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ORIGINAL PAPER

Response of soil organic matter fractions and composition of microbial community to long-term organic and mineral fertilization

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Abstract The effects of organic and mineral fertilization on four soil organic matter (SOM) fractions (non-protected, physically protected, chemically protected, and biochemically protected) and microbial community composition were investigated by sampling soil of a 35-year-long fertilization experiment. The SOM fractions were investigated by combined physical and chemical approaches, while microbial community composition was determined by phospholipid fatty acid analysis (PLFA). Organic C (SOC) was primarily distributed within the microaggregate-protected particulate organic matter (iPOM) and the hydrolysable and non-hydrolysable silt-sized (H-Silt, NH-Silt) fractions, which accounted for 11.6–16.9, 23.4–28.9, and 25.4–30.6% of the total SOC content, respectively. The contributions of these "slow" fractions (iPOM, H-Silt, NH-Silt) to the increased SOC were 178–293, 118–209, and 85–109% higher after long-term sole manure or manure in combination with inorganic N fertilization compared with unfertilized soil (control). The combination of manure and mineral fertilizers increased the coarse and fine non-protected C (cPOM and fPOM) contents much more (34.1–60.7%) than did manure alone. PLFAs, bacteria, G (+) bacteria, and actinomycete abundances were the highest in soil with manure, followed by soil treated with manure combined with mineral N. The addition of inorganic and organic fertilization both altered the microbial community composition compared with the control. All SOM fractions contributed to 81.1% of the variance of the PLFAs-related microbial community composition by direct and indirect effects. The change in coarse unprotected particulate organic matter (cPOM) was the major factor affecting soil microbial community composition ($p < 0.001$). Our study indicates that physical, chemical, and biochemical protection mechanisms are important in maintaining high SOC level after the addition of manure. A close linkage between soil microbial community composition and cPOM suggests that C availability is an important factor for influencing microbial composition after long-term inorganic and organic fertilization.

Keywords Long-term fertilization · Particulate organic matter · Soil aggregation · Soil microbial community composition · Physical and chemical fractionation

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Introduction

Fertilization is the most common worldwide management practice for improving soil quality, and it is often recommended to increase soil organic matter (SOM) and fertility in intensively managed agroecosystems (Majumder et al. 2007; Ai et al. 2012; Shang et al. 2014). The SOM cycling is mainly

mediated by microbial activity and community composition (Balser and Firestone 2005; Bowles et al. 2014). Fertilization impacts SOM content and quality and nutrient cycling by affecting the composition of microbial communities.

The effects of fertilizers (either organic or mineral) on SOM cycling have received extensive attention. A number of field experiments have revealed that long-term fertilization, especially the application of manure or in combination with mineral fertilizers, can increase SOM and the contents of organic matter of its fractions (Majumder et al. 2007; Purakayastha et al. 2008; Chai et al. 2015; Wang et al. 2015; Li and Han 2016). The positive effect of manure on C sequestration was also showed by a meta-analysis based on 95 long-term field experiments (Tian et al. 2015).

In most soil C and N models, SOM is considered to be composed of several functional pools that differ both in their intrinsic degradability and in the factors controlling the relative decomposition rates (Stevenson 1994; Von Lützow et al. 2007). For example, considering different protection mechanisms, SOM can be separated into physically, chemically, and biochemically protected pools and labile non-protected pools (Six et al. 2002). Relative increases in the SOM contents in particulate organic matter (POM), light fraction organic matter (LFOM) or soil aggregate size fractions after long-term fertilization were detected by physical fractionation (Su et al. 2006; Purakayastha et al. 2008; Bhattacharyya et al. 2010; Yan et al. 2012; Chai et al. 2015). The chemical stabilization of SOM through silt and clay particles is well established (Six et al. 2002). However, the relative importance of differing SOM stabilization mechanisms for C sequestration under the long-term effects of fertilization is still unclear. This is because only a few studies have used a combination of physical and chemical fractionation techniques, which can isolate conceptual chemical, physical, biochemical, and non-protected SOM pools simultaneously under long-term fertilization (Sleutel et al. 2006).

After long-term fertilization, greater SOC was observed, accompanied by higher microbial activity (Islam et al. 2011; Ai et al. 2012) or a shift in microbial community composition to more obligate anaerobes (Zhang et al. 2015), compared to unfertilized plots. In addition, compared to total SOM, certain fractions of SOM were more related to microbial community composition (Cookson et al. 2005; Huang et al. 2008; Cusack et al. 2011). In terms of the four functional SOM fractions, physical protection is indicated by the positive influence of aggregation on the accumulation of SOM, chemical protection is understood as the stabilization of SOM by occlusion with minerals, while biochemical protection by recalcitrance (Six et al. 2002). The non-protected fractions are not intimately associated with soil mineral particles and are not occluded within aggregates (Six et al. 2002). Compared with physically, chemically, and biochemically protected pools, the non-protected fractions, i.e., coarse and fine particulate organic matter (POM and light fraction), are mainly composed of newly plant residue input and thus decompose more easily (Six et al. 2002; Baldock et al. 1997). Previous study found that LFOM was an important C source for microorganisms (Chotte et al. 1998; Cookson et al. 2005). However, no studies have simultaneously tested the relationship between soil functional SOM fractions and composition of microbial communities related to the long-term addition of fertilizer. Therefore, better understanding the interactive mechanisms of long-term mineral fertilizers or manure additions affecting microbial community compositions associated with four functional SOM fractions are necessary.

We hypothesized that (1) physical, chemical, and biochemical protection mechanisms simultaneously play an important role in maintaining high SOC level after the addition of manure and (2) changes of contents of soil SOM fractions after long-term mineral fertilizers or manure additions are important factors for shaping microbial community composition. To test our hypotheses, we evaluated how mineral fertilizers and manure affected functional SOM fractions (unprotected, physically protected, chemically protected, and biochemically protected) and microbial community composition (using phospholipid fatty acid analysis, PLFA) based on a 35-year-long field experiment. We also evaluated the relationships between functional SOM fractions and shifts in microbial community composition in soils.

Materials and methods

Study site and field experiment

A long-term field experiment was established in 1978 in Laiyang, Shandong Province, China (120° 42′ E, 36° 54′ N). The climate for this region is characterized as a semi-humid warm-temperate continental monsoon climate with a mean annual temperature of 11.2 °C and an annual precipitation of 779 mm. The field experiment was a winter wheat (*Triticum aestivum* L.)–summer maize (*Zea mays* L.) rotation. The soil was classified as non-calcareous fluro-aquic soil according to Chinese classification and had developed on alluvial parent materials. It has a loam texture with 52.1% sand, 28.7% silt, and 19.2% clay. The basic soil characteristics (0–20 cm) at the start of the field experiment were 4.10 g kg^{-1} SOC, 0.5 g kg^{-1} total N (TN), 0.46 g kg^{-1} total P, 15 mg kg^{-1} Olsen-P, and a soil pH of 6.8.

Twelve treatments were laid out in a randomized complete blocked design with three replicates per each treatment. Each plot was 33 m^2 in size. Five treatments were selected for this study, including (1) control (CK, without any organic and mineral fertilizers), (2) N fertilization (N, urea 276 kg N ha^{-1} a^{-1}), (3) NPK fertilization (NPK, 276 kg N ha^{-1} a^{-1}, 90 kg P_2O_5 ha^{-1} a^{-1},

135 kg K_2O ha^{-1} a^{-1}), (4) manure application (M, pig manure), and (5) combined manure and N fertilizer application (MN; the same rates of chemical fertilizers as N treatment and same amounts of manures as M treatment). The fresh pig manure, with 70.0% water content, was applied into soil with an annual rate of 23.4 t ha^{-1} $year^{-1}$. The dry matter of pig manure contained 331 g kg^{-1} C, 19.7 g kg^{-1} total N (TN), 18.4 g kg^{-1} total P, and 12.3 g kg^{-1} total K. P, and K fertilizers and manure were applied as basal fertilization and were broadcasted evenly onto soil surface 2 days before sowing winter wheat and maize, and then the surface soil (0–15 cm) was tilled immediately. N fertilizer was split into three doses, 41.4 kg N ha^{-1} a^{-1} at the seeding time of winter wheat, 96.6 kg N ha^{-1} a^{-1} at the green returned stage of wheat and jointing stage of maize, and 138 kg N ha^{-1} a^{-1} at the jointing stage of wheat and the small bell mouth period of maize. All the crops were manually harvested and the aboveground crop residue removed from the field.

Soil sampling

After the winter wheat harvest in May 2014, soil was sampled from each plot by collecting eight randomly selected cores (0–10 cm deep), which were mixed to yield one composite sample per plot. The samples were then stored in airtight polypropylene bags, placed in a cooler box at 4 °C, and transported to the laboratory. Visible leaves, roots, and rock fragments were carefully removed, and the remaining soil samples were divided into several subsamples. Subsamples for SOM fractionation were air dried at room temperature. The subsamples for the microbial community analysis were stored at −80 °C.

Soil chemical analysis

Soil pH was measured using a pH meter after shaking the soil in deionized water (1:2.5 *w/v*) suspensions for 30 min. The SOC and TN contents were determined by a Vario EL III Elemental Analyzer (Elementar, Germany). Ammonium N (NH_4^+-N) and nitrate N (NO_3^--N) concentrations were determined by extracting the soil with 0.01 M KCL (1:10 *w/v*) for 30 min and were subsequently analyzed using an autoanalyzer (TRAACS-2000, BRAN+LUEBBE, Germany).

Soil SOM fractionation

Soil was fractionated using a combination of physical, chemical, and density methods (Six et al. 2002; Plante et al. 2006a; Stewart et al. 2008) (Fig. 1). The first step was to obtain three size fractions by the partial dispersion and physical fractionation of the soil (<2 mm) in the microaggregates isolator described by Six et al. (2000). The three size fractions were >250 μm (coarse unprotected particulate organic matter, cPOM), 53–250 μm (microaggregate fraction), and <53 μm (easily dispersed silt and clay, dSilt and dClay).

The second step was a further fractionation of the microaggregate fraction isolated in the first step. Fine unprotected POM (fPOM) was isolated by density flotation using 1.85 g cm^{-3} sodium polytungstate. After removing the fPOM, the heavy fraction was dispersed by shaking with 12 glass beads overnight and sieved (<53 μm), separating the microaggregate-protected POM (>53 μm in size, iPOM) and the microaggregate-derived silt- and clay-sized fractions (μSilt and μClay).

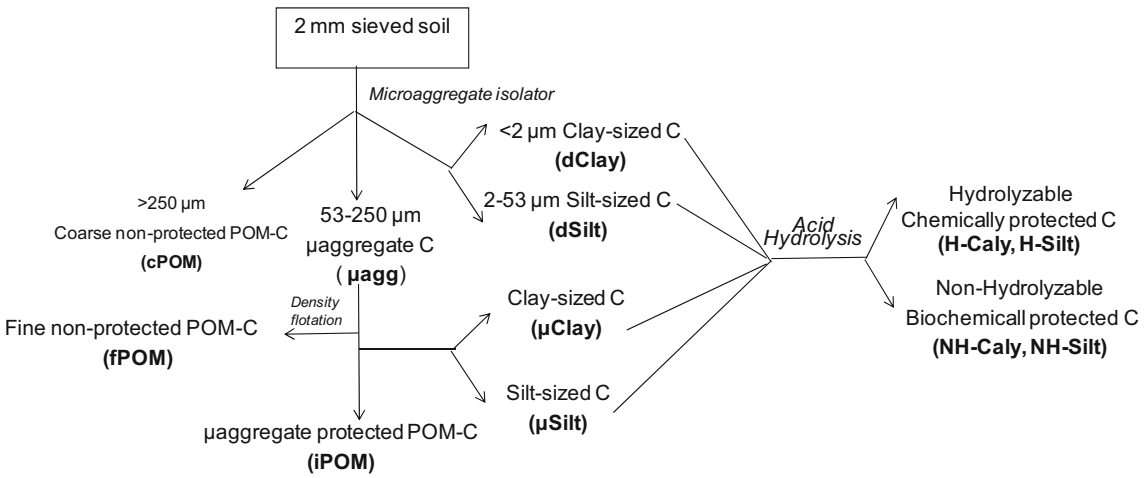

Fig. 1 A soil fractionation scheme that isolates the four hypothesized C fractions: non-protected (cPOM + fPOM), physically protected (iPOM), chemically protected (H-Silt + H-Clay), and biochemically protected fractions (NH-Silt + NH-Clay) (modified from Six et al. 2002; Plante et al. 2006a; Stewart et al. 2008). *cPOM* coarse unprotected particulate organic matter, *fPOM* fine unprotected POM, *iPOM* microaggregate-protected POM, *NH-μSilt* non-hydrolyzable microaggregate-derived silt-sized fraction, *NH-μClay* non-hydrolyzable microaggregate-derived clay-sized fraction, *H-μSilt* hydrolyzable microaggregate-derived silt-sized fraction, *H-μClay* hydrolyzable microaggregate-derived clay-sized fraction, *H-dSilt* hydrolyzable easily dispersed silt-sized fraction, *H-dClay* hydrolyzable easily dispersed clay-sized fraction, *NH-dSilt* non-hydrolyzable easily dispersed silt-sized fraction, *NH-dClay* non-hydrolyzable easily dispersed clay-sized fraction

The third step involved acid hydrolysis of the silt- and clay-sized fractions isolated in the first two steps, as described by Plante et al. (2006a). Acid hydrolysis consisted of refluxing at 95 °C for 16 h in 6 M HCl. After refluxing, the suspension was filtered and washed with deionized water over a glass fiber filter. Residues were dried at 60 °C, weighed, and analyzed for organic C content. These represented the non-hydrolyzable C fractions (NH-dSilt, NH-dClay, NH-μSilt, and NH-μClay). The hydrolysable C fractions (H-dSilt, H-dClay, H-μSilt, and H-μClay) were determined by the difference between the total organic C content of the whole fractions and the C contents of the non-hydrolyzable fractions.

All fractions were divided into four C pools based on the assumed link between the isolated fractions and the protection mechanisms. The non-protected C pool consists of the cPOM fraction and the fPOM fraction. The physically protected C pool was iPOM. The chemically protected pool corresponds to the hydrolyzable portion of the silt- and clay-sized fractions isolated during the initial dispersion (H-Silt and H-Clay). The biochemically protected pool corresponds to the non-hydrolyzable C remaining in the silt and clay fractions after acid hydrolysis (NH-Silt and NH-Clay).

Analysis of the composition of soil microbial communities

The composition of the microbial community was determined using PLFA analysis (Frostegård et al. 1991). The fatty acids were extracted from 8 g of dry-weight-equivalent fresh soil using a one-phase extraction mixture containing chloroform, methanol, and phosphate buffer. The amounts of fatty acid methyl esters (FAMEs) were analyzed on a GC-MS (TRACE GC Ultra ISQ). The individual compounds were identified by comparing their relative retention times to commercially available 37 FAMEs (FAME 37 47,885-U, Supelco, Inc.) and a mixture of 26 bacterial FAMEs (BAME 26 47,080-U, Supelco, Inc.). The individual compounds were quantified based on an internal standard.

We distinguished four microbial groups: Gram-positive bacteria (i14:0, i15:0, a15:0, a16:0, i17:0), Gram-negative bacteria (16:1ω7c, 18:1ω7c, cy17:0, cy19:0), fungi (18:2ω6c, 18:1ω9c), and actinomycetes (10Me18:0, 10Me20:0) (Zelles 1997, 1999; Bossio and Scow 1998; Frostegård et al. 1993).

Statistical analysis

Principal component analysis (PCA) was used to assess changes in the composition of soil microbial community. A permutational multivariate analysis of variance (PERMANOVA) was further used to reveal significant shifts in the community composition in any pair of samples (Anderson 2005).

To determine the significance of the effects of the analyzed soil variables (including SOM fractions and other chemical parameters) on microbial community composition and their relative contributions, canonical correspondence analysis (CCA) and variation partitioning analysis (VPA) were performed using the vegan package in R v.3.2.1.

The SOM fractions and other chemical properties were analyzed using a one-way ANOVA with SAS. Differences were considered significant at $p < 0.05$, and a post hoc least significant difference (LSD) test was carried out to compare differences between treatments.

Results

Organic C, total N, pH, and inorganic N

The SOC and TN contents were 11.0–155 and 51.2–208% higher, respectively, after long-term organic or mineral fertilization than in the CK soil ($p < 0.05$, Table 1). Compared to CK, the SOC/TN ratio decreased after long-term organic or mineral fertilization ($p < 0.05$, Table 1). Compared to CK, the inorganic N (nitrate and ammonium) concentrations were 5.55–16.8 and 1.83–2.24 times higher after the long-term application of organic manure, regardless of whether inorganic N fertilizer was added ($p < 0.05$, Table 1). The soil pH values were lowest after long-term inorganic fertilization (N, NPK) ($p < 0.05$, Table 1).

Table 1 Effects of long-term fertilization on soil chemical properties

Treatments	SOC (g C kg^{-1})	TN (g N kg^{-1})	SOC/TN	NO_3^--N (mg N kg^{-1})	NH_4^+-N (mg N kg^{-1})	pH
CK	5.71 (0.24)c	0.51 (0.05)c	11.4 (1.09)a	0.95 (0.04)c	0.43 (0.05)c	7.90 (0.04)a
N	6.71 (0.28)b	0.77 (0.06)b	8.77 (0.35)b	4.51 (0.12)b	1.16 (0.07)a	6.70 (0.22)c
NPK	6.34 (0.01)b	0.76 (0.06)b	8.38 (0.69)b	1.64 (0.14)c	0.78 (0.06)b	6.72 (0.01)c
M	14.3 (0.13)a	1.55 (0.03)a	9.22 (0.22)b	5.37 (1.25)b	0.79 (0.09)b	7.70 (0.02)a
MN	14.5 (0.31)a	1.56 (0.04)a	9.33 (0.009)b	15.9 (1.27)a	0.97 (0.14)ab	7.13 (0.08)b

Different lower case letters indicate significant differences among treatments and numbers in brackets represent standard error of means ($n = 3$)

CK control, *N* N fertilizer application, *NPK* NPK fertilizer application, *M* manure application, *MN* combined application of manure and N fertilizer

Organic C in soil functional SOM fractions

Non-protected C (cPOM and fPOM) and physically protected C (iPOM) were 102–348% higher after manure application (M, MN) than were in the CK and mineral-fertilized soils (N, NPK) ($p < 0.05$; Fig. 2). The combination of manure and mineral fertilizers (MN) increased non-protected C (cPOM and fPOM) contents much more (34.1–60.7%) than did manure alone ($p < 0.05$; Fig. 2). Mineral fertilizers (N, NPK) led to an increase in the OC content in fPOM, but not in cPOM, relative to CK ($p < 0.05$; Fig. 2). Organic C contents in biochemically protected fractions (NH-Silt and NH-Clay) and H-silt fraction were higher after the addition of manure (M, MN) relative to CK and mineral-fertilized treatments (N, NPK) ($p < 0.05$; Fig. 2). Organic C contents in H-clay were 1.13–1.48 times higher with manure (M, MN) than in CK ($p < 0.05$; Fig. 2).

Soil microbial community composition

The total PLFA abundance was the highest after the addition of manure alone ($p < 0.05$; Table 2). The sole application of manure also increased bacterial abundance, specifically increasing G (+) abundance by 116–135 and 112–143% compared to CK and mineral fertilization treatments (N, NPK), respectively ($p < 0.05$; Table 2). The combined application of manure and N fertilizer decreased total PLFAs, bacterial abundance, and G(+) abundance by 15.9, 18.9, and 19.4%, respectively, compared with the sole addition of manure ($p < 0.05$; Table 2). Independent of N addition, fungi and actinomycetes were 31.63–70.48 and 37.8–183.96% more abundant, respectively, after manure application (M, MN) than without manure application (CK, N, NPK) ($p < 0.05$; Table 1). The bacteria/fungi ratio was 1.34 times higher in the M treatment than in the CK treatment ($p < 0.05$; Table 2).

The PCA analysis confirmed that the pattern of microbial community composition was altered by the addition of fertilizer ($p = 0.0001$; PERMANOVA test; Fig. 3). The analysis showed that the PLFA patterns after fertilization (M, MN, N, and NPK) significantly differed from those following CK treatment (PERMANOVA test; Fig. 3). Further, when examining the entire soil microbial community, there was a significant difference between manure application (M, MN) and mineral fertilization (N, NPK) (PERMANOVA test; Fig. 3). The PC1 and PC2 components accounted for 67.4 and 16.8% of the total variance, respectively (Fig. 3).

Linking soil microbial community composition to SOC fractions and other soil chemical properties

The soil microbial community composition was only correlated with cPOM content ($p < 0.001$, CCA analysis) (Fig. 4a). VPA analysis was subsequently performed to evaluate the relative contribution of measured soil properties to microbial community composition (Fig. 4b). A total of 95.2% of the community variations were explained by all measured variables, among which the SOM fractions (cPOM, fPOM, iPOM, H-Silt, H-Clay, NH-Silt, and NH-Clay) contributed most (81.1%, including direct and indirect roles) to the variation of total PLFAs patterns (Fig. 4b). The total SOC and SOC

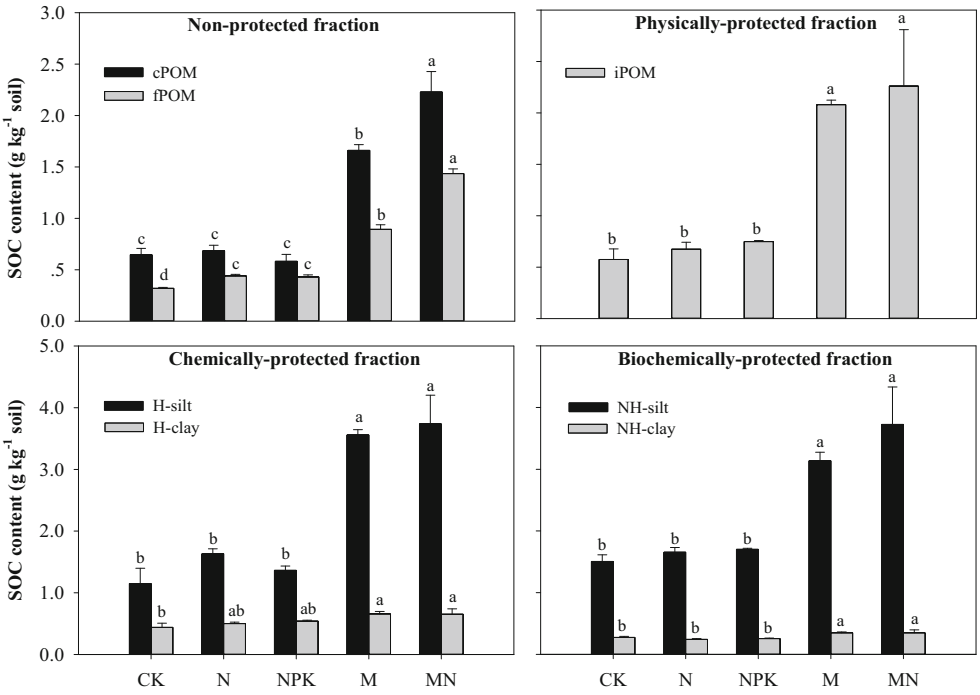

Fig. 2 OC contents in SOM functional fractions depending on long-term fertilization. *Different lower case letters* indicate significant differences among treatments and *numbers in brackets* represent standard error of the means ($n = 3$). *CK* control, *N* N fertilizer application, *NPK* NPK fertilizer application, *M* manure application, *MN* combined application of manure and N fertilizer

Table 2 Effects of long-term fertilization on abundance of microbial groups (nmol g^{-1})

Treatments	Total PLFAs	Bacteria	Fungi	Actinomycetes	G(+) bacteria	G(−) bacteria	Bacteria/fungi	G(+)/G(−)
CK	8.57 (0.67)b	1.63 (0.16)c	0.35 (0.35)b	0.26 (0.01)b	1.27 (0.09)c	0.36 (0.06)b	4.78 (0.28)b	3.67 (0.38)b
N	9.50 (1.11)b	1.75 (0.34)c	0.38 (0.38)b	0.20 (0.02)b	1.46 (0.27)c	0.29 (0.05)b	4.55 (0.68)b	4.99 (0.03)ab
NPK	9.02 (0.38)b	1.61 (0.04)c	0.35 (0.35)b	0.22 (0.01)b	1.40 (0.03)c	0.22 (0.02)b	4.66 (0.12)b	6.54 (0.50)a
M	12.1 (0.64)a	3.78 (0.14)a	0.59 (0.59)a	0.62 (0.13)a	3.09 (0.13)a	0.69 (0.05)a	6.40 (0.28)a	4.51 (0.39)ab
MN	10.1(0.47)ab	3.08 (0.11)b	0.50 (0.50)a	0.36 (0.03)b	2.49 (0.16)b	0.58 (0.10)a	6.24 (0.60)a	4.40 (1.05)ab

Different lower case letters indicate significant differences among treatments and numbers in brackets represent standard error of means (n = 3)
CK control, *N* N fertilizer application, *NPK* NPK fertilizer application, *M* manure application, *MN* combined application of manure and N fertilizer

fractions contributed an additional 26.3% of the total variance, which indicated that there were considerable interactions between them (Fig. 4b).

Discussion

Effects of long-term manure and mineral fertilization on SOM fractions

The increase in OC content in cPOM and fPOM after the long-term addition of manure, especially in combination with inorganic N fertilizer (Fig. 2), confirms previous findings (Gong et al. 2009; Chai et al. 2015; Li and Han 2016). The increase was partly attributed to the direct effects of the manure. This increase may also be caused by indirect crop effects after long-term organic and inorganic fertilizer amendment. The cPOM and fPOM, which are not occluded within microaggregates (53–250 μm), are mainly comprised of newly plant residue input (Six et al. 2002; Baldock et al. 1997). Therefore, the better crop growth (e.g., higher plant biomass production or larger amounts of root residues) may have increased the OC contents of cPOM and fPOM under long-term addition of manure by itself or in combination with mineral N fertilizer treatments. Indeed, the average aboveground biomass (grain + residue) from 1978 to 2012 was the highest after organic and inorganic fertilizer application (9741 kg ha^{-1} $year^{-1}$ for wheat, 15,724 kg ha^{-1} $year^{-1}$ for maize). Both cPOM and fPOM, especially cPOM (>250 μm), were very sensitive to changes in soil management (Cambardella and Elliott 1992; Six et al. 1998, 1999). Our study indicated that fPOM was more sensitive to fertilization than cPOM because organic and mineral fertilizer both induced a marked increase in the C contents of fPOM compared to cPOM (Fig. 2). Though cPOM and fPOM are mainly comprised of newly plant residue input (Six et al. 2002; Baldock et al. 1997), our study indicated that they are not equivalent fractions (Gregorich et al. 2006).

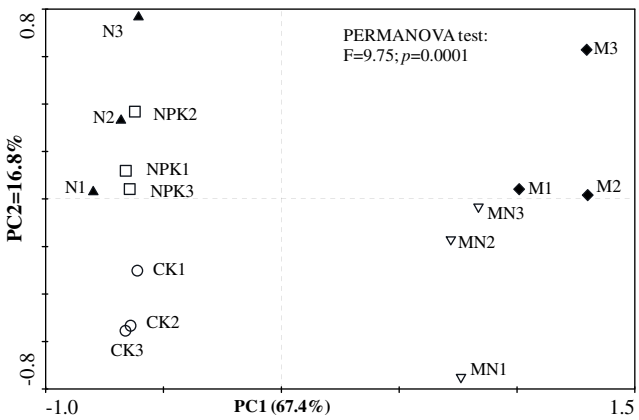

Fig. 3 Principal component analysis (PCA) of compositions of soil microbial communities under long-term fertilization. *Different lower case letters* indicate significant differences among treatments and *numbers in brackets* represent standard error of the means (n = 3). *CK* control, *N* N fertilizer application, *NPK* NPK fertilizer application, *M* manure application, *MN* combined application of manure and N fertilizer

The OC of occluded POM (iPOM) constituted 11.6–16.9% of the bulk soil OC and was 68.0–74.5% higher with manure than without manure, regardless of whether manure was combined with inorganic fertilizer (Fig. 2). Long-term manure input can improve microaggregation (Su et al. 2006; Sheng Zhe et al. 2012), which may decrease the OM turnover rate and increase the OC content of iPOM because of the enhanced physical protection (Pulleman et al. 2003; Marriott and Wander 2006; Hai et al. 2010). Microaggregate-within-macroaggregate fraction (iPOM in this study) was proved as a diagnostic of SOM storage induced by management practices across many soil types and climate regimes (Cambardella and Elliott 1992; Six et al. 2000; Six and Paustian 2014). Therefore, the increase in the OC content of iPOM indicated that long-term applications of manure alone or in combination with mineral fertilizer increased the total SOC storage. The long-term addition of manure increased both the chemically and biochemically protected C, thus confirming an increase in stable OM (Fig. 2). Chemically protected C (hydrolysable silt- and clay-sized fraction) is protected by association with mineral particles, whereas biochemically protected C is defined as the non-hydrolyzable fraction acquired through condensation and complexation reactions or through the inherent complex biochemical nature of the material (Six et al. 2002). The increase in the OC contents in non-protected SOM fractions and improved soil microaggregation after the long-term addition

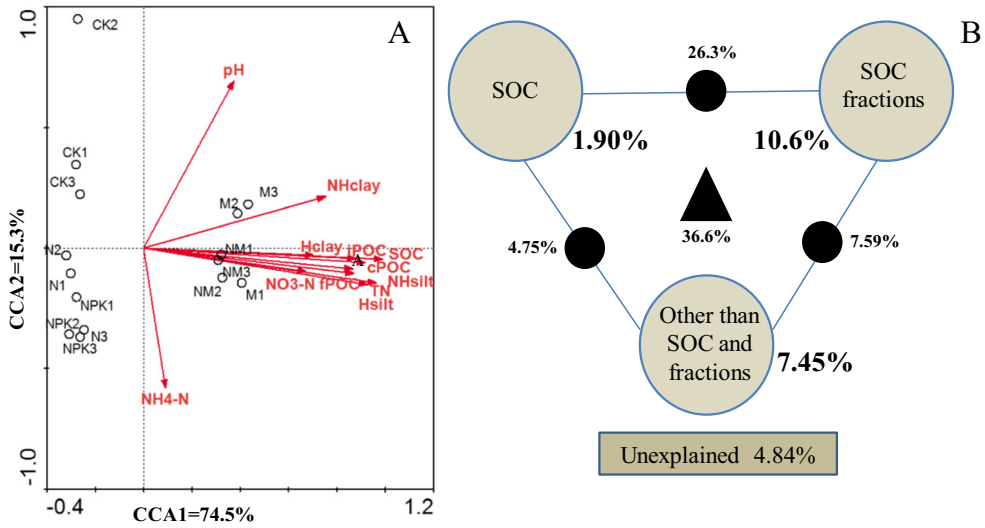

Fig. 4 **a** Canonical correspondence analysis (CCA) of compositions of microbial communities constrained by soil environmental variables. **b** Variation partitioning analysis (VPA) of microbial community explained by SOC, SOC fractions (including cPOM, fPOM, iPOM, H-Silt, H-Clay, NH-Silt, and NH-Clay) and other environmental properties (including TN, pH, NH_4^+-N, and NO_3^--N) and their interactions. The values beside parentheses are percentages explained by the selected environmental variables

of manure may provide more opportunity to enhance the mineral-associated soil OM (Ludwig et al. 2003; Hai et al. 2010). In addition, the application of manure each year also contributed to the observed increases in OC in chemical and biochemical fractions. The higher OC content in silt-sized fraction than clay-sized fraction was observed (Fig. 2). Furthermore, the proportion of non-hydrolysable C in silt (21.8–28.1%) was greater than clay-sized fractions (1.32–1.70%). This supported reports in the literature that concluded that silt-associated OC is more stable fraction than clay-associated OC (Plante et al. 2006a, b). The greater proportion of non-hydrolyzable C in silt than clay fraction may be attributed to greater lignin and less carbohydrate concentrations in silt-sized fraction (Kiem and Kögel-knabner 2003; Plante et al. 2006b). Further, biochemical analyses of these fractions before and after acid hydrolysis will be necessary to confirm this. The apparent increase in amounts of OC in iPOM, hydrolysable and non-hydrolysable silt fractions after the addition of manure as compared to the unfertilized plots in our study, indicates that physical, chemical, and biochemical protection mechanisms all played important roles in maintaining high SOC levels after long-term manure addition.

Effect of long-term manure and mineral fertilization on composition of soil microbial communities

The abundances of total PLFAs and of specific microbial groups and microbial community composition were significantly altered by long-term manure amendment compared to the control (Table 2). The input of manure can provide both relatively stable and labile substrate to support microbial growth, which may increase the total PLFAs abundances (Marschner et al. 2003; Ai et al. 2012; Zhang et al. 2015). Differences in manure and SOM composition and, thus, substrate availability are likely to be the reasons for the differences in the abundance of specific microbial groups and community composition observed in the present study. The easily degradable compounds, such as organic acids, in the insoluble or soluble organic matter may favor the growth of copiotrophic compared to oligotrophic organisms (Marschner et al. 2003). This coincides with the increase in G (−) abundances in our study because they are sensitive to available organic substances (Esperschütz et al. 2009; Tian et al. 2012). The more recalcitrant material in manure and SOM may increase the competitive ability of G (+) bacteria and actinomycetes as they are well adapted to soils with low substrate availability (Griffiths et al. 1998; Marschner et al. 2003; Zhang et al. 2015). Changes in manure composition can lead to a succession of G (−) and G (+) bacteria after the addition of compost (Marschner et al. 2003). The temporal dynamics in decomposition depend on the chemical composition of the organic matter added to the soil (Northup et al. 1998). Changes in the microbial community composition can be driven by C availability released by SOM fractions (Cusack et al. 2011; Bowles et al. 2014; Ng et al. 2014). The relatively labile fractions of SOM were more strongly related to microbial community composition than was total SOM (Cookson et al. 2005; Tian et al. 2016). This agrees with that change the content of cPOM influenced microbial community composition more ($p < 0.001$, Fig. 4). Microbial biomass and microbial residues are associated with LFOC (Cookson et al. 2005; Gong et al. 2009; Martins et al. 2012), and this fraction generally included non-protected and occluded LF after density fractionation, corresponding to cPOM and iPOM fractions of our study (Cookson et al. 2005; Gong et al. 2009; Martins et al. 2012). Our study indicated that cPOM was a good predictor of microbial community composition. This again suggested that the sources of cPOM and fPOM for soil microbe were similar, but are not equivalent fractions (Gregorich et al. 2006). Therefore, future biochemical analyses of these fractions need to be done.

Long-term inorganic fertilization significantly altered microbial community composition, but not the abundance of specific microbial groups (Table 1; Fig. 4). Past bibliography showed that increase (Rinnan et al. 2007), decrease (Zhong et al. 2010), or no change (Zhang et al. 2015) the abundance of microbial groups after inorganic fertilization. A meta-analysis based on 107 datasets from 64 long-term trails around the world revealed that mineral fertilizer application lead to a 15.1% increase in microbial biomass relative to the unfertilized control (Geisseler and Scow 2014). Presumably, the relatively small increase in total and labile SOC may be unable to support the substantial growth of microorganisms under mineral fertilization in our study (Geisseler and Scow 2014). Furthermore, the choice of taxonomic level to address the microbial community is a crucial issue (Nannipieri et al. 2003; Blaud et al. 2015). It should be noted that the limited taxonomic resolutions of PLFAs technique did not allow us to identify the specific microbial groups that shift in abundance. Therefore, future detailed taxonomic surveys of microbial abundance after long-term inorganic fertilization are necessary. Several long-term studies have found that mineral fertilizer leads to changes in microbial community composition (Zhong et al. 2010; Hu et al. 2011; Chen et al. 2016), but it also cannot or cause small changes (Esperschütz et al. 2007; Börjesson et al. 2012). These results are consistent with those of Allison and Martiny (2008), who found that 84% of studies with an average period of 8.2 years reported that microbial community composition was sensitive to N, P, and K fertilization. Soil pH was observed to play an important role in shaping microbial communities after long-term inorganic fertilization (Marstorp et al. 2000; Zhong et al. 2010). In our study, although the pH decreased 1.2 units after the addition of mineral fertilizer compared to the control, it did not exert a strong influence on composition of microbial community (Table 1; Fig. 4). Consistent with our study, an analysis of the microbial community composition suggested that observed differences could be mainly attributed to differences in C availability and, to a lesser degree, shifts in soil pH after long-term N application (Fierer et al. 2012; Geisseler and Scow 2014).

Conclusions

The long-term application of manure increased the amounts of OC in non-protected fractions (cPOM and fPOM), especially in combination with N fertilizer. Independent of N addition, physically protected (iPOM), chemically protected (H-Silt, H-Clay), and biochemically protected fractions (NH-Silt, NH-Clay) increased after the addition of manure alone or in combination with inorganic fertilizer. Organic C was mainly distributed in microaggregate-protected particulate organic matter (iPOM) and in hydrolysable and non-hydrolysable silt-sized C (H-Silt, NH-Silt) fractions, which accounted for 11.6–16.9, 23.4–28.9, and 25.4–30.6% of the total SOC, respectively. The contributions of increased SOC from these "slow" fractions (iPOM, H-Silt, NH-Silt) were 178–293, 118–209, and 85–109% higher after long-term addition of manure or in combination with inorganic N fertilizer compared to the unfertilized plots. Soil microbial community composition was significantly different after long-term inorganic and organic fertilization compared to the unfertilized plot. The cPOM content was related to the variation in microbial community composition ($p < 0.001$). These results indicate that long-term fertilization had clear effects on SOM functional fractions, and the effects on the content of SOM fractions were important factors for shaping microbial community composition. Future studies are required to investigate whether soil microbial community composition was changed by inorganic fertilization through detailed phylogenetic and taxonomic surveys like high throughput sequencing. Future biochemical analyses of four SOM fractions are also necessary.

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