolyzable nitrogen; AP, available phosphorus; AK, available potassium.

‡ For each variable, different letters between treatments indicate significant difference at $P = 0.05$ ($n = 3$).

Statistical Analyses

Statistical analyses were performed using SPSS version 17.00 (SPSS Inc., Chicago, IL). The *F*-tests from one-way analysis of variance were used to separate the means of the two treatments ($P = 0.05$). Canonical correspondence analysis (RDA) with the Monte Carlo permutation test (999 permutations) was applied to test which environmental variables best explained the variation in soil C and N transformation, soil microbial functional diversity, soil nutrient and heavy metal concentrations, and soybean yield and quality. The Monte Carlo permutation test is a method for testing the significance of the variables by automatic selection. The analysis was performed with Canoco 4.5 software (Biometry, Wageningen, Netherlands). The figures were created with Origin 8.5 software.

RESULTS

Soil Nutrient Concentrations

Organic farming significantly increased OM by 14%, total N by 12%, available N by 15%, Ca by 72%, Mg by 265%, and Mn by 6.0% compared with CF (Table 2). In comparison, OF significantly limited total P by 23% and Se by 33%. There was no significant difference between OF and CF in total K, available P, available K, and Fe.

Soil Heavy Metal Concentrations

Heavy metal concentrations in both CF and OF were below the limits set by the People's Republic of China for organic products (Table 3) (Certification and Accreditation Administration of People's Republic of China, 2005). Compared with CF, OF significantly limited Pb by 57%, As by 14%, and Cd by 44%. In contrast, OF significantly increased Cu by 29% and Ni by 15%. There were no significant differences between farming practices in Zn, Cr, and Hg.

Table 3. Heavy metal concentrations in the conventional farming (CF) and organic farming (OF) treatments

Heavy metal	CF	OF	GB† Soil pH 6.5–7.5	GB† Soil pH >7.5	Heavy metal	CF	OF	GB Soil pH 6.5–7.5	GB Soil pH >7.5
				mg kg^{-1}					
Cu	24.9b‡	31.1a	100	100	Pb	43.8a	19.4b	300	350
Zn	89.9a	87.3a	250	300	As	14.4a	12.3b	30	25
Ni	27.4b	31.5a	50	60	Hg	0.004a	0.005a	0.5	1.0
Cr	72.7a	73.2a	200	250	Cd	0.21a	0.08b	0.3	0.6

† GB indicates the maximum allowable concentration for organic production in China (Document GB/T19630–2005).

‡ For each variable, different letters between treatments indicate significant difference at $P = 0.05$ ($n = 3$).

Table 4. Aggregate size distribution in the conventional farming (CF) and organic farming (OF) treatments.

Treatments	Soil mass <0.053	Soil mass 0.053–0.25	Soil mass 0.25–2.0	Soil mass >2	MWD†
			mm		
CF	58.1a‡	15.6b	25.8a	0.67a	0.11b
OF	47.3b	23.2a	27.9a	0.84a	0.13a

† MWD, mean weight diameter.

‡ For each variable, different letters between treatments indicate significant difference at $P = 0.05$ ($n = 3$).

Soil Aggregation and Aggregate-associated Nutrient Concentrations

Aggregate Size Distribution

The distribution of soil mass among aggregate size fractions followed similar patterns in both treatments, with the <0.053-mm fraction having the highest percentage of soil followed by the 0.25 to 2.0, 0.053 to 0.25, and then the >2.0-mm fractions (Table 4). Compared with CF, OF had significantly (19%) less soil mass in the <0.053-mm fraction but significantly (49%) more soil mass in the 0.053- to 0.25-mm fraction. There were no significant differences between the two treatments either in the 0.25 to 2.0 fraction or in the >2.0-mm fraction. The MWD, which reflects aggregate stability, was significantly greater in OF than in CF.

Aggregate C and N concentrations

Organic farming significantly increased organic C and total N concentrations in each size fraction (Fig. 1a, b). Organic C concentrations in the <0.053-, 0.053- to 0.25-, 0.25- to 2.0-, and >2.0-mm fractions were 14.0, 12.0, 14.4, 24.1% greater, respectively, in OF than in CF. Similarly, OF increased total N concentrations in the <0.053-, 0.053- to 0.25-, 0.25–2.0, and >2.0-mm fractions by 11.7, 9.4, 9.5, 17.0%, respectively.

Organic farming also significantly increased the organic C and total N concentrations of microaggregates occluded within macroaggregates (Fig. 1c, d). Organic C concentrations in the <0.053 and 0.053 to 0.25 microaggregates were 14.0 and 13.8% greater, respectively, in OF than in CF. The total N concentrations in the <0.053 and 0.053 to 0.25 microaggregates were 23.1 and 19.4% greater, respectively, in OF than in CF.

Soil C and N Storage

Combining the four aggregate size classes, the total amount of soil organic C storage was 20% greater in OF (5.60 kg m^{-2}) than in CF (4.67 kg m^{-2}) (Fig. 2*a*). Total N storage was 18.5% greater in OF (0.58 kg m^{-2}) than in CF (0.46 kg m^{-2}) (Fig. 2b). In both treatments, organic C and total N storage were greatest in the <0.053-mm fraction and least in the >2-mm fraction. Organic farming significantly increased organic C storage in the 0.053 to 0.25 and >2.0 fractions, but had no significant effect on the <0.053-mm and 0.25 to 2.0 fractions. In comparison, OF significantly increased total N storage in all except the <0.053-mm fraction.

Microbial Biomass, Respiration, Nitrification, and Denitrification

The OF treatment significantly increased SMC by 60%, SMN by 233%, and the microbial quotient by 41% compared with CF (Table 5). The OF treatment also increased the rates of soil respiration, nitrification, and denitrification by 56, 51, and 75%, respectively. There was no significant difference in CO_2 flux between the two treatments; however, N_2O flux was 31% greater in CF than in OF. There was no significant difference in NH_4^+–N concentration between the two treatments, whereas NO_3^-–N concentrations were 31% greater in OF than in CF.

Carbon Utilization and Microbial Diversity

Average Well Color Development

The AWCD reflects the ability of microbial communities to utilize various C sources. The AWCD values increased slowly between 0 and 24 h and then rapidly between 24 and 156 h (Fig. 3). This re after a period of adjustment, the metabolic activity of the microbes was intense in either treatment. The trends were similar in both treatments; however, AWCD was generally greater in OF than in CF.

Microbial Species Diversity

The C utilization patterns of the microbial communities in OF and CF were compared using 31 different C sources. The C sources were divided into six types based on their functional groups: polymers (four compounds), carbohydrates (eight compounds), carboxylic acids (nine compounds), amino acids (six compounds), amines (two compounds), and phenols (two compounds). The optical density values of carboxylic acids and amino acids were significantly greater in OF than in CF, whereas those of phenols were significantly less (Table 6). This result means that compared with the microbial community in CF, microorganisms in OF had greater ability to utilize carboxylic acid and amino acids but less ability to utilize phenols. Organic farming significantly increased species diversity (i.e., Shannon, Simpson, or Pielou indexes) compared with CF (Table 6).

Soybean Yield and Quality

Soybean yield was significantly (14%) greater in OF than in CF (Table 7). The differences in yield can be attributed to

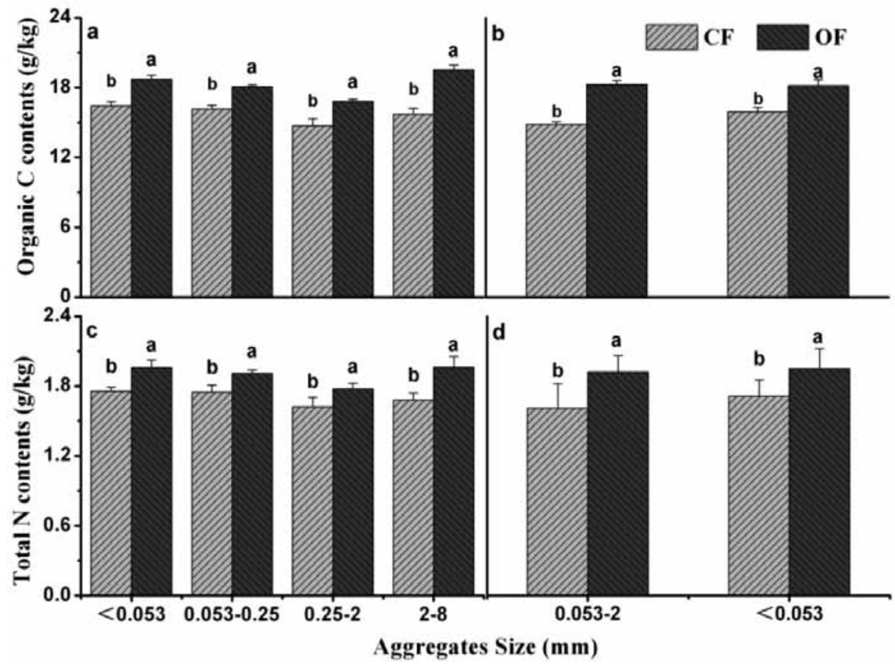

Fig. 1. Soil organic C and total N concentrations in aggregates (panels *a* and *c*) and in microaggregates occluded within macro-aggregates (panels *b* and *d*) under conventional farming (CF) and organic farming (OF). Different letters within a size fraction indicate significant differences between the two treatments ($P = 0.05$).

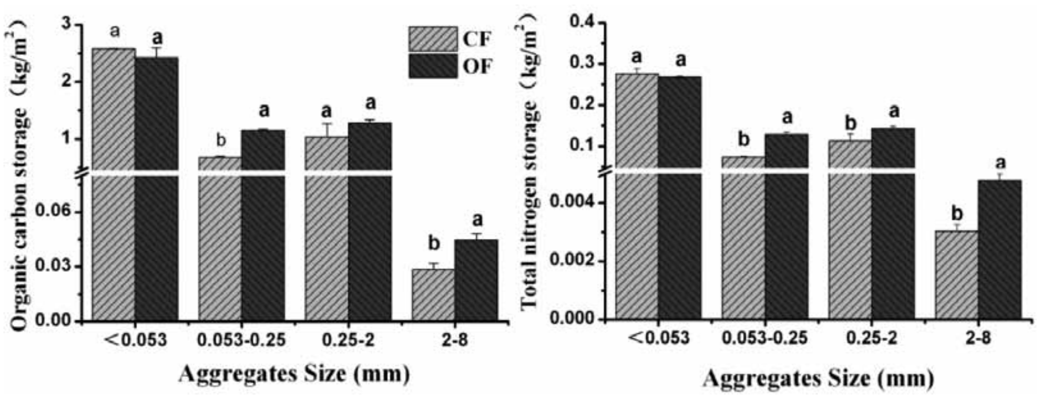

Fig. 2. Total organic C and total N in aggregate fractions as influenced by conventional farming (CF) and organic farming (OF). Different letters within a size fraction indicate significant differences between the two treatments ($P = 0.05$).

significantly (12%) greater hundred-grain weight in the OF treatment. There was no significant difference in pod number between the two treatments. It was interesting to note that at harvest, the plant density was significantly less in OF than in CF. In regard to grain quality, OF increased soybean protein content by 2.9% but suppressed oil content by 4.2%.

Redundancy Analysis

In this study, the following factors were considered to be indicators of soil C and N transformations: (i.e., total organic C storage in each size fraction, total N storage in each size fraction, nitrification rate, denitrification rate, N_2O flux, CO_2 flux, NH_4^+–N, and NO_3^-–N). Figure 4*a* shows the redundancy analysis between the indicators of soil C and N transformation and microbial functional diversity. The RDA1 explained 69% of the variation. Microbial functional diversity was correlated with total N storage in the 0.053- to 0.25-mm fraction (Explains% = 67.3, Pseudo-$F = 8.25$, $P = 0.012$), nitrification rate (Explains% = 52.2 Pseudo-$F = 4.361$, $P = 0.04$) and organic C storage in the <0.053-mm fraction (Explains% = 39.0, Pseudo-$F = 2.555$, $P = 0.016$). Among these three factors, total N storage in the 0.053- to 0.25-mm fraction and nitrification rate influenced SMC and SMN. Organic C storage in the <0.053-mm fraction influenced the ability of the microbial community to utilize polymers and amines as their sole C source.

Figure 4*b* shows how the indicators of soil C and N transformations were related to soil nutrient and heavy metal concentrations. Soil nutrient and heavy metal concentrations were significantly correlated with total N storage in the 0.053- to 0.25-mm fraction (Explain% = 66.7, Pseudo-$F = 8.009$, $P = 0.004$) and the 0.25- to 2.0-mm fraction (Explain% = 49.3, Pseudo-$F = 3.883$, $P = 0.044$), denitrification rate (Explain% = 59.1, Pseudo-$F = 5.770$, $P = 0.01$), and soil organic C storage in the <0.053-mm fraction (Explain% = 35.8, Pseudo-$F = 2.231$, $P = 0.016$). Among these factors, total N storage in the 0.053 to 0.25 and 0.25- to 2.0-mm fractions and denitrification rate significantly influenced soil Fe, Mg, Ca and Cu. Soil organic C storage in the <0.053-mm fraction significantly influenced

Table 5. Soil C and N transformations in the conventional farming (CF) and organic farming (OF) treatments

Treatment	Respiration rate	Nitrification rate	Dentrification rate	N_2O flux	CO_2 flux
	μg C kg ·h^{-1}	μg N kg ·h^{-1}	μg N kg ·h^{-1}	μmol h^{-1}	
CF	433b†	368b	376b	4.2a	7.6a
OF	674a	555a	658a	2.9b	7.4a
	SMC‡	SMN	Microbial quotient	NH_4^+–N	NO_3^-–N
	mg/kg		%	mg/kg	
CF	90.7b	14.6b	0.31b	6.13a	18.8b
OF	145a	48.5a	0.43a	6.10a	24.7a

†For each variable, different letters between treatments indicate significant difference at $P = 0.05$ ($n = 3$).
‡ SMC, soil microbial biomass C; SMN, soil microbial biomass N.

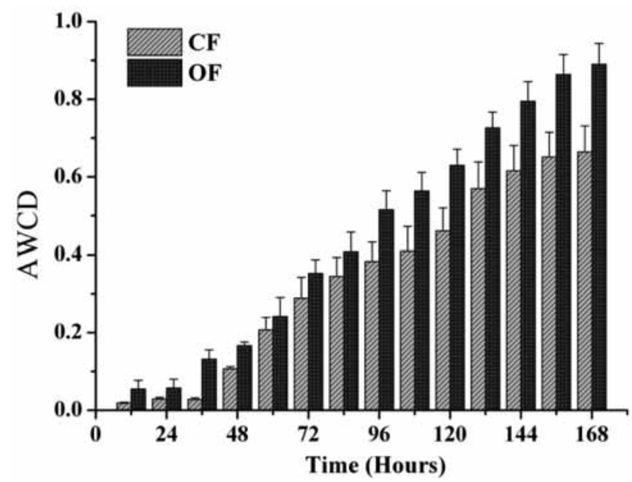

Fig. 3. Kinetics of average well color development (AWCD) as influenced by conventional farming (CF) and organic farming (OF). Error bars represent SE.

soil Pb. These results show that in CF, total N storage in the <0.053-mm fraction was closely linked to Pb. In OF, total N storage in the 0.053 to 0.25 and 0.25- to 2.0-mm fractions and denitrification rates were closely correlated with Fe, Mg, Ca, and Cu.

Figure 4*c* shows the relationship between indicators related to soil C and N transformations and soybean yield and quality. The RDA1 and RDA2 explained 77 and 12% of the variation, respectively. Soybean yield, hundred-grain weight, grain moisture content, and grain protein content were correlated with soil organic C storage in the 0.053- to 0.25-mm fraction (Explain% = 77.5, Pseudo-F = 12.303, $P = 0.042$), total N storage in the 0.053- to 0.25-mm fraction (Explain% = 76.4, Pseudo-F = 12.965, $P = 0.016$), dentrification rate (Explain% = 66.7, Pseudo-F = 8.774, $P = 0.01$), and respiration rate (Explain% = 56.1, Pseudo-F = 5.118, $P = 0.048$). These results indicate that soybean yield and quality in OF are influenced by C and N storage in the 0.053- to 0.25-mm fraction and by soil respiration and denitrification.

DISCUSSION

Soil C and N transformations

Soil aggregation is an important characteristic affecting soil quality. Organic matter acts as glue that binds sand, silt, and clay particles together to form soil aggregates. Aggregate stability generally increases as soil organic C concentrations increase (Zhou et al., 2013). The results of this study showed that OF increased the proportion of 0.053 to 0.25 mm aggregates in the soil and suppressed the proportion of <0.053-mm aggregates compared with CF. The MWD was greater in the OF treatment than in the CF treatment. Aggregate-associated C and N concentrations were also greater in OF than in CF. These results, which are similar to those of Six et al. (2000) and Mikha et al. (2005), are consistent with the hierarchical model of aggregate formation and stabilization (Tisdall and Oades, 1982). Specifically, OF increased soil organic matter which cemented microaggregates together to form macroaggregates (0.053–0.25 mm). It is also likely plant roots and microbial mycelium wrapped around microaggregates to form new macroaggregates that were enriched in organic C and N.

Soil C and N mineralization is a vital link in nutrient cycling and an essential factor determining the capacity of soil to supply nutrients. Nutrient availability is generally greater when soil C and N mineralization rates are rapid (Huygens et al., 2008). This study showed that compared with CF, OF greatly increased respiration, nitrification, and dentrification, the microbial quotient, SMC, SMN, and NO_3^-–N. As a source of C, organic fertilizers can promote denitrification directly by providing energy and substrates for denitrifying bacteria (Böhme et al., 2005; Hayakawa et al., 2009). Organic fertilizers also can stimulate autotrophic and heterotrophic nitrification (Cheng et al., 2012; Zhang et al., 2014). In this study, N_2O emission rates were significantly greater in CF than in OF. Long-term use of chemical fertilizers in CF can lead to soil acidification (Bhattacharya et al., 2016; Guo et al., 2010). A reduction in soil pH can significantly increase the contribution of denitrification to N_2O emission (Rochester, 2003; Saggar et al., 2013 Wicht, 1996).

The RDA analysis indicated that SMC and SMN in the OF treatment were affected by N concentrations in the 0.053- to 0.25-mm fraction and by denitrification rates. This test indicated that increased N concentrations in the 0.053- to 0.25-mm fraction of the OF treatment were beneficial to the growth and reproduction of soil microorganisms (Wang et al., 1998).

Soil Chemical Properties

Melero et al. (2006) compared soil properties after four to six years of OF and CF. The authors reported that soil organic C, available N, available P, and microbial biomass were all greater under OF than under CF. Bending et al. (2000) reported that OF increased soil Ca, Mg, and Mn compared with CF. In contrast, Gard et al. (2006) observed that OF had no significant effect on Fe and Se. The results of this study were similar to those described above. Specifically, OF increased soil organic matter, total N, available N, total Ca, total Mg, and total Mn compared with CF. The reasons are that (i) the biological effectiveness of nutrients in organic fertilizer is comparatively strong and (ii) organic matter decomposition activates soil micronutrients (Baumann et al., 2013). Total soil P and Se concentrations

Table 6. Soil microbial diversity in the conventional farming (CF) and organic farming (OF) treatments.

Treatment	Shannon index	Simpson index	Pielou index	Carbohydrates†	Carboxylic acids	Polymer	Amino acids	Amines	Phenols
CF	2.40b‡	0.88b	0.85b	2.62a	4.37b	2.18a	3.36b	1.61a	1.50a
OF	2.74a	0.92a	0.89a	3.66a	7.74a	2.77a	6.90a	1.21a	0.23b

† The 31 carbon sources were divided into six groups. The values in columns 5–10 represent the sum of the optical density values for all of the C sources within a group.

‡ Values in the same column followed by a different letter are significantly different ($P = 0.05$).

Table 7. Yield formation factors and soybean quality in the conventional farming (CF) and organic farming (OF) treatments.

Treatment	Pod number	Plant number	Hundred-grain weight	Yield	Moisture	Protein	Oil
	$\times 10^6$ ha	$\times 10^4$ plant ha^{-1}	g	kg ha^{-1}		%	
CF	2.49a†	65.2a	22.8b	3100b	6.5a	37.3b	19.7a
OF	2.45a	61.6b	25.5a	3525a	6.6a	38.4a	18.9b

† Values in the same column followed by a different letter are significantly different ($P = 0.05$).

were significantly less in OF than in CF. The effects of OF on soil nutrient content varies widely depending on the type of organic fertilizer that is used (e.g., livestock manure, straw, and charcoal). Long-term OF can result in the accumulation of some nutrients and the loss of others.

Many studies have shown that long-term manure application increases the concentrations of Cd, Cu, and other heavy metals in agricultural soils (Fan et al., 2013; Zaccone et al., 2010; Zhao et al., 2014). In some areas, the concentrations of these heavy metals exceed the national standards for soil environmental quality. The results of this study showed that the application of composted manure in the OF treatment greatly increased soil Cu and Ni, but did not increase soil Pb, As, and Cd. It should be noted that Cu and Ni concentrations in OF were still far below the quality standards for organic production. Other researchers have reported that OF increased soil Cu concentrations (Bending et al., 2000), but had no significant effect on soil Ni concentrations (Gard et al., 2006). The organic fertilizer in this study consisted of commercially available, composted animal manure. Although the application of livestock or poultry manure from large-scale farms can result in soil heavy metal contamination (Pérez-López et al., 2007), organic fertilizers also can limit heavy metal activity by increasing soil organic matter, which forms complexes with heavy metals (Karlsson et al., 2006). The return of crop residue to the soil is a valuable means of improving soil quality in OF without the risk of heavy metal contamination.

Zhang and Ke (2004) showed that Cu, Zn, and Cd concentrations increased as aggregate size decreased. Balabane and Van (2002) compared soil Cd concentrations among different aggregate size classes in a lightly polluted grassland soil and in an undisturbed natural soil. The largest difference in Cd concentration between the two soils was in the smallest aggregate size class (i.e., 0.10–0.05 mm fraction). In this study, RDA analysis showed that (i) denitrification rates and N storage in the 0.053 to 0.25 or 0.25- to 2.0-mm fractions contributed significantly to the accumulation of Fe, Mg, Ca, and Cu in soil and (ii) C storage in the <0.053 mm size class was associated closely with the heavy metal Pb. Compared with macroaggregates, microaggregates have greater specific surface area and higher concentrations of clay minerals and iron-manganese oxide. As a result, microaggregates have strong affinity for heavy metals (Quenea et al., 2009). Microaggregates are also relatively stable and therefore can be increasingly enriched across time.

Carbon Source Utilization

Carbon utilization by soil microorganisms can be influenced by fertilizer type. Average well color development tests can show the microbial utilization rate of different C sources. The proper application of organic fertilizers like animal manure or green manure is conducive to maintaining the diversity and activity of soil microbes (Dick et al., 1998). Manure application promotes soil biological activity by adding organic matter to the soil, increasing soil enzyme activities (Böhme et al., 2005), and improving the metabolic characteristics of soil microbial communities (Tian et al., 2016). The results of this study demonstrated that OF significantly influenced the diversity and metabolic structure of the soil microbial community. The OF treatment increased the ability of the microorganism to utilize amino acids and carboxylic acids as C sources and limited their ability to utilize phenols. The RDA analysis showed that the AWCD values of phenols and amines were related with C storage in the <0.053-mm fraction of CF. Overall, the results showed that organic fertilizer provides energy and nutrients for microorganisms, increasing their populations and metabolic activity (Huang et al., 2012). In addition, organic fertilizer can foster the growth of certain microbial populations whereas other microbial populations are inhibited, thus bringing about changes in microbial community composition (Lin et al., 2014). In addition, organic fertilizer can foster the growth of certain microbial populations whereas other microbial populations are inhibited (Tong et al., 2014), thus bringing about changes in microbial metabolic functions.

Soybean Yield and Quality

Previous studies indicated that manure increased soybean yield and biomass compared with a chemical fertilizer treatment or an unfertilized treatment (Bandyopadhyay et al., 2010; Mandal et al., 2009). Melero et al. (2006) reported that four to six years after conversion to OF, yields were higher on organic farms than on conventional farms. Studies have shown that manure and straw can both produce phytotoxins during decomposition, which may explain why plant populations were significantly less in OF than in CF (Bhattacharya et al., 2016; Prochazkova et al., 2002; Roy et al., 2010). However, prolonged nutrient release from organic fertilizer meant that nutrient availability later in the growing season was greater in OF than in CF. As a result, the hundred-grain weight, yield, and protein content were significantly greater in OF than in CF. Kumudini

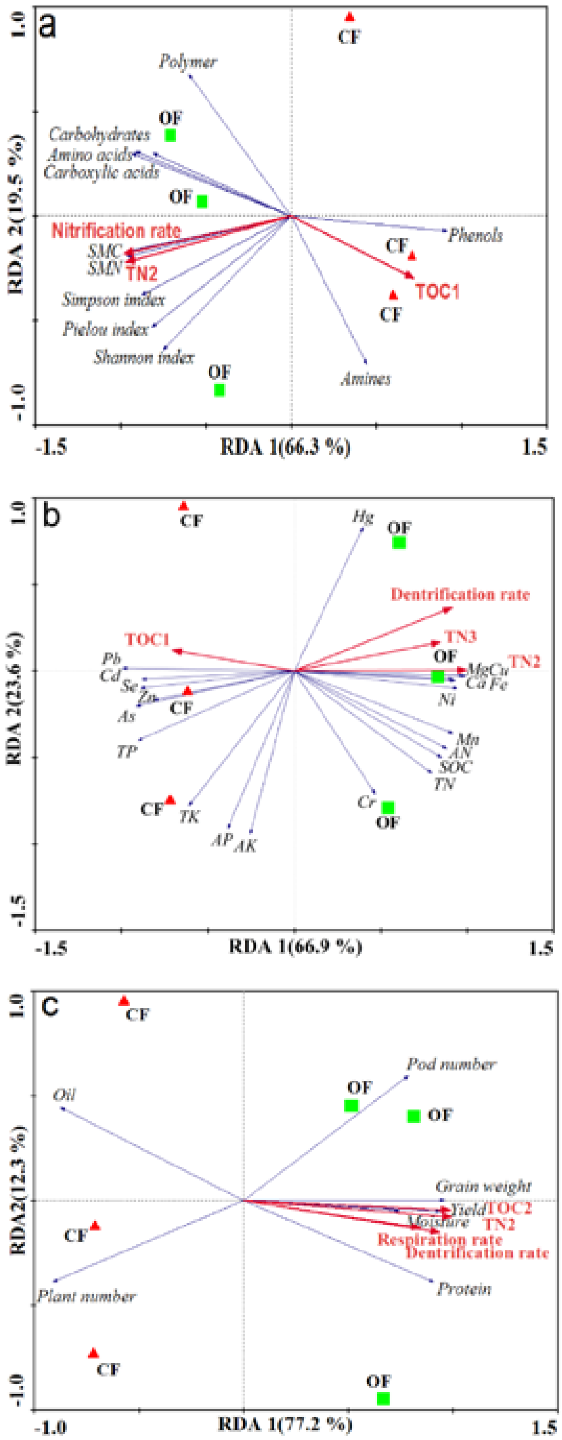

Fig. 4. Redundancy analyses (RDA) showing how factors related to soil C and N transformation are related to soil microbial functional diversity (panel *a*), soil nutrient and heavy metal concentrations (panel *b*), and soybean yield and quality (panel *c*). The factors related to soil C and N transformation included the following: total organic C storage in each size fraction, total N storage in each size fraction, nitrification rate, denitrification rate, N_2O flux, CO_2 flux, NH_4^+–N, and NO_3^-–N. Only the significant relationships ($P \leq 0.05$) are shown. Abbreviations: OF, organic farming; CF, conventional farming; TOC1, organic C storage in the <0.053 mm fraction; TOC2, organic C storage in the 0.053 to 0.25 mm fraction; TOC3, organic C storage in the 0.25 to 2.0 mm fraction; TOC4, organic C storage in the >2.0 mm fraction. TN1, organic N storage in the <0.053 mm fraction; TN2, organic N storage in the 0.053 to 0.25 mm fraction; TN3, organic N storage in the 0.25 to 2.0 mm fraction; TN4, organic N storage in the >2.0 mm fraction.

et al. (2007) reported that the nutrient requirements of soybeans is high during the seed-filling period, when nutrient metabolism influences the final soybean yield. The RDA analysis showed that soybean yield and quality was related to the following factors affecting soil C and N transformation: respiration rate, denitrification rate, and C and N storage in the 0.053- to 0.25-mm fraction. This is similar to the results of Saikia et al. (2015) who observed that C and N transformation have a major influence on soybean yield and quality.

CONCLUSION

Conversion to OF can provide a better soil environment for microbial growth and reproduction, which in turn lead to increases in microbially-mediated transformations of soil C and N, as evidenced by increases in soil respiration, nitrification, dentrification, the microbial quotient, SMC, SMN, and NO_3^-–N. Organic farming increased soil organic matter content, nutrient content, and yield compared with CF; however, OF also increased soil Cu and Ni. This increase is a potential risk even though soil Cu and Ni concentrations in this study were still less than the maximum allowable limits for organic products. The development of OF is of great significance to sustainable agricultural production. Although OF has many positive effects on the soil, it is necessary to monitor soil properties at a fixed point over an extended time to ensure the safety of organic foods.

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