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Subtropical maize production and soil microbial communities show minimal response to earthworm bio-tillage

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Conservation tillage has become an increasingly important practice for addressing soil degradation in agricultural systems, but its effectiveness on crop production remains uncertain, especially under humid conditions. Besides, earthworm bioturbation, a natural form of bio-tillage, can impact soil quality and crop production, but previous studies have failed to isolate and quantify the specific contribution of earthworm bioturbation in conservation tillage systems. The aim of this study was to investigate the effects of conservation tillage on subtropical farmland, with the goal of evaluating its suitability and recommending appropriate practices for humid conditions. A two-year field experiment was conducted on subtropical Ultisols and tested three tillage types (no-tillage, earthworm bio-tillage, and traditional rotary tillage) along with three organic matter inputs (none, straw return, and composted cow manure addition). Maize (*Zea mays* L.) yield and soil samples (0–10 cm) were collected after four growing seasons to determine the maize production, soil properties and microbial communities. Results showed that tillage type and organic matter input generally did not impact yield or aboveground biomass, except for a 14.4% and 33.5% reduction, respectively, under earthworm bio-tillage compared to traditional tillage under the straw input condition ($p < 0.05$). Tillage mainly affected soil phosphorus dynamic, while organic matter influenced pH and nitrogen dynamics. Both no-tillage and earthworm bio-tillage increased soil pH, organic carbon, nitrogen and phosphorus contents, especially with cow manure additions, and increased the general bacteria and gram-positive bacteria under none input. No-tillage significantly increased microbial biomass carbon, especially with none and straw inputs (over 6.3 times, $p < 0.05$). This study addresses a critical gap by evaluating the effects of no-tillage and earthworm bio-tillage with organic matter inputs. Here we show that conservation tillage practices, such as no-tillage and earthworm bio-tillage combined with appropriate organic matter inputs, are effective strategies for enhancing soil quality without adversely affecting crop production in subtropical sustainable agriculture.

Keywords Conservation agriculture, Earthworm bio-tillage, Maize production, Organic matter input, PLFAs

Increases agricultural production is a major driver of soil degradation[1]. Conservation tillage practices, such as reducing tillage, no-tillage (NT) combined with residue return, is recommended for improving soil quality and crop production[2–4]. However, many previous studies reported that conservation tillage do not always lead to positive results, especially in tropical and subtropical areas[5–9]. The adaptability of conservation agriculture still needs further study in the humid conditions.

Earthworms, recognized as soil ecosystem engineers, are skilled at burrowing to form holes, especially the anecic earthworms[10–12], and burrow channels are conducive to soil air and water permeability and crop production[13,14]. Additionally, earthworms accelerate surface straw decomposition and integrate it into the soil[15,16], underscoring their role as agents of bio-tillage. Previous studies indicated that NT have positive effects on earthworm population[17]. However, prior studies have failed to isolate and quantify the specific contribution of earthworm bioturbation in conservation tillage systems. Although evidence confirms that earthworms can

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boost plant growth and crop yields[13,18], further research is required to elucidate the effects of earthworm bio-tillage on soil properties and crop production under humid field conditions.

Soil microbes play crucial roles in soil biogeochemistry and are commonly used as indicators of soil quality[19–21]. Phospholipid fatty acids (PLFAs) are key components of the cellular membranes of all living soil microbial cells and can be used to fingerprint the structures of soil microbial communities[22,23]. PLFAs are generally used as bioindicator in environment monitoring and in the assessment of soil ecosystems since they are sensitive to land management practices and environmental stresses[24]. For example, the ratio of gram-positive to gram-negative bacteria (G+/G-) reflects soil carbon availability, while the fungi-to-bacteria ratio (F/B) is linked to carbon sequestration[19,21]. However, studies on conservation tillage's effects on soil microbial communities have yielded inconsistent results[21,25,26].

Organic matter (OM) input practices, such as straw return and composted cow manure (CM) addition, are integral to conservation tillage and have positive effects on soil properties and microbial biomass[9,27,28]. Spedding et al.[27]and Wang et al.[29] reported that the effect of OM input on microbial biomass was greater than that of tillage. Wang et al.[30] reported that tillage and OM input explained 76.1% and 0.6%, respectively, of the variation in soil microbial communities. Nevertheless, the interactive effects of tillage intensities and OM inputs require further systematic investigation, particularly in relation to earthworm bio-tillage process.

In this study, a two-year field experiment involving various tillage practices (including NT, earthworm bio-tillage [ET] and traditional rotary tillage [RT]) and OM inputs (including none return [None], straw [Straw] and CM addition) was conducted to assess the effects of conservation tillage on maize (*Zea mays* L.) production, soil properties and the microbial community in a subtropical Ultisols. The objectives of this study were (1) to examine the effects of tillage and OM input on maize yield, aboveground biomass, soil properties, microbial biomass and PLFAs and (2) to investigate the relationships between soil properties, the microbial community, and crop production in a subtropical region. We hypothesized that (1) conservation tillage would enhance soil quality by improving soil pH, nutrients, microbial biomass and PLFAs and that (2) conservation tillage would not significantly affect maize production in a subtropical climatic regime.

Materials and methods

Site description and experimental design

The field experiment was performed at the Zengcheng Experimental Station of the Guangdong Academy of Sciences (23°16′16′N, 113°34′56′E) in a subtropical climate with a mean annual temperature of 21.6°, sunshine duration of 1710 h and precipitation of 1968 mm (80% from April to September). The soil was developed from granite parent materials and classified as an Ultisol according to the USDA Soil Taxonomy System. The soil in this region has a pH of 5.66, a soil total organic carbon (OC) content of 14.1 g/kg, a soil total nitrogen (TN) content of 1.6 g/kg, a soil total phosphorus (TP) content of 1.29 g/kg, a mean weight diameter of the soil aggregates of 0.97 mm and a clayey texture (44% clay and 44.3% silt particle contents). The region has a long history of maize cultivation (over 20 years) under RT treatment with a ridge width of 1.2 m and a ridge spacing of 0.4 m. Maize (Mangu No. 1 variety) was grown twice each year (generally in the first season from March to June and in the second season from August to December), and the field was left fallow in the winter. The planting density of maize was 34,500 plants per hectare.

The experiment was established in July 2020 and followed a 3 tillage type × 3 OM input full factorial design with four replicates. The tillage treatments included NT, ET, and RT. The OM inputs included None, Straw, and CM. Plots were 1.2 m wide by 2.4 m long, with 0.4 m spacing between them for access. Polyvinyl chloride (PVC) panels were installed around plots (NT and ET treatments) to prevent the added earthworms from escaping (Fig. S1). The heights of the PVC panels were 0.8 m, with 0.6 m of the panel buried underground and 0.2 m exposed. To facilitate the operation of small rotary tillers, the RT plots were built without the use of PVC panels.

For the ET treatments, the earthworms used were anecic *Amynthas aspergillum*[10,15], which is widely distributed in the subtropical areas of China. Considering earthworm mortality and application difficulty[12], epigeic and endogeic species was not used in this study. Before the experiment, earthworms were removed from the entire study area by electroshock treatment. Twenty sexually mature *A. aspergillum* with similar body size (average weight of 8.0 g), collected from an adjacent vegetable field as the density of earthworms was low due to tillage in the cornfield, were added to each ET plots at the beginning of the experiment. The density of earthworms was determined arbitrarily since the effects of earthworm density was not considered in this study. To eliminate earthworm effects in the NT and RT plots, electroshock treatments were applied annually after the second season of maize harvest, and the collected earthworms were uniformly added to the ET plots. Earthworms collected in the NT and RT plots were less than 2 earthworms per plots during the experiment, therefore, the bio-tillage effects of earthworms in NT and RT plots could be neglected when compared with the ET treatments.

In the straw treatments, harvested straw was crushed (approximately 2 cm long) and uniformly applied to the soil surface at a rate of 4 t/ha, equivalent to the annual straw yield in this subtropical region. Similarly, for the CM treatment, fermented cow manure containing 222 g/kg OC, 12.1 g/kg TN and 5.5 g/kg TP, was surface-applied at the same rate of 4 t/ha, maintaining consistency in OM input across treatments as this study did not investigate dose effects. Both straw and CM amendments were initially maintained on the soil surface. However, under the RT management, these surface-applied OM were subsequently incorporated into the soil profile through rotary tiller operations. All the OM input treatments were performed once before the start of the experiment (July 2020) and once a year after the second season of maize harvest (including 2020). Field managements, including irrigation, weeding and fertilization, were same and standardized across all plots. The fertilization regime consisted of an initial basal application of 180 kg/ha compound fertilizer before planting, followed by two subsequent applications of 225 kg/ha and 375 kg/ha at monthly intervals. Irrigation (approximately 5 mm) was conducted at two-week intervals when no precipitation occurred for seven consecutive days. Three centimeters of maize stubble was left on the ground during harvest.

Maize sampling, soil sampling and analysis
Maize was harvested after four growing seasons in June 2022. Three plants were randomly sampled from each plot to measure maize production (maize yield and aboveground straw biomass). Corn kernels and straw were oven-dried at 60 °C to a constant mass. The maize yield and aboveground straw biomass were calculated by multiplying the weight of the corn kernels and aboveground straw with the maize density (34500 plants per hectare), respectively.

The soil was sampled simultaneously at the time of maize harvest, and soil samples (0–10 cm soil depth) were collected from five random points in each plot to prepare composite samples. One subsample was air-dried and sieved through 0.25- and 1-mm meshes to measure soil pH and soil total nutrient and available nutrient contents. The soil pH was measured using an electrometric method (1:2.5 w/v). OC was determined using the potassium dichromate oxidation method. TN was determined using the Kjeldahl method. The TP content was determined colorimetrically after digestion with sulfuric acid and perchloric acid. Soil inorganic available nitrogen (AN) was the sum of ammonium nitrogen and nitrate nitrogen and was determined by an AA3 continuous flow analyzer after potassium chloride extraction. Soil Olsen available phosphorus (OlsenP) was determined using the Olsen 0.5 M sodium bicarbonate method. The detailed experimental procedures for soil nutrient analysis were based on those used for soil agro-chemistry analysis[31].

The other subsample was stored at 4 °C and analyzed within one week to measure the soil microbial biomass and microbial community properties. Microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) were determined by chloroform fumigation-extraction[32]. The nonfumigated and fumigated soil samples were extracted with potassium sulfate and then subjected to TOC analysis (LiquiTOC II, Elementar). The PLFAs were determined to assess the soil microbial community structure[21]. The phospholipids were separated, derivatized to their fatty acid methyl esters, analyzed by gas chromatography (GC 6890), and identified with MIDI Sherlock peak identification software (v6.2, MIDI Inc., USA. www.midi-inc.com). The PLFAs included general bacteria, fungi, G+, G- and actinomycetes. The general bacteria included the following: 16:0, 17:0, 18:0 and 20:0[33–35]; fungi included 18:1w9c, 18:2w6c and 18:3w6c[33,35,36]; G + included i15:0, a15:0, i16:0, i17:0 and a17:0; G- included 16:1w7c, 16:1w9c, 17:1w8c, 18:1w7c, 18:1w9c, cy17:0 and cy19:0[33,35–37]; and actinomycetes included 10Me16:0, 10Me17:0 and 10Me18:0[33,34,37]. The prefixes 'i', 'a', and 'Me' indicate iso-, anteiso-, and mid-chain methyl branching, respectively, and the prefix 'cy' refers to cyclopropyl rings[33,35]. The ratios of fungi to general bacteria (F/B) and G+/G- were then calculated.

Statistical analysis
A two-way analysis of variance (ANOVA) was used to evaluate the effects of tillage type and OM input on maize production, soil properties, microbial biomass and PLFAs, and a general linear model was used when the variables satisfied normality (Shapiro-Wilk test) and homogeneity of variance (Levene's test), otherwise a generalized linear model was used. The means between different tillage practices under the same OM input or between different OM inputs under the same tillage treatment were compared using Tukey's multiple comparisons test in the agricolae package when the variables satisfied normality and homogeneity of variance[38], otherwise Nemenyi's all-pairs rank comparison test in the PMCMRplus package was used[39]. Principal component analysis (PCA) was used to reduce the dimensionality of the PLFAs in the FactoMineR package[40]. A principal component (PC) was selected when the eigenvalue was greater than 1. Partial least squares path modeling (PLS-PM) was conducted to explore the relationships between land management practices (tillage intensity and OM input), soil properties, microbial biomass, PLFAs and crop production with the plspm package[41]. The tillage practice intensities were arbitrarily set to 0, 1 and 2 for NT, ET and RT, respectively, in the PLS-PM. The degrees of soil disturbance caused by OM input were set to 0, 1 and 2 for the None, Straw and CM treatments in the PLS-PM, respectively, as composted manure generally has nominally great nitrogen and phosphorus contents than straw[42]. All statistical analyses and plotting were performed in R software (v4.1.0, R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/).

Results
Maize production
The maize yield and aboveground biomass are presented in Fig. 1. OM input had an independent effect on corn yield ($p = 0.039$; Table 1). Tillage type significantly influenced corn yield ($p = 0.041$) and biomass ($p = 0.003$; Table 1), and the means of maize yield and biomass under ET were 14.4% and 33.5% significantly lower than those under RT under the OM treatment of Straw, respectively ($p < 0.05$; Fig. 1).

Soil properties
The soil properties were presented in Fig. 2. Generally, RT had the lowest soil indices under the OM treatment of CM, which were significantly lower than those of NT for soil pH, TN, TP, and AN ($p < 0.05$) and lower than those of ET for OC, TN, TP and AN ($p < 0.05$), except for OlsenP ($p > 0.05$). The OlsenP under NT was significantly greater than that under ET under the OM treatment of Straw ($p = 0.032$, Fig. 2F). CM significantly increased soil pH under ET ($p = 0.006$) and RT ($p = 0.033$) compared with the OM treatment of None (Fig. 2A) and increased soil pH under NT ($p = 0.019$), OC under ET ($p = 0.029$), TN under ET ($p = 0.004$), and TP under ET ($p = 0.049$) compared with the OM treatment of Straw (Fig. 2A, B, C and D). AN in the Straw treatment was significantly greater than that in the None and CM treatments under the NT treatment ($p = 0.005$), and AN in the CM treatment was less than that in the None and Straw treatments under the RT treatment ($p = 0.003$, Fig. 2E).

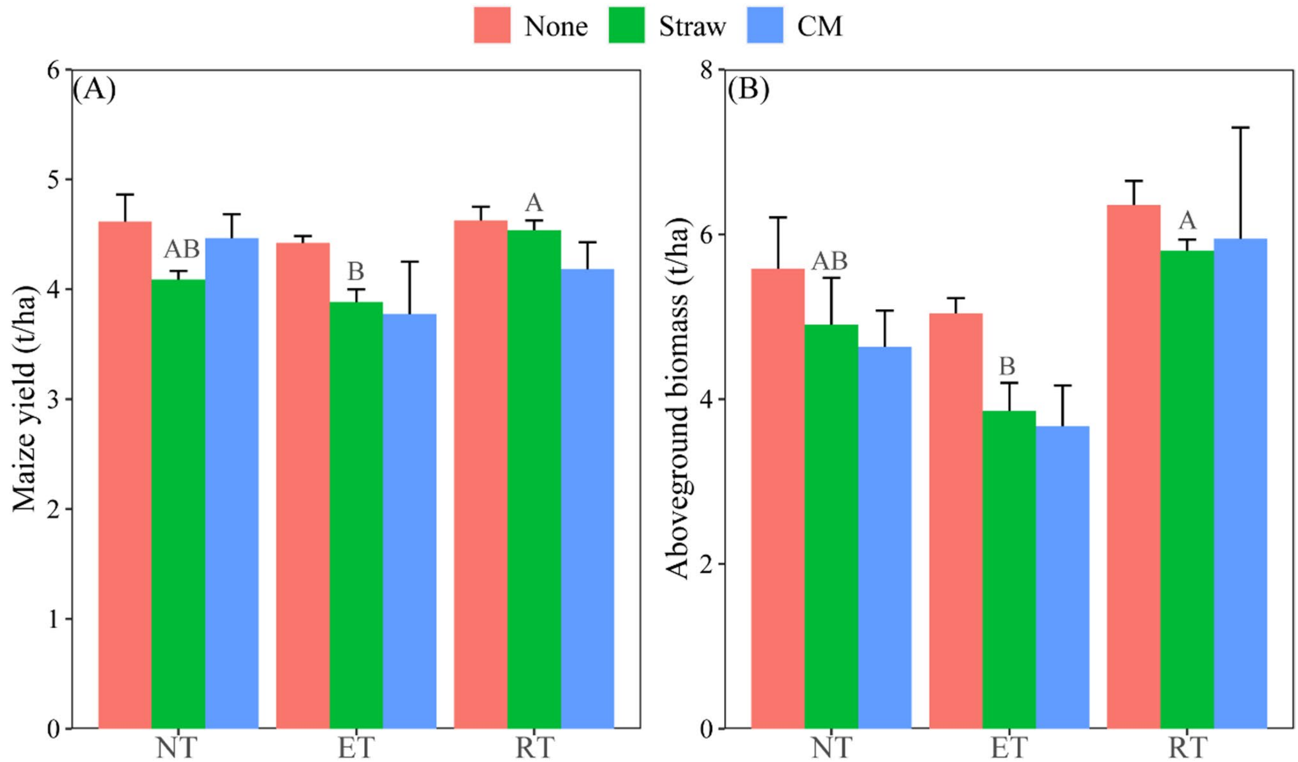

Fig. 1. Maize yield and aboveground biomass (single season, mean ± standard error) under various tillage and organic matter (OM) input practices. NT, no-tillage; ET, earthworm bio-tillage; RT, traditional rotary tillage; None, without OM input; Straw, straw return; CM, cow manure addition. Different uppercase letters indicate significant differences between tillage practices under the same OM input ($n = 4$, $p < 0.05$). The difference between various OM inputs under the same tillage practice was not significant ($n = 4$, $p > 0.05$).

Soil microbial biomass and PLFAs

The tillage and OM input treatments had a significant influence on MBC ($p < 0.001$, Table 1; Fig. 3A) but had no influence on MBN ($p > 0.05$, Fig. 3B). The MBC of None and Straw under NT were 31.2 and 7.3 times greater than those under RT, respectively ($p > 0.038$; Fig. 3A). The MBC of the None treatment was the highest and was significantly greater that of the CM treatment under the NT treatment ($p = 0.004$).

The characteristics of PLFAs are presented in Fig. 4. Under the OM treatment of None, the RT group had fewer bacteria than did the ET group ($p = 0.020$) and fewer G + than did the NT and ET groups ($p = 0.010$; Fig. 4A and C). Compared with the OM treatment of None, Straw generally increased the abundance of soil bacteria ($p = 0.013$), G+ ($p = 0.009$) and actinomycetes ($p = 0.029$; Fig. 4A, C and E). OM treatment of CM generally increased the F/B ratio under RT ($p = 0.049$) and decreased the G+/G- ratio under ET ($p = 0.038$) and RT ($p = 0.049$; Fig. 4F and G). Tillage and OM input treatments had no significant influence on fungi or G- ($p > 0.05$; Table 1; Fig. 4B and D). To further analyze the characteristics of the PLFAs, we carried out dimension reduction analysis via the PCA (Table 2; Fig. 5). The first two PCs cumulatively explained 90.1% of the variance in the PLFAs. The variances of bacteria, fungi, G + and G- were mainly represented by PC1 and were positively correlated with PC1 (Table 2; Fig. 5). PC2 mainly represented the variances of F/B and G+/G- and had a positive relationship with F/B and a negatively relationship with G+/G- (Table 2; Fig. 5). The actinomycetes content was positively correlated with PC1 and PC2.

Relationships between land management, soil properties, microbial community, and crop production

The PLS-PM explained 65.7%, 26.4%, 64.9%, 60.6% and 67.1% of the variation in soil pH, total nutrients, available nutrients, microbial biomass and PLFAs, respectively (Fig. 6A). Tillage had a significant and direct negative effect on soil pH ($p = 0.022$), total nutrients ($p = 0.033$) and available nutrients ($p = 0.002$) and a positive effect on microbial biomass ($p = 0.001$). OM input had a significant and direct positive effect on soil pH ($p < 0.001$) and microbial biomass ($p = 0.003$). Soil pH had a direct effect on soil available nutrients and PLFAs, with path coefficients of -0.71 ($p < 0.001$) and 0.52 ($p = 0.030$), respectively. Soil total nutrients positively affected soil available nutrients ($p < 0.001$), and available nutrients positively affected soil microbial biomass ($p = 0.033$). Crop production had no significant relationships with land management, soil properties or the microbial community ($p > 0.05$). Overall, tillage directly decreased soil total and available nutrients and indirectly increased soil available nutrients, and the total effects of tillage on soil nutrients were negative (Fig. 6B). OM input had no effect on soil total nutrients and indirectly decreased soil available nutrients. Tillage and OM input directly increased

Variable	Tillage	OM	Tillage × OM
Maize yield	**0.041**	**0.039**	0.461
Aboveground biomass	**0.003**	0.144	0.946
pH	**< 0.001**	**< 0.001**	**< 0.001**
OC	0.060	**0.016**	0.072
TN	0.065	**0.010**	**0.034**
TP	**0.001**	0.260	0.169
AN	0.089	**< 0.001**	**0.003**
OlsenP	**0.004**	0.263	0.307
MBC	**< 0.001**	**< 0.001**	**< 0.001**
MBN	0.519	0.059	0.631
Bacteria	**0.047**	**0.035**	**0.028**
Fungi	0.382	0.444	0.236
G+	**0.012**	0.109	**0.024**
G-	0.070	0.369	0.079
Actinomycetes	**0.048**	**0.002**	0.271
F/B	0.142	0.050	0.842
G+/G-	0.555	**< 0.001**	0.793

Table 1. Two-way analysis of variance (ANOVA, *p*-values) for crop production, soil properties, microbial biomass, and phospholipid fatty acids (PLFAs) following various tillage and organic matter (OM) input practices. OC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; AN, inorganic available nitrogen; OlsenP, Olsen-available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; G+, gram-positive bacteria; G-, gram-negative bacteria; F/B, the ratio of fungi to bacteria; G+/G-, the ratio of G + to G-. Significant effects ($p < 0.05$) are indicated in bold.

and indirectly decreased soil microbial biomass, and the total effects of tillage and OM input on soil microbial biomass were positive. PLFAs were both significantly affected by tillage and OM input indirectly, with negative and positive total effects, respectively.

Discussion

Generally, conservation tillage has positive effects on soil physicochemical properties[2,42]. In the present study, NT and ET treatments increased soil indices, such as pH, soil OC, and soil total and available nutrients, especially under NT with CM input (Figs. 2 and 6). Moreover, one-way ANOVA indicated that tillage mainly affected soil phosphorus dynamics, while OM input mainly affected soil pH and nitrogen dynamics (Figs. S2 & S3). Surface soil is prone to hardening under rainy conditions with subtropical weather, and our previous study showed that the sampled soil exhibited microbial phosphorus limitation[43]. Therefore, the improved aeration and mixing of soil and OM caused by rotary tillage are conducive to soil phosphate mineralization and result in lower TP and OlsenP in the presence of crop P uptake (Figs. S2B & S2C). Our previous study also indicated that the combined addition of *A. aspergillum* and straw could increase soil enzymatic activities and alleviate microbial phosphorus limitations[43]; as a result, OlsenP was lower in ET than in NT and RT under straw addition (Fig. 2F). In the present study, CM application significantly increased soil pH (Figs. 6A & S3A), which was consistent with a previous meta-analysis showing that manure application increases soil pH when the initial soil pH is lower than 7.47[42]. The PLS-PM model showed that OM input had an indirect negative effect on soil available nutrients (Fig. 6B), which was mainly reflected in the reduction in soil AN, especially under the CM treatment (Fig. S3D). Liu et al.[42] reported that manure application increased the risk of nitrogen loss due to its strong effect on nitrogen mineralization. In addition, the inorganic nitrogen forms, such as nitrate nitrogen, are susceptible to losses through runoff and leaching[44].

To date, there are no consistent conclusions on the effect of conservation tillage on crop production[5,9,37]. In a 3-year field experiment in semiarid Northeast China, Sui et al.[8] reported that no-tillage decreased yield by 16.1% compared with rotary tillage ($p > 0.05$). However, a meta-analysis conducted in the arid and semi-arid Loess Plateau region indicated that compared with conventional tillage, NT with straw mulching increased wheat and pea yields by 14.3% and 29.5%, respectively, and straw mulching was more effective at improving crop yield than NT[9]. Based on an eight-years' field experiment in semi-arid North China Plain, Teng et al.[4] reported that conservation agriculture (NT with straw mulching) results in an average 21% increase in soil health and enhanced similar levels of crop production after long-term warming compared to conventional agriculture. Some studies have also indicated that tillage practices have no significant impact on crop yield[45]. A long-term (15-year) tillage field study in semi-humid areas indicated that compared with traditional mouldboard plough tillage, NT significantly increased maize yield by18.2% in a normal year but significantly decreased yield by 90.3% in a dry year[5]. In this study, the differences in maize yield and aboveground biomass between NT and RT were not significant under any of the OM input conditions ($p > 0.05$; Fig. 1). This may have occurred because there was no significant difference in soil moisture in the subtropical region studied (Fig. S4)[5]. Previous meta-analyses conducted by Fonte et al.[13]and van Groenigen et al.[18] indicated that earthworms had significant positive

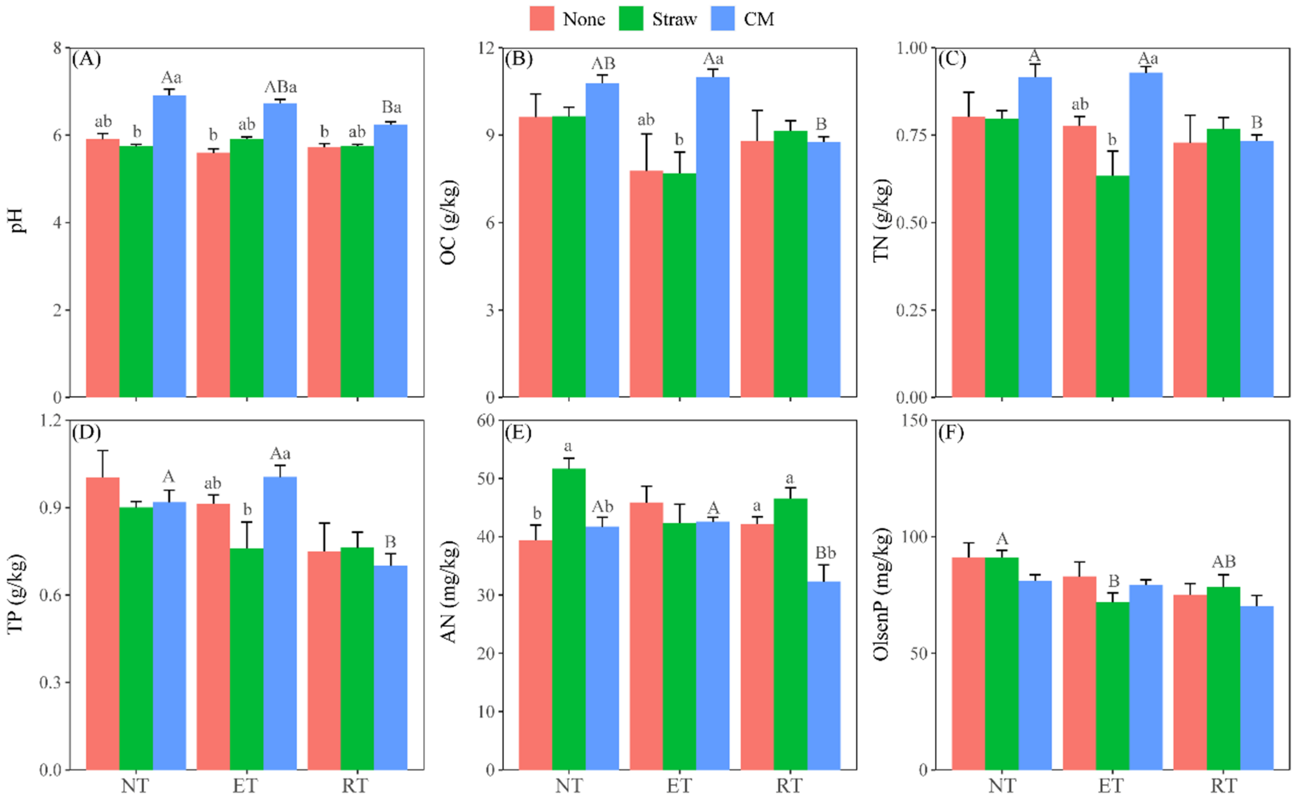

Fig. 2. Soil properties (mean ± standard error) at 0–10 cm depth under various tillage and organic matter (OM) input practices. NT, no-tillage; ET, earthworm bio-tillage; RT, traditional rotary tillage; None, without OM input; Straw, straw return; CM, cow manure addition. OC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; AN, inorganic available nitrogen; OlsenP, Olsen-available phosphorus. Different uppercase letters indicate significant differences between tillage practices under the same OM input ($n = 4$, $p < 0.05$). Different lowercase letters indicate significant differences between OM inputs under the same tillage treatment ($n = 4$, $p < 0.05$).

effects on crop yield and aboveground biomass. However, in the present study, compared with RT, ET decreased the crop yield and aboveground biomass by14.4% and 33.5%, respectively, under the straw mulching treatment ($p < 0.05$), and had no influence on maize production under OM inputs of None and CM conditions ($p > 0.05$; Fig. 1). The decrease in crop production may be due to available phosphorus limitations under the ET and Straw conditions (Fig. 2F), and earthworms may also consume crop roots in soils and further affect crop growth[46]. Besides, excessive bioturbation may cause uneven nutrient distribution, and crop yield reduction can occur when nutrient-rich drilosphere zones are spatially mismatched with root distribution patterns. Overall, the direct effects of tillage intensities and OM input on crop production were not significant ($p > 0.05$; Figs. 6A, S2 & S3).

Generally, conservation tillage has positive effects on soil microbial biomass and PLFA contents[3,5,28]. Consistent with previous studies[47], soil MBC significantly increased under NT conditions in this study (increased by 30.2 and 6.3 times under OM input conditions of None and Straw, respectively; Figs. 3A and 6B & S2D). Straw return also significantly increased microbial biomass[27]. Spedding et al.[27] reported that residue addition increased MBC by 61% and MBN by 96%, and the effect of organic matter addition was more pronounced than that of tillage[29]. In the present study, soil MBN had no relationship with tillage or OM input (Table 1), but increased under CM addition (Fig. S3E). Cow manure may serve as a source of nitrogen in soils, which is conducive to microbial growth. However, some previous studies also showed that there was no significant change in soil microbial biomass[20,21].

In the present study, conservation tillage had a positive effect on bacteria, especially without organic matter input (Figs. 4A and 6B), which was consistent with previous studies[26]. The increase in bacteria may be due to the increase in the available nutrient content, which can be verified by the positive relationships between bacteria and TP and OlsenP (Fig. S5). Previous studies have shown that fungal hyphal length and abundance increased under NT[25]. However, this study found no significant relationship between fungi and land management practices (tillage and OM input) (Table 1; Fig. 4B). This discrepancy is likely due to the strong association of fungi with soil moisture and oxygen conditions, which did not differ significantly between treatments in this study (Fig. S4)[28]. Based on a meta-analysis, Li et al.[48] reported that NT increased soil bacterial diversity but had no significant effect on soil fungal diversity. Previous studies have shown that conservation tillage can increase[5,25], decrease[21], or have no effect on F/B[26]. In this study, land management practices had no influence on F/B (Fig. S4).

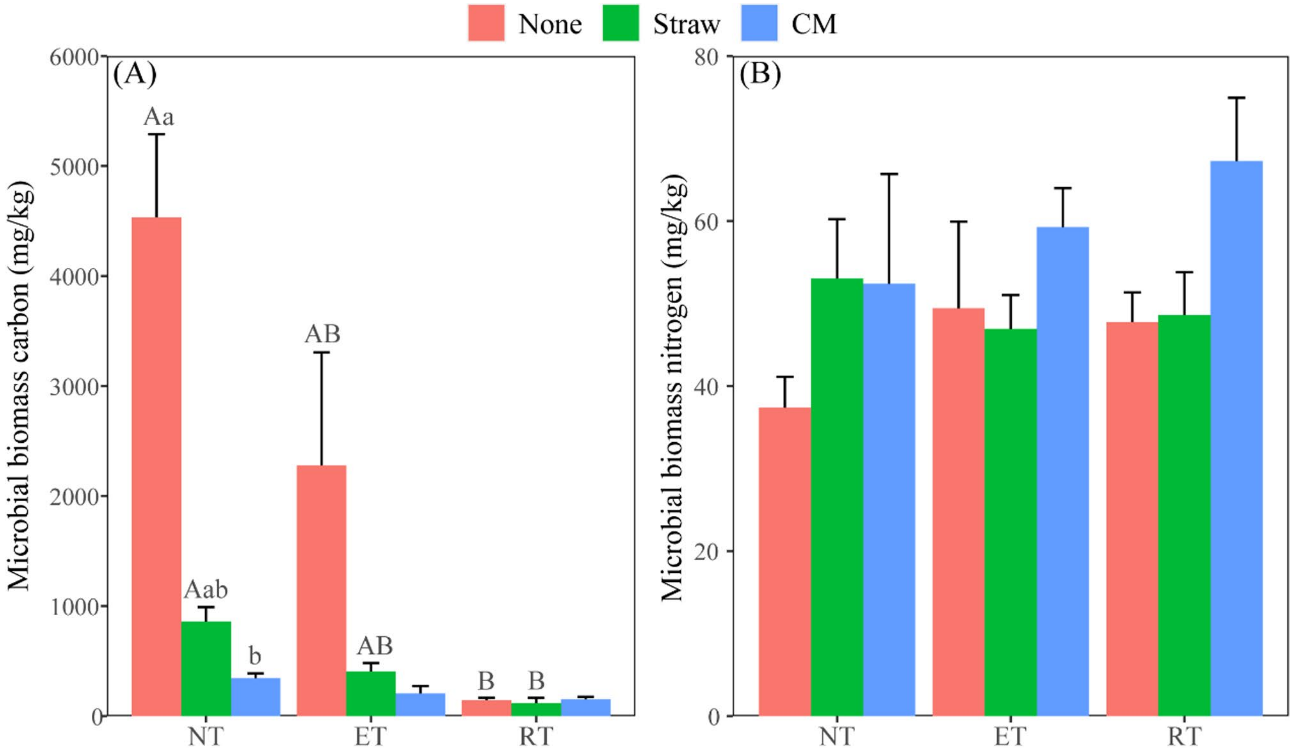

Fig. 3. Microbial biomass carbon and nitrogen (mean ± standard error) at 0–10 cm depth under various tillage and organic matter (OM) input practices. NT, no-tillage; ET, earthworm bio-tillage; RT, traditional rotary tillage; None, without OM input; Straw, straw return; CM, cow manure addition. Different uppercase letters indicate significant differences between tillage practices under the same OM input ($n = 4$, $p < 0.05$). Different lowercase letters indicate significant differences between OM inputs under the same tillage treatment ($n = 4$, $p < 0.05$).

G + and actinomycetes are known to utilize many recalcitrant compounds, and a shift in the G+/G- ratio is related to anaerobic soil conditions and a lack of nutrients[21,28,49]. Based on a 31-year field study, Mbuthia et al.[21] reported that NT increased the abundance of G + and actinomycetes. Feng et al.[49] also reported that deeper soil layers with more recalcitrant aromatic carbon had greater abundances of G + and actinomycetes. In the present study, reduced tillage (NT and ET) increased the G + concentration, while the OM input decreased the actinomycetes and G+/G- ratio (Fig. 4C, E and G, S2E, S3F & S3G). In addition, the G+/G- was influenced mainly by OM input rather than by tillage in this study (Table 1).

The effects of earthworms on PLFAs were inconsistent within previous studies[50–52]. Clapperton et al.[50] reported that the total PLFAs and the activity of G- increased in the presence of earthworms. Hedenec et al.[51] also reported that earthworms could increase the biomass of bacteria and fungi. However, after 90 days of incubation, Zheng et al.[52] reported that earthworms decreased the abundance of fungi, G+, G+/G-, and actinomycetes and total PLFAs. In the present study, ET had a positive effect on bacteria when no organic matter was added, and resulted in a greater abundance of G + than traditional rotary tillage (Figs. 4A & S2E). This was mainly due to earthworms facilitating a direct supply of readily assimilable nutrients and soil microbial metabolism, which may enhance bacteria growth and ultimately lead to the production of more resistant compounds[43,53]. The PLS-PM results indicated that tillage and OM influenced PLFAs indirectly through their effects on soil pH (Fig. 6), which has been found in previous studies[54]. The most likely mechanism underlying the impact of soil pH on PLFAs was the mediation of nutrient availability as soil pH is strongly related to soil enzyme potential[55].

Agricultural practices are major drivers of soil carbon loss and soil degradation[1], but soil quality loss can be reversed by adopting sustainable farming practices, such as conservation tillage[2,4,56]. In the present study, NT and OM addition generally had positive effects on soil properties and microbial biomass and no influence on crop yield or biomass (Fig. 6). Conservation tillage can also increase the ability of soil ecosystems to cope with drought stress[5]. In addition, a transition from conventional tillage to NT does not affect crop yield stability[6]. Conservation tillage (NT with straw return) could also increase soil health and crop production under future climate warming conditions[4]. Therefore, NT practices are recommended for subtropical sustainable agriculture. Earthworms decreased crop production under straw return but had no effect on crop production under CM addition or without OM addition (Fig. 1). Thus, considering the beneficial effects of earthworms on soil quality, earthworm bio-tillage under suitable OM inputs is also recommended[12,13,57].

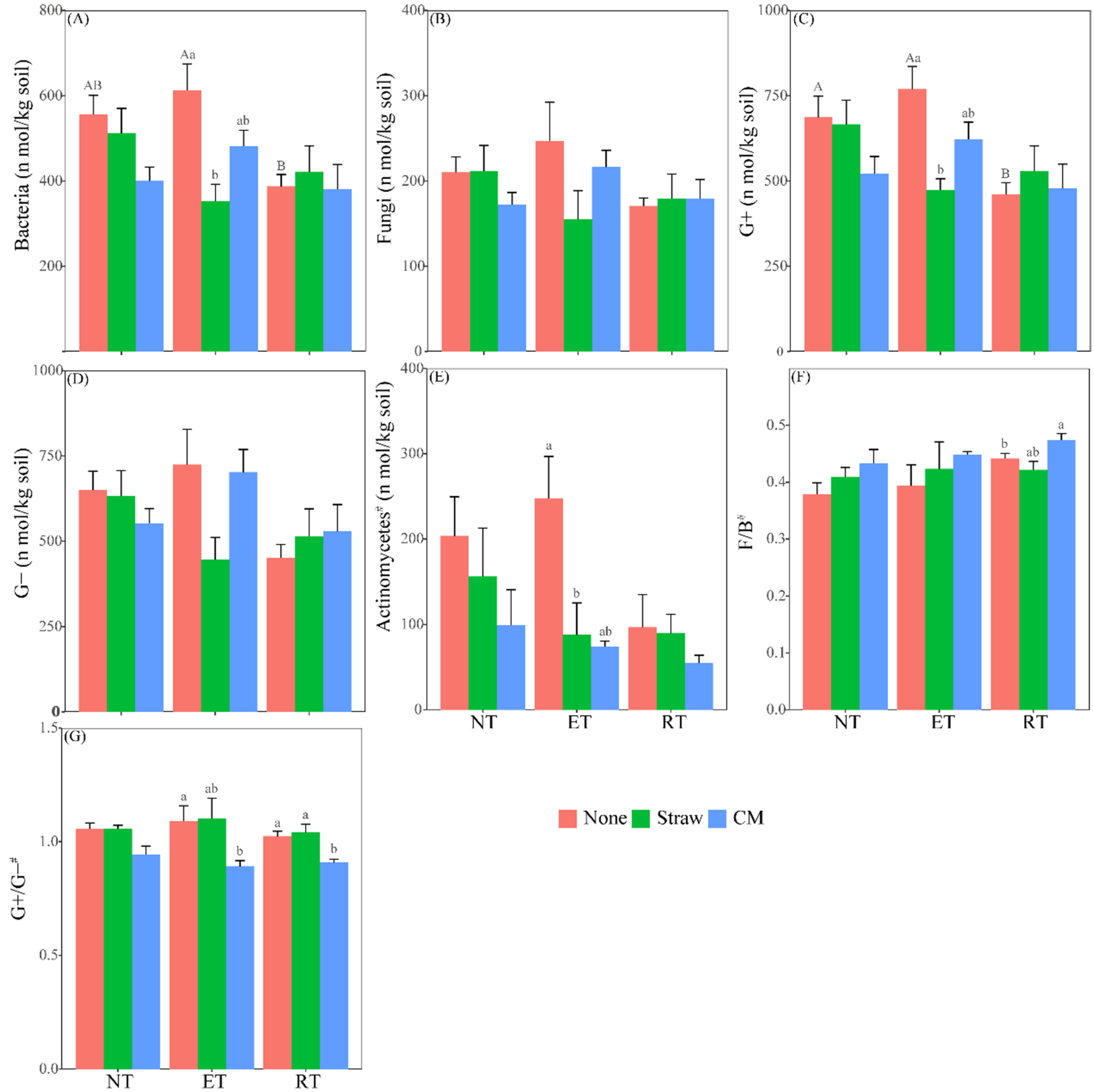

Fig. 4. Phospholipid fatty acids (PLFAs, mean ± standard error) at 0–10 cm depth under various tillage and organic matter (OM) input practices. NT, no-tillage; ET, earthworm bio-tillage; RT, traditional rotary tillage; None, without OM input; Straw, straw return; CM, cow manure addition. G+, gram-positive bacteria; G-, gram-negative bacteria; F/B, the ratio of fungi to bacteria; G+/G-, the ratio of G + to G-. Different uppercase letters indicate significant differences between tillage practices under the same OM input ($n = 4$, $p < 0.05$). Different lowercase letters indicate significant differences between OM inputs under the same tillage treatment ($n = 4$, $p < 0.05$).

Conclusions

In this two-year field experiment, we evaluated the effects of conservation tillage practices on crop production, soil properties, microbial biomass and PLFAs and explored their relationships in a subtropical area. Overall, conservation tillage did not significantly impact crop yield or aboveground biomass, except for a reduction in yield under combined earthworm bio-tillage and straw return. Conservation tillage positively affected soil pH and total nutrient content, although it reduced soil inorganic nitrogen under cow manure treatment. No-tillage significantly increased soil MBC, while tillage and OM inputs had no notable effect on MBN, fungi, gram-negative bacteria, or the F/B. Both no-tillage and earthworm bio-tillage increased soil bacteria and gram-positive bacteria in the absence of OM inputs. The positive effects of conservation tillage on PLFAs

Variable	PC1	PC2
Bacteria	0.980	0.151
Fungi	0.951	-0.248
G+	0.962	0.213
G-	0.977	-0.119
Actinomycetes	0.540	0.607
F/B	0.093	-0.943
G+/G-	-0.272	0.889
Eigenvalue	2.03	1.48
Cumulative variance%	58.8	90.1

Table 2. The first two principal components (PCs) of phospholipid fatty acids (PLFAs). G+, gram-positive bacteria; G-, gram-negative bacteria; F/B, the ratio of fungi to bacteria; G+/G-, the ratio of G + to G-.

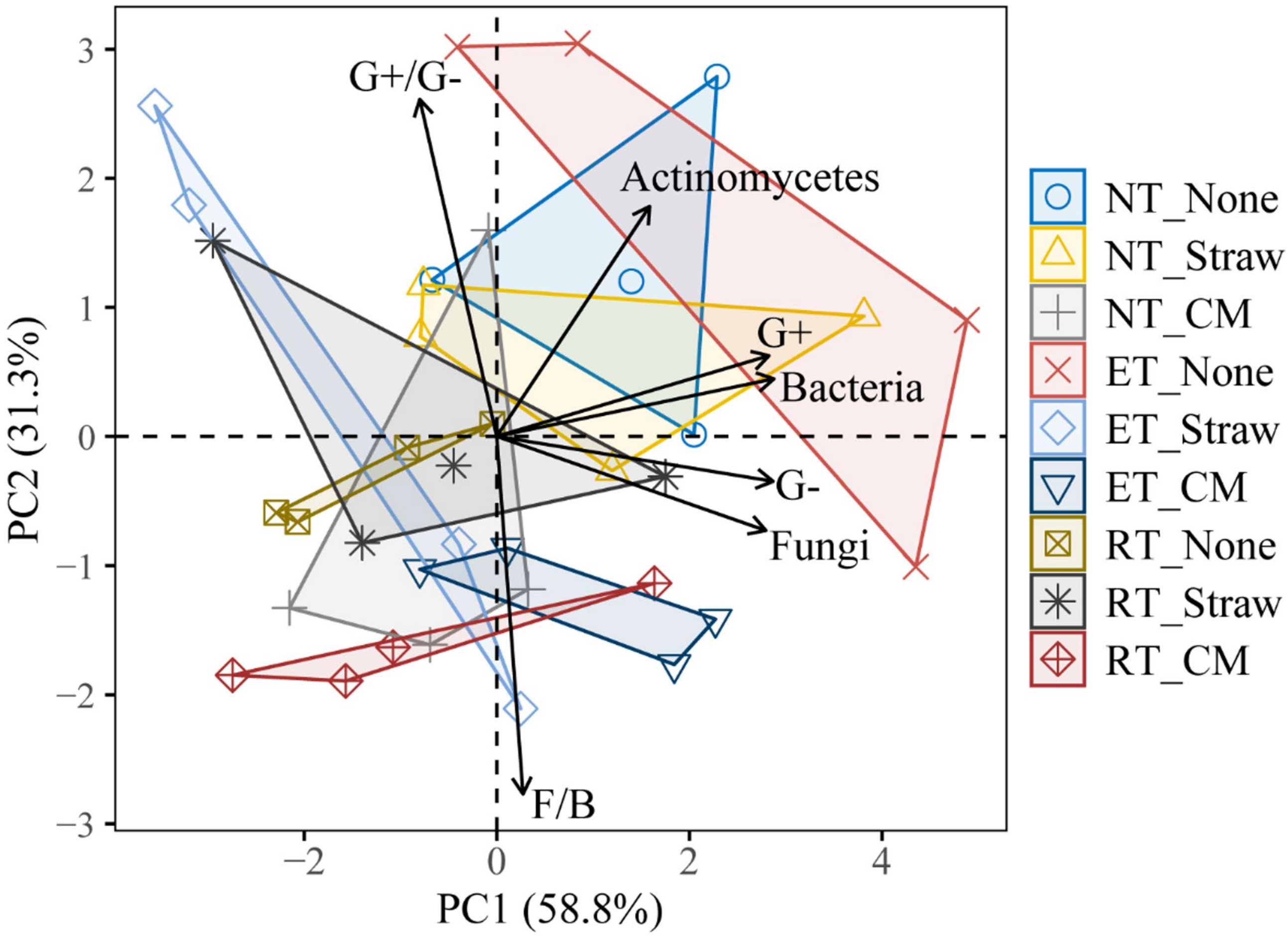

Fig. 5. Diagram of the principal component analysis (PCA) of the phospholipid fatty acids (PLFAs). NT, no-tillage; ET, earthworm bio-tillage; RT, traditional rotary tillage; None, without OM input; Straw, straw return; CM, cow manure addition. G+, gram-positive bacteria; G-, gram-negative bacteria; F/B, the ratio of fungi to bacteria; G+/G-, the ratio of G + to G-.

were mediated by improvements in soil pH. Given these benefits for soil quality, conservation tillage practices such as earthworm bio-tillage with appropriate OM inputs and no-tillage are recommended for subtropical regions. Furthermore, further studies should prioritize the implementation of long-term experimental studies to systematically elucidate the mechanistic pathways through which earthworm bioturbation directly regulates crop growth dynamics and developmental processes.

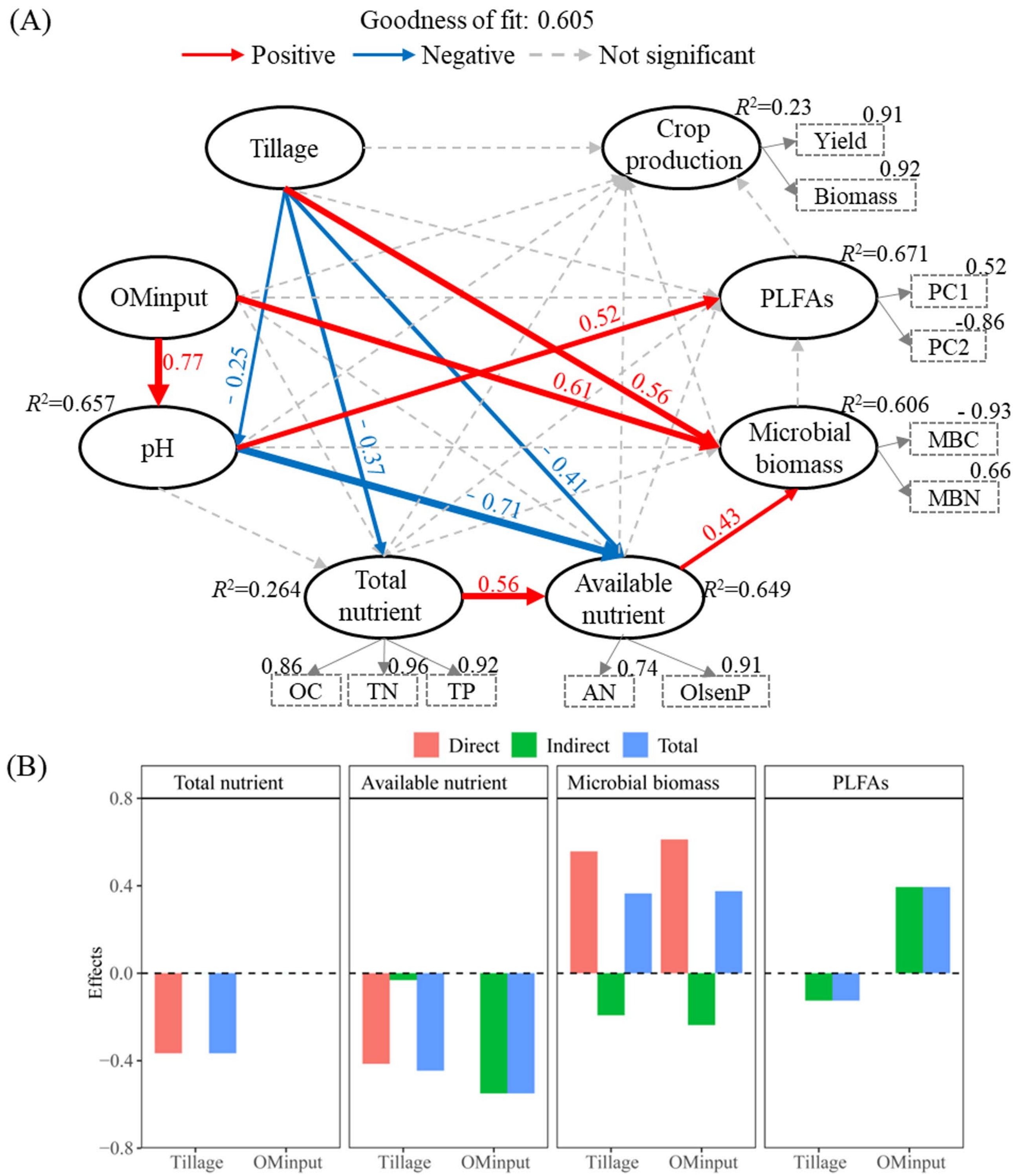

Fig. 6. Paths and effects of tillage and organic matter (OM) input on soil properties, microbial biomass, phospholipid fatty acids (PLFAs) and crop production according to partial least squares path modeling (PLS-PM). OC, soil organic carbon; TN, soil total nitrogen; TP, soil total phosphorus; AN, inorganic available nitrogen; OlsenP, Olsen-available phosphorus; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; PC1 and PC2 were the first two principal components of PLFAs. A goodness of fit > 0.6 indicated that the PLS-PM model was acceptable. The numbers on the arrows are the standardized path coefficients and the widths of the arrows are proportional to the strengths of the path coefficients. Continuous red and blue arrows indicate significant positive and negative relationships ($p < 0.05$), respectively. The gray dashed arrows indicate no significant relationships ($p > 0.05$). R^2 indicates the proportion of variation explained. The numbers on the rectangles are the loadings between the latent variables and observed variables.

Data availability

Data is available at https://data.mendeley.com/datasets/tn35b9wmrm/1.

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Author contributions

X.D. Li and N. Mao conceived the idea. X.D. Li, N. Mao, T. Liu and H. Jiang established the field experiment and performed field and lab work. X.D. Li and N. Mao performed data analyses and drafted the manuscript. All authors reviewed the manuscript.

Declarations

Competing interests

The authors declare no competing interests.

Additional information

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