Soil & Tillage Research 184 (2018) 235–242

Contents lists available at ScienceDirect

Soil & Tillage Research

journal homepage: www.elsevier.com/locate/still

Effects of tillage practices and microbial agent applications on dry matter accumulation, yield and the soil microbial index of winter wheat in North China

Huarui Gong[a,b,1], Jing Li[a,1,*], Junhua Ma[a], Fadong Li[a], Zhu Ouyang[a,*], Congke Gu[a,b]

[a] *Yucheng Comprehensive Experiment Station, Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China*
[b] *University of Chinese Academy of Sciences, Beijing 100049, China*

ARTICLE INFO

Keywords:
Tillage practices
Microbial agents
Soil microbial index
Winter wheat

ABSTRACT

Using microbial agents combined with chemical fertilizers is a promising approach to maintain the soil microbiota balance in continuous wheat–crop rotation fields. Most previous studies focused on microbial agents applied in the topsoil, with limited studies investigating the effects microbial agents under different tillage practices on soil quality and crops growth. In this study, a field experiment was conducted using a two-factor randomized block design (tillage practices and fertilizer applications) with three replicates, the effects of conventional rotary tillage and deep plowing when applying two different types of microbial agents (ETS and JS) on the dry matter accumulation, yield, soil microbial index and soil respiration of winter wheat (*Triticum aestivum* L.) in north China. The results indicated that wheat grain yield was not decreased after the application of different microbial agents plus 70% of the normal amount of chemical fertilizer and that the straw yield was decreased by 19.1% and 16.4% when ETS and JS, respectively, were individually applied. Additionally, there were no differences in grain and straw yields between the different tillage practices. The aboveground dry matter accumulation increased by 47.5% with ETS application under conventional rotary tillage compared with applications of chemical fertilizer. Under conventional rotary tillage, applications of different microbial agents decreased the microbial biomass C (MBC) concentration by 35.2–42.3% compared with applications of chemical fertilizer, while, the microbial biomass N (MBN) concentration increased by 10.0–18.5% in the 0–20-cm soil layer. With deep plowing, the soil respiration rate was greater than under conventional rotary tillage. In addition, the soil respiration rate after the application of the ETS plus JS combination with deep plowing was greater than after the microbial agents were individually applied during wheat's growth period.

1. Introduction

In the past 30 years, China's grain production has achieved significant growth, and in 2016, China's total grain output reached 616.239 million tons. The increase in grain production is partially due to the large-scale use of chemical fertilizers. According to statistics, the amount of chemical fertilizers used per ha of crops in China was 328.5 kg in 2015, which was much greater than the national average of 120 kg per ha, and was 2.6 times that of the USA and 2.5 times that of the European Union (Zhao and Yin, 2015). The long-term and excessive use of chemical fertilizers causes lower fertilizer efficiencies and increases production costs. Currently, the fertilizer efficiency rate in China is only ∼30% (Zhao et al., 2008), about half those of developed countries, such as the USA and those in the European Union. In addition, chemical fertilizer use creates secondary problems, such as environmental pollution, soil compaction, soil fertility declines, ecological deterioration and crop quality reductions (Guo et al., 2010). The status quo, if continued in China, will reduce the quality of agricultural products and also destroy the balance between the coordinated development of agricultural production and the environment (Chen, 1982; Han et al., 2018).

The application of a chemical fertilizer mixed with a microbial agent is regarded as an efficient way of regulating soil microbial community structure by promoting beneficial bacteria and suppressing

---

[*] Corresponding authors at: Key Laboratory of Ecosystem Network Observation and Modeling, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Sciences, Beijing 100101, China. Tel.: 86 10 64889300.
E-mail addresses: jingli@igsnrr.ac.cn (J. Li), ouyz@igsnrr.ac.cn (Z. Ouyang).

[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.still.2018.07.002
Received 28 December 2017; Received in revised form 1 June 2018; Accepted 2 July 2018
0167-1987/

pathogens (Zhang et al., 2013; Raza et al., 2017). Microbial fertilizers are living organisms undergoing life activities, and their products lead to specific fertilizer effects on crops. Because of the different microorganisms residing in microbial agents, the use of a particular microbial agent will affect the richness of the soil, change the nutrient circle and optimize the structure (including composition and temporal-spatial characteristics) of soil ecosystems, thereby affecting soil health (Wu, 2017). As early as the beginning of the 20th century, organic fertilizers containing azotobacter have been studied (Bowen and Rovira, 1999; Amarger, 2001). At present, microbial agents have been shown to improve soil structures, alter soil microflora, and control soil-borne diseases and agricultural nonpoint pollution (Yao et al., 2010; Tao et al., 2015; Xiong et al., 2017; Yilmaz and Sönmez, 2017). He et al. (2015) isolated plant growth-promoting bacteria from saline soil in a cotton field, and develop efficient slow-release biofertilizer formulations with this special bacteria. Pishchik et al. (2015) investigated the effects of a fertilizer having *Bacillus subtilis* as the main component on the physiological status of wheat. Wheat had an enhanced resistance to the adverse impacts of high rates of nitrogen (N) fertilizer owing to the rearrangement of bacteria in the rhizosphere's ecological niches by the applied microbial agent. Andreev et al. (2016) applied microbial fertilizer based on a Lacto-fermented mix of feces on maize planting experiment. The height of corn plants after the application of microbial fertilizer significantly increased, but there was no significant difference in the corn yield. Applying a microbial fertilizer (seed inoculation with *Azotobacter chroococcum* and *Peudomoance fluorescence*) plus half the normal amount of chemical fertilizer was successfully used for fenugreek, a legume, production, minimizing the consumption of chemical fertilizers and improving fenugreek yield, especially under deficit irrigation regimes (Dadrasan et al., 2015).

Currently, microbial agents are generally used in traditional methods of fertilization by combining with organic fertilizers and straw return. Some antibiotic microbial agents have been used in seed dressing. These traditional methods place microbial agents only on the soil surface and do not show distinct effects on deeper soil. Long-term traditional tillage compacts soil, which maintains the stability of soil structures to some extent but increases the bulk density of deep soils and hinders root growth (Zhang et al., 2011). Deep plowing could take advantage of the nutrient subsoil and improve the adaptability of crops by destroying the surface soil structure (Schneider et al., 2017). Different tillage practices may have relatively high impacts on the use efficiencies of different fertilizers and on crop growth (Wang et al., 2015; Seddaiu et al., 2016). However, there are limited reports on the effects of using microbial agents in different tillage practices on soil quality and crops growth.

At present, most microbial agents are applied to the topsoil and produce excellent results, but limited effects focused on the subsoil. In this study, a field experiment was conducted using a two-factor randomized block design – fertilization and the tillage practice. The aim of this study was, (1) to investigate the effects of the microbial agents on the dry matter accumulation (DMA), yield and soil respiration of winter wheat; (2) to compare the effects of microbial agents under conventional rotary tillage and deep plowing practices; (3) to discuss the feasibility of partially replacing chemical fertilizers with microbial agents under different tillage practices on the production of an efficient and dependable winter wheat crop in northern China

2. Material and methods

2.1. Study site

The field experiment was conducted from October 2016 to June 2017 at the Yucheng Comprehensive Experiment Station of the Chinese Academy of Sciences (116°36′E, 36°57′N, 21.2 m above sea level), Dezhou, Shandong Province, northern China. This site is representative of agriculturally intensive areas of the North China Plain that have an annual mean temperature of approximately 13.1 °C and precipitation of 593.2 mm. The soil type is fluvo-aquic, and the soil texture is silt loam (sand, 12%; silt, 66%; clay, 22%). Detailed soil characteristics at the experimental site are shown in Table 1.

Table 1
Soil characteristics at the experimental site.

Parameter	Values
Bulk density ($g{\cdot}cm^{-3}$)	1.46
$EC_{1:5}$($ds{\cdot}m^{-1}$)	0.17
Ph^{water}(1:2.5)	8.00
Total organic matter (%)	1.50
Total N ($g{\cdot}kg^{-1}$)	0.64
Total P ($g{\cdot}kg^{-1}$)	0.84
Total K ($g{\cdot}kg^{-1}$)	19.99

Values are means ($n \geq 3$) with standard deviations.

2.2. Experimental design

Winter wheat was used in our experiment. A field experiment was conducted using a two-factor randomized block design with three replicates. The main factors were the tillage practices of conventional rotary tillage and deep plowing (DT). The secondary factors were the fertilizer applications, which consisted of the normal amount of chemical fertilizer and a reduced chemical fertilizer level plus two types of microbial agents (used individually). The microbial agents were provided by ETS Biotechnology Development Company, Ltd. (Tianjin, China). The two tested microbial mixtures were a microbial organic fertilizer agent (ETS; Fertilizer registration certificate No. 2011-0801 according to the Chinese Ministry of Agriculture) and a microbial decomposition agent (JS). Seven treatments were designed for winter wheat planting, TF (rotary tillage with chemical fertilizer), TE (rotary tillage with ETS plus 70% the normal amount of chemical fertilizer), TJ (rotary tillage with JS plus 70% the normal amount of chemical fertilizer), TEJ (rotary tillage with the combination of ETS and JS, plus 70% the normal amount of chemical fertilizer), DTE (deep plowing with ETS plus 70% the normal amount of chemical fertilizer), DTJ (deep plowing with JS plus 70% the normal amount of chemical fertilizer), DTEJ (deep plowing with the combination of ETS and JS, plus 70% the normal amount of chemical fertilizer). Details of the application rates of the chemical fertilizer and microbial agents per treatment are presented in Table 2.

Winter wheat (*Triticum aestivum* L.) 'Jimai 22' was planted on October 18, 2016 and harvested on June 16, 2017. A combined chemical fertilizer (N 26%, P 12% and K 10%, respectively) was used at a normal amount of 865 $kg{\cdot}ha^{-1}$ (225 kg $N{\cdot}ha^{-1}$). This was divided into two equal parts, one was applied as the base fertilizer and the other was applied at the jointing stages. Tillage was carried out before winter wheat sowing, and the experimental area (5 m × 5 m, 25 m^2) was fixed with a cell spacing of 50 cm A rotocultivator was used for conventional

Table 2
Details of the seven tillage and microbial agent application treatments used in the field experiment.

Treatment	Tillage practices	Straw returning depth	Chemical fertilizer	ETS($kg\ ha^{-1}$)	JS($t\ ha^{-1}$)
TF	Conventional rotary tillage (working depth of 10-15 cm)	Uniformly distributed in 0-15 cm	225 kg N ha^{-1}		
TE			70% TF	3000	
TJ			70% TF		30
TEJ			70% TF	3000	30
DTE	Deep plowing (over 35 cm)	Uniformly distributed in 0-35cm	70% TF	3000	
DTJ			70% TF		30
DTEJ			70% TF	3000	30

rotary tillage, at a working depth of 10–15 cm. Deep plowing was to 35 cm. Corn straw applications were 7.5 t·ha^{-1}, which was based on approximately 7.5 t·ha^{-1} of corn stalk being harvested in wheat–maize double-cropping systems in Shandong Province, with ~500 kg·ha^{-1} grain yield. After crushing, the corn stalk was buried under the ground with soil tillage.

The microbial agents contained 61 beneficial microorganisms, 40% aerobic and 60% anaerobic, which could work in the subsoil. The aerobic microorganisms were composed mainly of archaebacteria extracted from nature by Thomas (1917), and the anaerobic microorganisms were rich high-quality strains from the Institute Pasteur (Paris, France). The average carbon-nitrogen (C/N) ratio of ETS approximately was 7. The total C and N concentrations were 13.25% and 1.78%, respectively. JS was a farm-oriented liquid microbial decomposition agent based on ETS microflora. It included actinomycetes, decomposers, *B. subtilis* and other functional microflora, which were able to efficiently decompose fiber and other macromolecular organic matter, and synergistic effects existed among these microflora. ETS (1.78% N) was supplied at 3,000 kg·ha^{-1}, contained the same N concentration as 30% the normal amount of chemical fertilizer, and was applied as a base fertilizer (Cong et al., 2017). JS was supplied at 30 L·ha^{-1}, and sprayed on the soil surface in accordance with the 1:150 ratio of JS:water before wheat sowing. ETS and JS were both applied before fertilization and irrigation, with tillage occurring at the same time to guarantee the uniform use of the fertilizer and microbial agent. Irrigation measures and other management practices were followed as usual.

2.3. Sample collection and analysis

2.3.1. Plant sample collection

In each plot, 0.5-m high consecutive plants from the same inside row were cut at ground level for the determination of aboveground dry matter accumulation at the regreening and harvest stages, and then oven dried at 70 °C until they reached constant weights. Additionally, three winter wheat root' sampling points were randomly selected in each plot at two depths (0–20 and 20–40 cm). A large-bore soil auger (10 cm in diameter) was used. The soil remnants attached to the root samples were removed by washing with water. Roots were then sorted and oven dried at 70 °C until they reached a constant weight. The weights were converted to the root area per unit area of dry matter (g·m^{-2}).

Plots were harvested when approximately 70% of the seeds were brown, and the grain and straw yields were measured. From each plot, 20 plants were randomly sampled to determine the yield components and seed yield per plant. The following measurements and observations were made for each plant: plant height (cm), spike length (cm), number of grains per plant and thousand-seed weight.

2.3.2. Soil sample collection, and MBC and MBN analyses

When winter wheat was harvested, three soil sampling points were randomly selected in each plot at two depths (0–20 and 20–40 cm). To measure MBC and MBN, the chloroform-fumigation method using fresh soil samples (Vance et al., 1987) and the potassium dichromate-volumetric method using a Kjeldahl apparatus, respectively, were followed. Total organic C (TOC) in soil was analyzed from dry soil samples using a TOC Analyzer (Vario TOC, Elementar, Germany).

2.3.3. Soil respiration analysis

Measurements of CO_2 fluxes using a static chamber system were conducted in each plot on the 15th of every month (Parkin and Venterea, 2010). The system consisted of a circular stainless steel base (0.19-m inner diameter and 0.22-m external diameter) surrounded by a trough (0.03-m width and 0.05-m height) and a cylindrical opaque chamber made of polyvinyl chloride (0.20-m inner diameter and 0.15-m height). Each chamber was fitted to the stainless steel base that was permanently inserted up to 5 cm into the soil below the infrared or "dummy" heater and was only removed for tillage following sowing. Moreover, the stainless steel base was installed between two rows of wheat plants, and small living plants were removed inside the base at least 1d before measurements were taken to avoid the effects of plants on the sampling gas (Tu and Li, 2017). Gas samples (10 ml per sample) in each plot were collected at 0, 10, 20 and 30 min after chamber closure. These gas samples, representing the daily CO_2 fluxes, were collected between 9:00 and 11:00 a.m. once every month for laboratory analysis. Additionally, the chamber, atmospheric, and soil temperatures at two depths (0–5 and 5–10 cm) were recorded. All of the samples were analyzed within 24 h using a gas chromatograph (Agilent 7890 A, Agilent Technologies, USA).

CO_2 flux rates were calculated from the measured slope of the linear change in the gas concentration within the chamber over time after chamber closure, as follows (Liu et al., 2014):

$$F = K \times (273 \times (273 + T_a)^{-1}) \times (M \times V^{-1}) \times H \times (dc \times dt^{-1}),$$

where F represents the CO_2 flux rate (mg·m^{-2} h^{-1}), K represents a conversion coefficient (1 for CO_2), T_a (°C) represents the air temperature within the chamber, M represents the molecular weight (44 CO_2 mol^{-1}), V represents the volume under standard atmospheric conditions (22.4 L·mol^{-1}), H (m) represents the chamber headspace height, and $dc \times dt^{-1}$ (μL·L^{-1} h^{-1}) represents the change in the CO_2 concentration.

2.4. Data analysis

Multiple comparisons were performed using Duncan's new multiple-range test (*i.e.*, smallest significance ranges) in SPSS (ver.23.0, IBM, USA). All figures were drawn using Sigmaplot (ver.12.5, Systat Software, USA).

3. Results

3.1. DMA

DMA under different tillage practices and microbial agent applications during regreening and harvest stages of winter wheat are shown in Fig. 1. At the regreening stage, the aboveground DMA under TEJ was the greatest (21.04 g·plant^{-1}), and was significantly different than that under DTEJ (16.69 g·plant^{-1}; $P < 0.05$). The aboveground DMA values under TEJ and DTEJ were greater than those from other treatment groups under the same respective tillage practices, but no significant differences were observed among the treatment groups under different tillage practices. At the harvest stage, the aboveground DMA values of wheat receiving TE, TJ and TEJ were significantly greater than those receiving TF (49.63 g·plant^{-1}) and all of the deep-plowing treatment groups ($P < 0.05$). The aboveground DMA of wheat receiving TE (73.21 g·plant^{-1}) was significantly greater than those receiving TEJ (60.10 g·plant^{-1}; $P < 0.05$). A comparison between the two microbial agents revealed no significant differences between DMA values of wheat plants grown under TE and TJ. The aboveground DMA levels from plants receiving DTJ (55.29 g·plant^{-1}) were not significantly different than those receiving DTEJ (47.40 g·plant^{-1}), but significantly greater than those receiving DTE (44.25 g·plant^{-1}; $P < 0.05$).

The peak root DMA alues of wheat receiving TEJ (109.12 g·m^{-1}) and DTEJ (122.36 g·m^{-1}) in the 0–40-cm soil layer occurred at the regreening stage. The root DMA values under TE and DTE conditions were significantly greater than those under TF and TJ conditions ($P < 0.05$), but no significant differences were observed with applications of the same microbial agent under different tillage practices. Additionally, the root DMA value under DTJ conditions (24.19 g·m^{-1}) was extremely significantly greater than the DMA values in the 20-40-

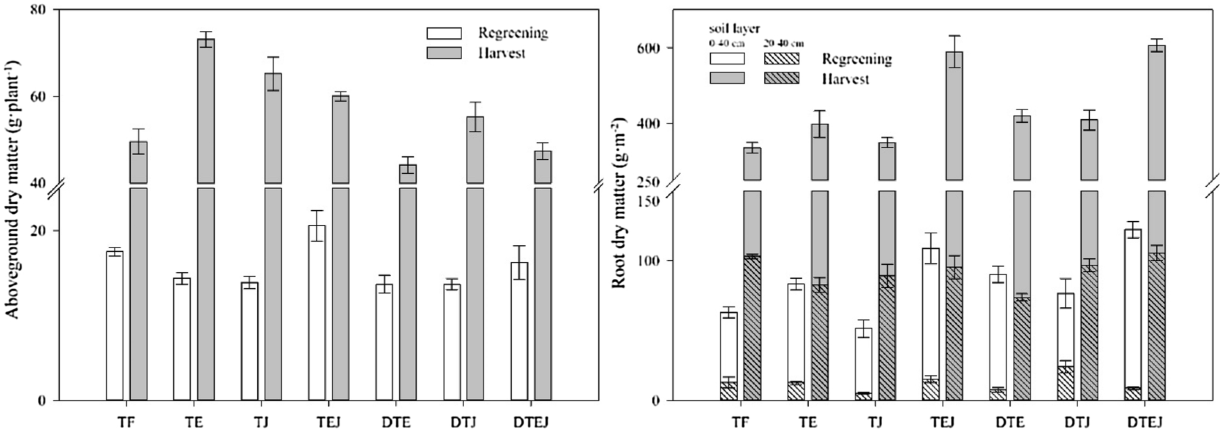

Fig. 1. Aboveground and root dry matter of winter wheat under different treatments.

cm soil layer from other treatment groups ($P < 0.01$). At the harvest stage, the root DMA values in the 0–40-cm soil layer of wheat receiving TEJ (589.26 $g{\cdot}m^{-1}$) and DTEJ (606.19 $g{\cdot}m^{-1}$) were significantly greater than those from other treatment groups ($P < 0.05$), but no significant differences were observed among the other treatment groups. The root DMA values in the 20–40-cm soil layer under TEJ and DTEJ conditions were significantly greater than those under TE and DTE conditions ($P < 0.05$), respectively.

3.2. Wheat yield

Yield and biological characteristics of winter wheat under different tillage practices and microbial agent applications are shown in Table 3. The straw yield of winter wheat grown under TF (12.36 $t{\cdot}ha^{-1}$) conditions was significantly greater than wheat grown under TE, TJ, DTE and DTJ conditions ($P < 0.05$) but not significantly different from those grown under TEJ (10.8 $t{\cdot}ha^{-1}$) conditions and DTEJ (10.79 $t{\cdot}ha^{-1}$) conditions. The peak straw grain ratio (1.44) occurred under TF, indicating that, with the same aboveground DMA level, the greater the straw grain ratio, the lower the grain yield. The thousand-grain weight under TJ (49.37 g) and DTJ (49.49 g) was significantly greater than that under TEJ (46.93 g, $P < 0.05$). Peak spike lengths occurred under TF (8.04 cm) conditions and were significantly greater than the lengths of spikes from other treatment groups ($P < 0.05$). The spike lengths from wheat receiving TEJ (7.9 cm) conditions was significantly greater than that from TE and TJ (7.5 and 7.53 cm, $P < 0.05$), and DTEJ (7.67 cm) conditions was significantly greater than that from DTE and DTJ (7.36 cm and 7.2 cm, $P < 0.05$). However, the spike length under TJ (7.53 cm) conditions was significantly longer than the length under DTJ conditions (7.2 cm, $P < 0.05$). Within other treatment groups, no significant differences were found between the different tillage practices. Additionally, there were no significant differences in grain numbers per ear among the microbial agent applications, except under DTJ conditions (28.0), compared with those under TF conditions (31.73), and the plant height (77.19 cm) under DTJ conditions was also the lowest among all of the treatment groups.

3.3. MBC and MBN

TOC, MBC and MBN concentrations under different tillage practices and microbial agent applications in the 0–40-cm soil layer of winter wheat are shown in Table 4. In the 0–20-cm soil layer, the MBC concentrations under different tillage practices were ranked as follows: TF (532.48 $mg\,kg^{-1}$) > conventional rotary tillage > deep plowing, and the MBN concentrations were ranked as follows: conventional rotary tillage > TF (41.86 $mg\,kg^{-1}$) > deep plowing. The MBC/MBN ratio under TF was the greatest among the treatment groups. The difference between the TOC concentrations under microbial agent and chemical fertilizer applications was not significant. In the 20–40-cm soil layer, the TOC concentration under TEJ conditions (6.34%) was significantly greater than that under TJ conditions (4.61%), but no significant differences occurred among the other treatment groups ($P < 0.05$). The peak MBC concentration was under TEJ treatment (496.98 $mg\,kg^{-1}$), and no significant differences were observed between conventional rotary tillage and deep plowing for the same treatment. Under the same tillage practices, the MBC concentrations from the combined ETS and JS application was significantly greater than those from independent ETS and JS applications. Thus, the concentration under TEJ conditions was greater than under either TE or TJ conditions, and the concentration under DTEJ conditions was greater than under either DTE or DTJ conditions. The MBN concentration under TEJ had a peak value of 32.65 $mg\,kg^{-1}$. No significant differences were observed between the MBC/MBN ratios under TF conditions and those of the other treatment groups undergoing conventional rotary tillage (TE, TJ and TEJ). The ratios of wheat grown under DTE and DTEJ were significantly greater than under TF condition ($P < 0.05$), and the MBC/MBN ratios from

Table 3
Yield and biological characteristics of winter wheat under each treatment.

Treatments	Grain yield ($t{\cdot}ha^{-1}$)	Stover yield ($t{\cdot}ha^{-1}$)	Straw grain ratios	Thousand-grain weight (g)	Spike length (cm)	Grain number per ear	Plant height (cm)
TF	8.59 ± 1.38a	12.36 ± 1.3a	1.44	48.05 ± 0.46ab	8.04 ± 0.66a	31.73 ± 7.88ab	82.56 ± 5.54a
TE	8.19 ± 1.3a	10 ± 0.98b	1.22	48.23 ± 1.29ab	7.5 ± 0.79 cd	29.92 ± 8.1bc	81.75 ± 4.13a
TJ	9.1 ± 0.21a	10.33 ± 0.37b	1.14	49.37 ± 0.44a	7.53 ± 0.79 cd	30.85 ± 7.14abc	82.65 ± 5.83a
TEJ	8.22 ± 0.68a	10.8 ± 0.65ab	1.31	46.93 ± 1.58b	7.9 ± 0.7ab	33.27 ± 8.24a	80.52 ± 5.59a
DTE	8.65 ± 0.88a	10.16 ± 0.55b	1.18	49.49 ± 0.37a	7.36 ± 0.81de	29.47 ± 8.66bc	80.65 ± 6.42a
DTJ	8.81 ± 0.99a	9.49 ± 1.51b	1.08	49.49 ± 1.87a	7.2 ± 0.77e	28 ± 8.75c	77.19 ± 8.48b
DTEJ	8.73 ± 0.7a	10.79 ± 0.74ab	1.24	48.64 ± 0.75ab	7.67 ± 0.65bc	30.43 ± 6.82abc	81.62 ± 6.53a

Values are means (n ≥ 3) with standard deviations. Different letters within the same column indicate significant differences between the treatment and control with the same salt content at $P < 0.05$.

Table 4
MBC and MBN concentrations in soil from each treatment.

Treatments	Soil layer: 0-20 cm				Soil layer: 20-40 cm			
	TOC (%)	MBC ($mg{\cdot}kg^{-1}$)	MBN ($mg{\cdot}kg^{-1}$)	MBC/MBN	TOC (%)	MBC ($mg{\cdot}kg^{-1}$)	MBN ($mg{\cdot}kg^{-1}$)	MBC/MBN
TF	8.93a	532.48a	41.86abc	13.47a	6.34ab	176.43c	25.01ab	7.06b
TE	7.89a	345.08b	49.59a	7.48b	7.42ab	335.4b	29.02ab	11.93ab
TJ	8.18a	344.82b	49.57a	7.74b	4.61b	170.77c	21.99b	7.92b
TEJ	6.78a	307.01b	45.95ab	6.77b	9.33a	496.98a	32.65a	15.21ab
DTE	8.22a	116.32c	38.96bc	3.02b	5.58b	360.72b	21.78b	18.02a
DTJ	9.63a	114.1c	28.72d	3.95b	5.43b	179.47c	22.48ab	7.84b
DTEJ	9.62a	150.02c	35.46cd	4.49b	5.16b	420.41ab	22.67ab	20.27a

Different letters within the same column indicate significant differences between treatment and control with the same salt content at $P < 0.05$.

wheat grown under TJ and DTJ conditions were the lowest under conventional rotary tillage and deep plowing, respectively.

3.4. Soil respiration

The dynamics of the soil respiration and temperature of winter wheat under different tillage practices and microbial agent applications are shown in Fig. 2. In the 0–5-cm soil layer, the soil temperature under TF condition was the lowest (4.3°C) at the seedling stages, and the soil temperature under DTEJ, DTJ and DTE conditions were significantly higher than those under TEJ TJ and TE conditions, respectively ($P < 0.05$). At the regreening stage, the soil temperature under TF condition was the lowest (4.2°C), and the soil temperature under DTEJ (5.0°C) was significantly higher than those under TF, TE and TEJ ($P < 0.05$), but no significant differences were observed among different treatment at other growth stage. In the 5–10-cm soil layer, there were no significant differences in the temperatures of soil undergoing the same tillage practices at wheat-growing stages after the application of microbial agents. In this soil layer, the soil temperatures under TF condition (1.2–10.2°C) and other undergoing conventional rotary tillage treatments were significantly higher than those that occurred under deep plowing at the seedling to regreening stages ($P < 0.05$).The temperatures in the 5–10-cm soil layer under deep plowing were significantly higher than those of other treatment groups from the regreening stage until harvest ($P < 0.05$).

The lowest soil respiration rate among treatment groups was recorded during the overwintering stage (January), and the greatest soil respiration rate varied from 404.2 to 538.1 $mg\,m^{-2}\,h^{-1}$ during April. During the seedling stage, the soil respiration rates under deep plowing were significantly greater than those under conventional rotary tillage and TF (57.3–90.6 $mg\,m^{-2}\,h^{-1}$, $P < 0.05$), and the soil respiration rates under TEJ (60.2–95.2 $mg\,m^{-2}\,h^{-1}$) and DTEJ (75.6–131.5 $mg\,m^{-2}\,h^{-1}$) conditions were the highest for the two tillage practices. There were no significant differences among treatment groups during the overwintering stage. With increasing temperatures, the soil respiration rates under DTJ and DTEJ conditions in February increased by 183.7% and 167.8% compared with in January, respectively, and were significantly greater than those of the other treatment groups ($P < 0.05$). The soil respiration rate under different tillage practices in April were ranked as follows: TF (538.1 $mg\,m^{-2}\,h^{-1}$) > deep plowing > conventional rotary tillage, and no significant differences were observed among those undergoing the same tillage practices. The soil respiration rates from all of the treatment groups in May significantly decreased compared with those from April, and those under TEJ (314.5 $mg\,m^{-2}\,h^{-1}$) and DTEJ (323.5 $mg\,m^{-2}\,h^{-1}$) conditions were significantly greater than those of the other treatment groups undergoing the same tillage practice ($P < 0.05$). The respiration rates of the treatment groups' soil continued to fall at harvest time, but no significant differences were observed between soils undergoing conventional rotary tillage or deep plowing and TF (255.0 $mg\,m^{-2}\,h^{-1}$) condition. Meanwhile, the respiration rate under TE (269.0 $mg\,m^{-2}\,h^{-1}$) condition was significantly greater than that exposed to TJ (202.2 $mg\,m^{-2}\,h^{-1}$, $P < 0.05$) condition, but the rates were not significantly different among soils exposed to different microbial agent applications under deep plowing.

4. Discussion

4.1. *The combinedapplication of microbial agents under conventional rotary tillage increased the DMA of winter wheat but not increased the grain yield*

DMA is the basis of crop yield. The soil moisture contents and nutrient utilization efficiencies of crops are directly related to the DMA at different crop growth stages, which affects the formation of winter wheat grains (Ma et al., 2015; Bei et al., 2018). Under conventional rotary tillage, according to our study, applied microbial agents increased the aboveground DMA values of winter wheat compared with applied chemical fertilizers. The use of a microbial agent alone had no effect on root DMA, but combinations could promote root growth significantly ($P < 0.05$), indicating that ETS and JS had synergistic effects on root growth. Similarly, in this study, the microbial agents were applied after reducing the amount of total chemical fertilizer by 30%. The grain yield of winter wheat was not reduced, but the straw yield decreased by 12.6–19.1%. Zhang et al. (2013) applied two bacterial groups that had synergistic effects, *Bacillus* and *Funneliformis,* in pot experiments, and the combination of bacterial flora promoted plant growth and reduced the amount of chemical fertilizer required by 50%. The decrease in chemical fertilizer did not affect crop growth or yield, similar to the results of this study. Here, the straw yield, but not the grain yield, was reduced after applications of microbial agents plus 70% the normal amount of chemical fertilizer. The application of the microbial agent may have affected the subsoil moisture transportation by changing the soil aggregate and the stability of organic C fractions. Because the water utilization levels in the wheat flowering and grain filling stages affect the distribution of wheat dry matter (Guo et al., 2015), they directly affect the distribution of dry matter in the grain.

Here, when applying the same microbial agent, deep plowing influenced the aboveground DMA, but it did not affect root growth in the 20–40-cm soil layer compared with conventional rotary tillage. Deep plowing did not affect the yield of winter wheat; however, the spike lengths and grain numbers per ear of the wheat were significantly lower than those of wheat grown under conventional rotary tillage ($P < 0.05$). Tillage practices and the depth of the plow play important roles in altering soil porosity and infiltration capacity, which affects surface runoff and soil water availability. Here, the tillage method did not significantly affect the average grain or biomass yield under conditions that met the crop's water demand (Berhe et al., 2013), which may explain the lack of differences in average grain or biomass yield between the two tillage practices. However, the different tillage practices did result in differences in spike length and other biological characteristics, which may be caused by the deep plowing and result in

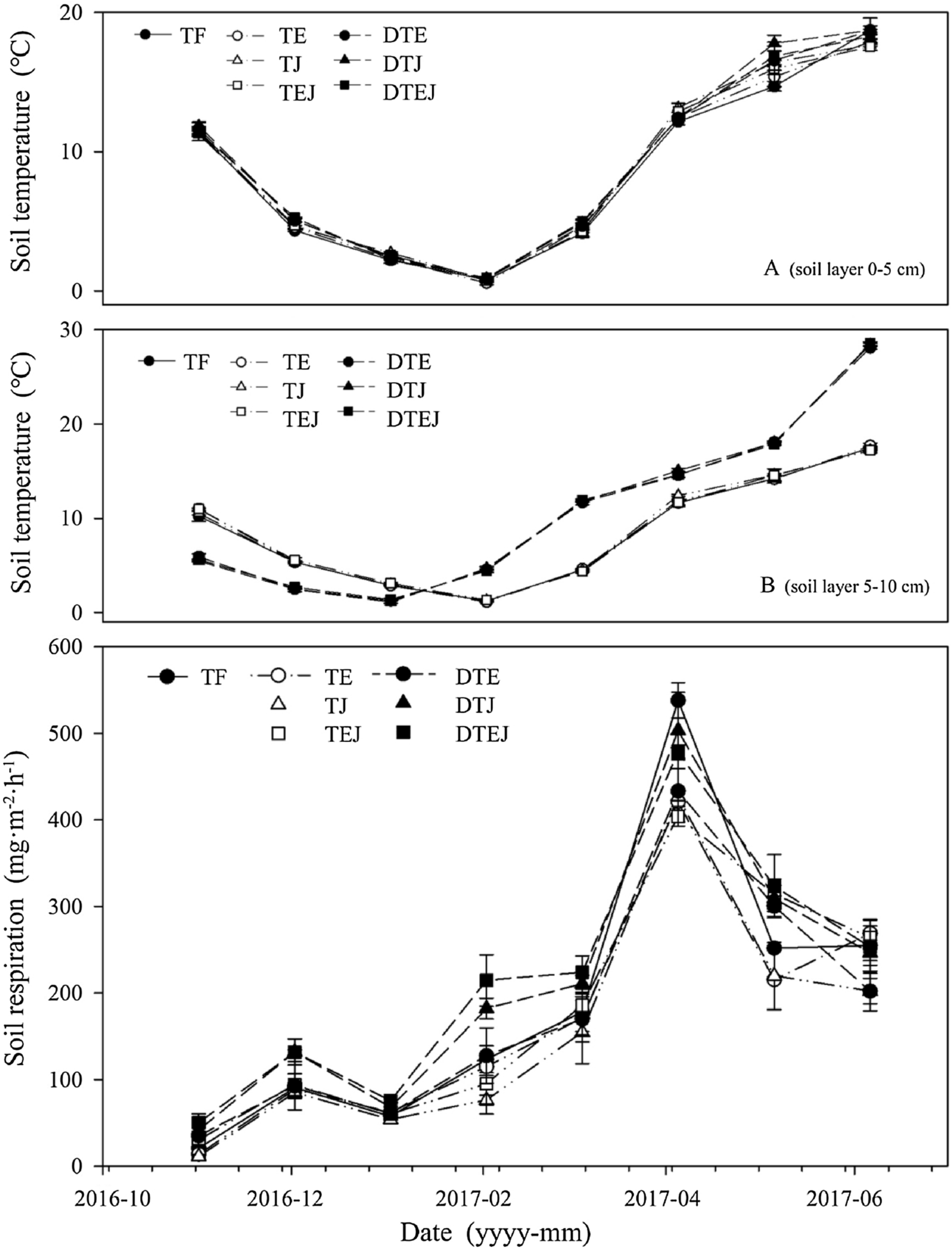

Fig. 2. Dynamics of soil respiration and temperature in winter wheat under different treatments.

the delayed root growth of winter wheat and the lower booting grain rate compared with conventional rotary tillage (Wang et al., 2014). The grain yields of the winter wheat under deep plowing are greater than those under conventional rotary tillage; however, the yield-increasing effects were not observed in the initial experimental stages (Šíp et al., 2013; Shi et al., 2016; Zhai et al., 2017).

4.2. The combined application of microbial agents under different tillage practices increased the MBC and MBN concentrations in the subsoil

MBC and MBN are important indicators of the degree of soil interference and microbial activity. In this study, under conventional rotary tillage, the MBC content in the 0–20-cm soil layer significantly decreased with the application of microbial agents ($P < 0.05$), while the MBN content increased. In the 20–40-cm soil layer, the MBC and MBN concentrations after JS and total chemical fertilizer applications were significantly lower ($P < 0.05$) than after ETS or the combined ETS and JS applications, and the TOC concentration showed the same trend. Meanwhile, the combination of ETS and JS had synergistic effects on microbiological quantity and TOC concentration. In Mahmood et al. (1997), when the total N content was 9 kg·ha^{-1}, there was no significant difference in the MBC after the application of farmyard manure or urea, but when the amount of total N was doubled, the MBC after the application of farmyard manure was significantly greater than that after the application of urea. This indicates that the application of an exogenous materials, whether organic or a chemical fertilizer, has a significant impact on MBC and MBN. MBC represents an important component of unstable C, and dynamic variations in microbial C-use efficiency and MBC were observed in different depths of the subsoil (Spohn et al., 2016). Thus, the application of a microbial agent may have increased the turnover time of the microbial biomass, which decreased the MBC at harvest in comparision with the use of a conventional fertilizer treatment. However, the MBN increased after the application of microbial agents, which indicated that they enhanced the capacity of soil microbes for soil N conservation. The results of this study still need to be verified by investigating the changes in the soil microbial community structure. Bacterial flora with decomposing functions have been used in soil to accelerate the decomposition of organic materials, which may be antagonistic or synergistic with the native flora of the soil (Lang et al., 1995; Qiu et al., 2009; Song and Zhang, 2015). This may be related to the nutrient environment of the soil, and this may explain why the MBC concentration after JS application was lower than that after ETS application.

The MBC and MBN concentrations after deep plowing in the 0–20-cm soil layer were significantly lower than those after conventional rotary tillage when the same microbial agent was applied ($P < 0.05$), but there was no significant difference in the MBC and MBN concentrations in the 20–40-cm soil layer between the two tillage practices. The soil disturbance caused by tillage practices does not significantly influence the soil TOC concentration, which was corroborated in the present study. Although the MBC concentration was significantly affected by soil disturbance, this did not occur during the early part of the year when tillage practices were changed. However, the amplitudes of the changes in MBC and soil enzymes increased with time after the adoption of tillage practices that caused greater level of soil disturbance than conventional rotary tillage (Kabiri et al., 2016).

4.3. Deep plowing with applied microbial agents increases the soil respiration rate

Soil respiration, which is the CO_2 produced by the biological activities of soil organisms, is the source of a major flux within the global C cycle (Elias, 2013). Soil respiration after conventional rotary tillage and microbial agent applications, rather than chemical fertilizer applications, was lower during the winter wheat growth stage. Additionally, there were no significant differences in soil respiration among ETS, JS and their combined treatments, which was consistent with there being lower MBC concentrations in the 0–20-cm soil layer compared with after chemical fertilizer application. Additionally, the soil temperature after the microbial agent application was not significantly different than after the microbial agent and chemical fertilizer application. The total amounts of MBC gained through chloroform fumigation-based extraction methods may not indicate the total amount of C substrate in soil respiration (Wang et al., 2003). However, the specific community structures of soil microorganisms were significantly correlated with the TOC, the total amount of dissolved organic C and the intensity of soil respiration (Zheng et al., 2009; Nazaries et al., 2015; Ozlu and Kumar, 2018). In this study, there were no significant differences in the TOC concentrations among treatment groups, and there were no significant differences in the soil temperatures between the applications of microbial agents and chemical fertilizers. Thus, the soil microbial community structure and soil-dissolved organic C concentration mainly caused a decrease in soil respiration after microbial agent applications compared with after chemical fertilizer applications under conventional rotary tillage.

Here, the soil under deep plowing was more sensitive to temperature changes in the external environment than those under conventional rotary tillage, and the temperature of the soil after the wheat regreening stage increased more rapidly and was higher than after conventional rotary tillage. Additionally, the soil respiration rate under deep plowing was greater than under conventional rotary tillage when the same microbial agent was applied. The soil respiration rate under the combined ETS and JS application with deep plowing was the greatest. Soil respiration is mainly controlled by temperature and soil moisture, and it can be limited by the soil's pore system (Schwen et al., 2015). Thus, the soil respiration rate under deep plowing was greater than under conventional rotary tillage in this study. Xu et al. (2001) found that deep plowing could increase the large pores of the soil and promote the exchange of air between the soil and atmosphere. This was corroborated by our results.

5. Conclusions

The study focused on the effects of tillage practices and microbial agents' applications on dry matter accumulation, yield and the soil microbial index of winter wheat in North China, while still to explore the feasibility and benefit of using combination of microbial agents to partially replacing chemical fertilizers. The practices in this paper could reduce the applications of chemical fertilizers and improve soil properties. Our findings showed that, the wheat grain yield was not decreased after the application of different microbial agents plus 70% of the normal amount of chemical fertilizer and the aboveground DMA increased greatly with ETS application under conventional rotary tillage, compared with TF. In 20–40-cm soil layer, the combined application of ETS and JS significantly increased the MBC and MBN concentrations, respectively. With deep plowing, the soil respiration rates were greater than that under conventional rotary tillage, and the soil respiration rate after the application of ETS and JS combination was greater than that after the microbial agents were individually applied during wheat's growth period. Whilst, under conventional rotary tillage, compared with TF, applications of different microbial agents decreased the straw yield by 19.1% and 16.4% respectively. When ETS and JS were individually applied under conventional rotary tillage, MBC concentration was decreased by 35.2–42.3% in 0–20 cm. Thus, under the application of microbial agents, the carbon and nitrogen cycle processes in the farmland soil and the mechanism of microbial action need to be further investigation, and the joint action mechanism of ETS and JS should be still elucidated.

Conflict of interest

No conflict of interest exits in the submission of this manuscript.

Acknowledgments

This study was supported by the Program for the National Key Research and Development Program of China (2016YFD0300808 and 2017YFD0801404), Youth Innovation Research Team Project (LENOM2016Q0004), and Youth Innovation Promotion Association CAS (2017073).

References

Amarger, N., 2001. Rhizobia in the field. Adv. Agron. 73, 109–168.

Andreev, N., Ronteltap, M., Lens, P.N.L., Boincean, B., Bulat, L., Zubcov, E., 2016. Lacto-fermented mix of faeces and bio-waste supplemented by biochar improves the growth and yield of corn (Zea mays L.). Agric. Ecosyst. Environ. 232, 263–272.

Bei, S., Zhang, Y., Li, T., Christie, P., Li, X., Zhang, J., 2018. Response of the soil microbial community to different fertilizer inputs in a wheat-maize rotation on a calcareous soil. Agric. Ecosyst. Environ. 260, 58–69.

Berhe, F.T., Fanta, A., Alamirew, T., Melesse, A.M., 2013. The effect of tillage practices on grain yield and water use efficiency. CATENA 100, 128–138.

Bowen, G.D., Rovira, A.D., 1999. The rhizosphere and its management to improve plant growth. Adv. Agron. 66, 1–102.

Chen, J.-r., 1982. Fertilizer and development of an agricultural economy. Zeitschrift für Nationalökonomie 42, 395–409.

Cong, P., Ouyang, Z., Hou, R., Han, D., 2017. Effects of application of microbial fertilizer on aggregation and aggregate-associated carbon in saline soils. Soil Tillage Res. 168, 33–41.

Dadrasan, M., Chaichi, M.R., Pourbabaee, A.A., Yazdani, D., Keshavarz-Afshar, R., 2015. Deficit irrigation and biological fertilizer influence on yield and trigonelline production of fenugreek. Ind. Crops Prod. 77, 156–162.

Elias, S.A., 2013. Reference module in earth systems and environmental sciences. Ref. Module Earth Syst. Environ. Sci.

Guo, J.H., Liu, X.J., Zhang, Y., Shen, J.L., Han, W.X., Zhang, W.F., Christie, P., Goulding, K.W.T., Vitousek, P.M., Zhang, F.S., 2010. Significant acidification in major Chinese croplands. Science 327, 1008.

Guo, Z., Shi, Y., Yu, Z., Zhang, Y., 2015. Supplemental irrigation affected flag leaves senescence post-anthesis and grain yield of winter wheat in the Huang-Huai-Hai Plain of China. Field Crops Res. 180, 100–109.

Han, X., Xu, C., Dungait, J.A.J., Bol, R., Wang, X.J., Wu, W.L., Meng, F.Q., 2018. Straw incorporation increases crop yield and soil organic carbon sequestration but varies under different natural conditions and farming practices in China: a system analysis. Biogeosciences 15, 1933–1946.

He, Y., Wu, Z., Tu, L., Han, Y., Zhang, G., Li, C., 2015. Encapsulation and characterization of slow-release microbial fertilizer from the composites of bentonite and alginate. Appl. Clay Sci. 109–110, 68–75.

Kabiri, V., Raiesi, F., Ghazavi, M.A., 2016. Tillage effects on soil microbial biomass, SOM mineralization and enzyme activity in a semi-arid Calcixerepts. Agric. Ecosyst. Environ. 232, 73–84.

Lang, E., E'Uer, G., Kleeberg, I., Martens, R., Zadrazil, F., 1995. Interaction of white rot fungi and soil microorganisms leading to biodegradation of soil pollutants. In: Van Den Brink, W.J., Bosman, R., Arendt, F. (Eds.), Contaminated Soil' 95: Proceedings of the Fifth International FZK/TNO Conference on Contaminated Soil, 30 October–3 November 1995, Maastricht, The Netherlands. Springer, Netherlands, Dordrecht, pp. 1277–1278.

Liu, J., Shen, J., Li, Y., Su, Y., Ge, T., Jones, D.L., Wu, J., 2014. Effects of biochar amendment on the net greenhouse gas emission and greenhouse gas intensity in a Chinese double rice cropping system. Eur. J. Soil Biol. 65, 30–39.

Ma, S., Duan, A., Wang, R., Guan, Z., Yang, S., Ma, S., Shao, Y., 2015. Root-sourced signal and photosynthetic traits, dry matter accumulation and remobilization, and yield stability in winter wheat as affected by regulated deficit irrigation. Agric. Water Manage. 148, 123–129.

Mahmood, T., Azam, F., Hussain, F., Malik, K.A., 1997. Carbon availability and microbial biomass in soil under an irrigated wheat-maize cropping system receiving different fertilizer treatments. Biol. Fertil. Soils 25, 63–68.

Nazaries, L., Tottey, W., Robinson, L., Khachane, A., Al-Soud, W.A., Sørensen, S., Singh, B.K., 2015. Shifts in the microbial community structure explain the response of soil respiration to land-use change but not to climate warming. Soil Biol. Biochem. 89, 123–134.

Ozlu, E., Kumar, S., 2018. Response of surface GHG fluxes to long-term manure and inorganic fertilizer application in corn and soybean rotation. Sci. Total Environ. 626, 817–825.

Parkin, T.B., Venterea, R.T., 2010. USDA-ARS GRACEnet Project Protocols Chapter 3. Chamber-Based Trace Gas Flux Measurements. Ncaur.usda.gov. .

Pishchik, V.N., Vorobyev, N.I., Moiseev, K.G., Sviridova, O.V., Surin, V.G., 2015. Influence of Bacillus subtilis on the physiological state of wheat and the microbial community of the soil under different rates of nitrogen fertilizers. Eurasian Soil Sci. 48, 77–84.

Qiu, Y., Pang, H., Zhou, Z., Zhang, P., Feng, Y., Sheng, G.D., 2009. Competitive biodegradation of dichlobenil and atrazine coexisting in soil amended with a char and citrate. Environ. Pollut. 157, 2964–2969.

Raza, W., Mei, X., Wei, Z., Ling, N., Yuan, J., Wang, J., Huang, Q., Shen, Q., 2017. Profiling of soil volatile organic compounds after long-term application of inorganic, organic and organic-inorganic mixed fertilizers and their effect on plant growth. Sci. Total Environ. 607–608, 326–338.

Schneider, F., Don, A., Hennings, I., Schmittmann, O., Seidel, S.J., 2017. The effect of deep tillage on crop yield – what do we really know? Soil Tillage Res. 174, 193–204.

Schwen, A., Jeitler, E., Böttcher, J., 2015. Spatial and temporal variability of soil gas diffusivity, its scaling and relevance for soil respiration under different tillage. Geoderma 259, 323–336.

Seddaiu, G., Iocola, I., Farina, R., Orsini, R., Iezzi, G., Roggero, P.P., 2016. Long term effects of tillage practices and N fertilization in rainfed Mediterranean cropping systems: durum wheat, sunflower and maize grain yield. Eur. J. Agron. 77, 166–178.

Shi, Y., Yu, Z., Man, J., Ma, S., Gao, Z., Zhang, Y., 2016. Tillage practices affect dry matter accumulation and grain yield in winter wheat in the North China Plain. Soil Tillage Res. 160, 73–81.

Šíp, V., Vavera, R., Chrpová, J., Kusá, H., Růžek, P., 2013. Winter wheat yield and quality related to tillage practice, input level and environmental conditions. Soil Tillage Res. 132, 77–85.

Song, Z., Zhang, C., 2015. Anaerobic codigestion of pretreated wheat straw with cattle manure and analysis of the microbial community. Bioresour. Technol. 186, 128–135.

Spohn, M., Klaus, K., Wanek, W., Richter, A., 2016. Microbial carbon use efficiency and biomass turnover times depending on soil depth – implications for carbon cycling. Soil Biol. Biochem. 96, 74–81.

Tao, R., Liang, Y., Wakelin, S.A., Chu, G., 2015. Supplementing chemical fertilizer with an organic component increases soil biological function and quality. Appl. Soil Ecol. 96, 42–51.

Thomas, G.H.E. (1917) Bacterial product and process of preparing same. In. US1252332A, USA.

Tu, C., Li, F., 2017. Responses of greenhouse gas fluxes to experimental warming in wheat season under conventional tillage and no-tillage fields. J. Environ. Sci. 54, 314–327.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19, 703–707.

Wang, W.J., Dalal, R.C., Moody, P.W., Smith, C.J., 2003. Relationships of soil respiration to microbial biomass, substrate availability and clay content. Soil Biol. Biochem. 35, 273–284.

Wang, Y., Hu, W., Zhang, X., Li, L., Kang, G., Feng, W., Zhu, Y., Wang, C., Guo, T., 2014. Effects of cultivation patterns on winter wheat root growth parameters and grain yield. Field Crops Res. 156, 208–218.

Wang, H., Guo, Z., Shi, Y., Zhang, Y., Yu, Z., 2015. Impact of tillage practices on nitrogen accumulation and translocation in wheat and soil nitrate-nitrogen leaching in drylands. Soil Tillage Res. 153, 20–27.

Wu, Y., 2017. Chapter 9 - periphyton: a promising bio-organic fertilizer source in agricultural ecosystems. Periphyton. Elsevier, Boston, pp. 225–249.

Xiong, W., Guo, S., Jousset, A., Zhao, Q., Wu, H., Li, R., Kowalchuk, G.A., Shen, Q., 2017. Bio-fertilizer application induces soil suppressiveness against Fusarium wilt disease by reshaping the soil microbiome. Soil Biol. Biochem. 114, 238–247.

Xu, W., Liu, W., Liu, G., 2001. Potential effect of fertilising and tilling on N2O emission from upland soils analyzed by DNDC model. Chin. J. Appl. Ecol. 917–922.

Yao, L., Wu, Z., Zheng, Y., Kaleem, I., Li, C., 2010. Growth promotion and protection against salt stress by Pseudomonas putida Rs-198 on cotton. Eur. J. Soil Biol. 46, 49–54.

Yilmaz, E., Sönmez, M., 2017. The role of organic/bio–fertilizer amendment on aggregate stability and organic carbon content in different aggregate scales. Soil Tillage Res. 168, 118–124.

Zhai, L., Xu, P., Zhang, Z., Li, S., Xie, R., Zhai, L., Wei, B., 2017. Effects of deep vertical rotary tillage on dry matter accumulation and grain yield of summer maize in the Huang-Huai-Hai Plain of China. Soil Tillage Res. 170, 167–174.

Zhang, G., Chan, K.Y., Li, G.D., Huang, G., 2011. The effects of stubble retention and tillage practices on surface soil structure and hydraulic conductivity of a loess soil. Acta Ecol. Sin. 31, 298–302.

Zhang, R., Yan, C., Zhang, N., Li, J., Shen, Q., 2013. Studies on microbial fertilizer and its application prospects in improving arable land quality. J. Agric. Sci. Technol. 8–16.

Zhao, Y., Yin, Y., 2015. Key scientific problems on establishing green fertilizer ensurance system. Chin. Sci. Bull. 3527–3534.

Zhao, B., Lin, Z., Liu, Z., 2008. The future developing route for China's fertilizer industry: increasing the use efficiency and decreasing the consumption of fertilizer. Phosphate Compd. Fertil. 1–4.

Zheng, Z., Yu, G., Fu, Y., Wang, Y., Sun, X., Wang, Y., 2009. Temperature sensitivity of soil respiration is affected by prevailing climatic conditions and soil organic carbon content: a trans-China based case study. Soil Biol. Biochem. 41, 1531–1540.