Growing-season soil microbial respiration response to long-term no tillage and spring ridge tillage

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Abstract: No tillage (NT) and spring ridge tillage (SRT) are two common applications of conservation tillage. Although conservation tillage is known to exert major control over soil microbial respiration (SMR), the growing-season SMR response to these two applications remains elusive. In order to better understand the influence of conservation tillage practices, this experiment was conducted in an experimental field using NT and SRT for 17 years. In situ measurements of SMR, soil temperature and soil water content (SWC) were performed. Soil samples were collected to analyze soil porosity, soil microbial biomass (SMB) and soil enzymatic activities. Results show that the two conservation tillage systems had a significant difference (p<0.05) in terms of SMR; the SMR of NT was 14.7 mg·C/m^2·h higher than that of SRT. In terms of soil temperature and soil enzymatic activities, the two treatments were not significantly different (p>0.05). Despite SRT increasing the proportion of micro-porosities and meso-porosities, the soil macro-porosities for NT were 7.37% higher than that of SRT, which resulted in higher bacteria and fungi in NT. Owing to SRT damaged the hypha, which had disadvantage in soil microbe protection. Inversely, less soil disturbance was a unique advantage in NT, which was in favor of improving soil macro-pores and SWC. Redundancy analyses (RDA) showed SMR was positively correlated with soil macro-pores, SMB and SWC. Furthermore, the Pearson correlation test indicated that SMB and soil enzymatic activities did not have a significant correlation (p>0.05). This study results suggest that SRT is more conducive to carbon sequestration compared with NT in cropland.

Keywords: no tillage, spring ridge tillage, soil microbial respiration, microbial biomass, soil porosity, soil enzymatic activity

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1 Introduction

Conservation tillage is an effective method facilitating the development of sustainable agricultural systems, via promotion of microbial activity, retention of soil pore continuity, increasing soil organic carbon (SOC) and through decreased soil moisture loss, decreased mechanical structural breakdown, as well as less exposure of the microbial population to solar radiation[1,2]. Crop residue and stubble retention are core features of conservation tillage, although the benefits of stubble retention are regionally variable, depending on both agro-climatic and socioeconomic factors[3]. Positive effects of stubble retention on agricultural sustainability include increasing SOC, soil water retention and nutrient cycling together with decreasing soil loss[3-6]. In addition to stubble retention after harvesting, conservation tillage also includes no tillage (NT) and spring ridge tillage (SRT), NT refers to nothing will be done to the field before sowing, SRT refers to ridging the field before sowing, NT and SRT are widely utilized in China[7].

Soil respiration (SR) consists of heterotrophic respiration (HR) and autotrophic respiration, where the former is contributed by soil microbes, so it can be regarded as soil microbial respiration (SMR), while the latter is mainly contributed by plant roots. Crop SR is highest during the growth period (seedling to maturity growth phases)[8]. Numerous studies on soil-temperature interactions indicate that the SR rate has a close relationship to soil temperature[9-11]. When the temperature declines below 0 ℃, the majority of microbial activities ceases[12]. With the rising temperature, SR reaches a maximum and then declines above the optimum temperature (25 ℃ to 35 ℃)[13]. The soil temperature is also essential for crop production, the higher of soil temperature, the more benefit for crop development and ripe[14]. Soil water content (SWC) also controls soil respiration. The SWC is a type of essential water for microbes, and provides a reactant for SOC decomposition. Furthermore, SWC influences soil aeration, oxygen content in pores and consequently SMR[15]. Just as soil temperature, within an appropriate range, the higher of SWC, the more favorable for crop growth. Although the relationship between soil porosity and the microbial community is not well

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understood yet, some previous studies have indicated that O_2 fluxes are taken place mostly in macropores (≥75 μm), which is essential for crop metabolism so as to grow[16], actinomycetes and fungi live in soil pores with diameters between 15-60 μm. Smaller soil pores between 0.3-3 μm can prevent bacteria from being preyed upon, while the majority of microbes are unable to enter soil pores with a diameter of fewer than 0.2 μm[17,18]. In conclusion, the above literature suggests that in addition to the crop growth stage, soil respiration is affected by soil temperature, soil moisture, soil aeration condition, as well as the soil pore dynamics.

Soil microbes are the major contributors to decompose organic matters, so as to release crop essential nutrients[19]. The debate on the quantitative relationship between soil microbial biomass (SMB) and SMR within conservation tillage systems is ongoing. Soil microbial community, species characteristics, microbial abundance as well as their activities can all affect SMR. Condron et al.[20] found about 85% to 90% of organic carbon in soil was decomposed by soil bacteria and soil fungi, while Ran et al.[21], working in forests and high latitude meadows, found that there is positive correlation between soil bacteria content and SMR, so does soil actinomycetes content and SMR. The determination coefficient was 0.52 for the regression analysis between soil bacteria content and SMR, and it was 0.71 between soil actinomycetes content and SMR. Birge et al.[22] explored different controls for SMR to elucidate the drivers of respiration rate, whereby their results indicated that SMR was not limited by SMB, available soil organic matter was the actual driver. It is apparent that relationships between SMB and SMR are localized, with a significant level of uncertainty. Further work investigating SMB and SMR for specific applications will be required to understand localized soil function.

SMR is an enzymatic reaction, thus, within the niche ecological temperature range, the higher the temperature, the higher enzymatic activity, and vice versa[12]. Soil enzymes are key participants in biochemical reactions, with one of the most important functions being to mediate the recalcitrant components of SOC[23]. Therefore soil enzyme content controls the soil microbial metabolism, which should be reflected by SMR. On this basis, soil enzyme content has been identified as a very sensitive indicator of soil quality[24]. But soil enzyme has little impact on crop production, although crop needs essential nutrients decomposed by SMB, biochemical reaction do not require a high enzyme content[25]. Urease is distributed widely throughout the soil, urease activity is often used to represent organic nitrogen mineralization, which is the source for releasing NH_4^+-N[26]. Sucrase plays an important role in increasing soluble nutrients to the soil. Catalase catalyzes hydrogen peroxide, which protects organisms from toxic effects[27]. Owing to these, soil enzymes are important to plant and soil organisms, but a definite conclusion about the relationship between soil enzymatic activity and SMR is still lacking. Therefore, soil enzymatic activities were investigated in this study.

It is clear that agricultural management has a great influence on cropland SMR[28]. For instance, studies have reported that tillage reduces microbial biomass and their activities, and concurrently disadvantages soil enzymatic activities[29]. In contrast, NT has been demonstrated to protect soil microbes. For example, Roldan et al.[30] demonstrated that catalase activity is higher in NT treatment. Additionally, agricultural management could change soil physicochemical properties, with these changes particularly evident in the tillage layer[31,32]. Soil physicochemical changes also affect SMR through soil temperature, SWC, soil porosity and soil enzymatic activity[33,34].

SMR is an important part of SR, and SR comprises the second largest terrestrial carbon cycling[35], which is a key factor influences carbon sequestration. Understanding tillage influences on SMR remains a longstanding challenge in agroecosystems. To approach this objective, two fields with long term (17 years) tillage treatments (NT and SRT) were used in this study. Within the scope of conservation tillage, the major objective of this study was to compare the growing-season SMR between NT and SRT. We want to evaluate the changes in soil temperature, SWC, soil porosity, SMB and soil enzymatic activity, which caused by different tillage treatments. We hypothesized that different tillage treatments led to different variations in above soil-related parameters, which further affected SMR during growing seasons. Thus in situ measurements and laboratory analyses were combined to assess how tillage treatments affected above soil-related parameters and growing-season SMR.

2 Materials and methods

2.1 Study site description

The study site is located in Huashishan County, Siping City, Jilin Province, China (43.31°N, 124.62°E). The annual mean temperature is 4.8°C, the annual precipitation ranges from 522 to 615 mm, and the annual accumulative temperature is 2770°C to 2910°C. The study site covers about 25 hm^2, which had been fallowed naturally until the spring of 2000. According to the USDA soil taxonomy[36], the soil belongs to the Mollisols order, Ustolls suborder, Argiustolls great group and Calcidic Argiustolls subgroup. A basic soil test was conducted according to the description of Sumner[37] in the autumn of 2000; in light of the test records, it has a SOC concentration of 1.34%, soil nitrogen concentration of 0.12%, available phosphorus concentration of 0.25%, available potassium concentration of 0.16% within top 30 cm depth and soil pH values ranged from 6.5 to 7.1; sand, silt and clay concentrations were 57%, 13% and 30%, respectively.

2.2 Field experimental design

The study was initiated in the year 2000. Two zones in north-south oriented plots of 300 m in length and 200 m in width were chosen. A 50 m and 300 m long wide zone fallowed all the time acted as a buffer zone between the two treatments. Two zones were covered by maize (*Zea Mays* L.) stalk after harvesting. The maize stalk was shattered into small pieces (less than 5 cm) by maize combined harvesters. In order to quantify the maize stalk residue, five square blocks of 2.25 m^2 were selected randomly. The total maize stalk residues were collected and weighted every day from 25th October every year, until the average dry mass of two adjacent days lacked a significant difference (p>0.05). The average dry mass was used to calculate the maize stalk residue amount. The average dry mass (2000-2017) was 5639 kg/ha. The monoculture rain-fed maize was the only crop in this study site, the seedtime is within late April to early May, and the maize is harvested in early October. The growing season is from late May to mid-September, which corresponds to the maize elongation stage to maize dough stage. Manual weeding control was applied during the growing period. In order to limit the experimental factors, no chemical fertilizer or manure was used.

In the NT treatment, the maize was planted by a no-till planter, the row distance was 65 cm, and the intra-row distance was 22 cm. In the SRT, the ridges were maintained yearly with a cultivator before sowing, and a modified lister and scrubber were used to form and press the ridge (each 16 cm of height and 55 cm of bottom width). Subsequently, the maize seeds were planted by a conventional maize planter with the same row distance and intra-row distance as NT treatment. The two treatments were presented in Figure 1. Excluding the ridge tillage treatment, the other managements were all the same for the two treatments.

a. No tillage

b. Spring ridge tillage

Figure 1 No tillage and spring ridge tillage

2.3 Soil microbial respiration, soil temperature, soil water content and soil porosity

In situ measurements were performed for acquiring the SMR, soil temperature and SWC. As the growing season ranges from late May to middle September, measurements were initiated on 27th May 2017 and ended on 16th September 2017 once per week. The measurements were conducted from 9:00 to 11:00 on each measurement date. In order to establish 10 replicates, 10 plots 10 m in width and 20 m in length were randomly selected in each treatment. Inside each plot, four polyvinyl chloride (PVC) collars (33 cm inside diameter, 30 cm tall) were placed in a 2 m×2 m grid and four PVC collars formed a square with a length of two meters. The collars were inserted 15 cm into the soil and left undisturbed throughout the study. Isotopic ^{13}C partitioning and root trenching are two common methods to obtain HR from total SR. Biasi et al.[38] demonstrated that the results of these two methods were comparable during the growing season, and root trenching excludes CO_2 emission which caused by dead root decomposition[39], besides, root trenching is more cost effective compared with isotopic methods, so root trenching method was adopted to obtain HR (namely the SMR) in this study. As the method described by Bond-Lamberty et al.[40], trenching was performed by excavating the outside edges of a 1 m×1 m square, centered on each collar, to a depth of 50 cm. The interior edges of the trenches were lined with 6-mm plastic sheeting and backfilled. To exclude above-ground vegetation respiration, the vegetation inside the trench was removed manually.

Soil microbial respiration was measured by a Li-8100A Soil CO_2 Flux System (Li-Cor Inc., Lincoln, NE, USA). SMR rate was measured by CO_2 diffusion amount from per unit area within per unit time. In order to collect soil temperature and SWC, one probe (Omega Engineering Inc. USA) was inserted 10 cm beneath the soil surface, which was about 10 cm away from the survey chamber of Li-8100A, the Em-50 data logger (Decagon Devices, Inc. USA) was connected to the probe, and the monitoring frequency was every 5 min.

In order to determine soil porosity in the top 30 cm, three vertical-connect acrylic tubes (2 cm inner diameter, 10 cm length) were inserted 20 cm from the survey chamber but at the opposite direction of the probe into the soil to collect undisturbed soil cylinders utilized for soil porosity analysis. Soil cores (5 cm diameter, 10 cm length) inside the collars were collected for lab analyses using augers. For each replication, four soil cores were mixed to constitute one composite sample. These soil samples were brought to the lab immediately and then sieved (2 mm mesh) to remove rocks and debris[41]. The soil sample was divided into two subsamples, one subsample was kept at 4℃ for the SMB analyses. The other subsample (for determination of soil enzymatic activities) was air-dried at room temperature (25℃) and ground with a mill to pass through a 0.25 mm sieve.

2.4 Soil analyses

Soil cylinders were scanned by the Nanotom (Phoenix electric group, Cologne Germany) X-ray digital core analysis instrument. This equipment employs computerized tomography (CT) 3D scanning technology, and determines soil porosity. Combined with digital data process software Open Text (Open text corporation, Waterloo, Canada), this instrument determines the percentages of each specific dimension. In this study, the 3D scanning parameters were set as follows: maximum tube voltage was 180 kV, pixel size of flat panel detector image≤50 μm, pixel number was 2200×2200, smallest Meta pixel≤0.5 μm, the maximum sample was 120 mm in diameter and 150 mm in height. Limited by image resolution, only soil pores larger than 9 μm in diameter were taken into consideration in this study. According to pore size classification[42], soil pores were classified into macropores (≥75 μm), mesopores (30-75 μm) and micropores (≤30 μm), respectively. Soil porosity was calculated as the quotient between soil pore volume and total soil sample volume.

Phospholipid-fatty acids (PLFA) were determined for analysis of soil microbial communities and their abundances employing a Sherlock™ Chromatographic Analysis System (MIDI, Inc., Newark, DE, USA), PLFAs were extracted from the soil sample stored at 4℃, for details, see Bossio & Scow[43]. Fatty acid nomenclature used in this study follows Zak et al.[44] The dominant PLFAs were classified as bacteria (15:0, i15:0, a15:0, 16:0, i16:0, 16:1ω5, 16:1ω9, 16:1ω7t, 17:0, i17:0, a17:0, cy17:0, 18:1ω5, 18:1ω7, 18:1ω7t, i19:0, a19:0 and cy19:0), actinomycetes (10Me16:0, 10Me17:0, 10Me18:0), fungi (18:1ω9, 18:2ω6, 18:3ω6, 18:3ω3).

Urease, sucrose and catalase activities were obtained according to the description of Guan[45] and Burns[46]. The urea hydrolysis method was utilized to obtain urease activity. The colorimetry of 3, 5-dinitrosalicylic acid was applied to obtain the sucrase activity. Potassium permanganate titration was used to obtain the catalase activity.

2.5 Data analysis

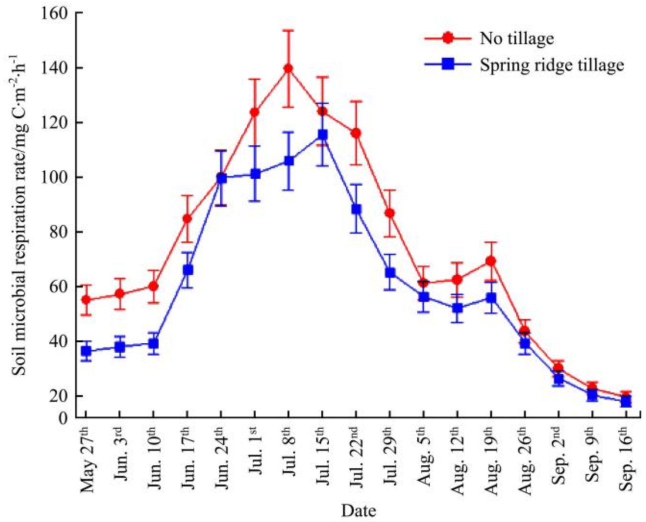
Software of SPSS 22.0 for Windows (IBM Inc., USA) was used in this study to carry out statistical analyses, student's *t* test was used to confirm whether the difference between NT and SRT was significant, comparative items included SMR, soil temperature, SWC, three kinds of soil porosities, SMB, soil enzymatic activity.

In addition, Pearson correlation test was performed to investigate the correlation between SMB and soil enzymatic activities. Multivariate regression analysis assumes independent relationships among variables, but there are correlations among intra-variables in this study, thus in order to examine the complex links between the response variables and explanatory variables, redundancy analysis (RDA) of type II (correlation plot) was utilized instead of the multivariate regression. Soil geochemical indicators were ascribed to the explanatory variables, which included the abundance of SMB, SMR, bacteria, actinomycetes, fungi, soil enzymes. Soil physical parameters were ascribed to the explanatory variables, which included soil temperature, SWC and three kinds of soil porosities. NT and SRT were designated as nominal variables.

3 Results

3.1 Soil microbial respiration

Figure 2 shows during the whole growing season, two treatments had significant differences (p<0.05) in average SMR, which were 79.9 mg·C/m^2·h and 65.2 mg·C/m^2·h for NT and SRT respectively. Their peak values were 150.8 mg·C/m^2·h and 124.8 mg·C/m^2·h for NT and SRT respectively. The SMR of NT was higher than that of SRT throughout the growing season, but the difference became less during the final stage of the growing season.



Note: Error bars indicated the standard deviations.

Figure 2 Soil microbial respiration of no tillage and spring ridge tillage

3.2 Soil temperature

The soil temperature was on the fluctuated-rising trend before late July, then the soil temperature began to decline. The average soil temperatures were 20.59℃ and 20.92℃ for NT and SRT, although the soil temperature of SRT was consistently slightly higher compared with NT, there was no significant difference (p>0.05) between two treatments.

3.3 Soil water content

Figure 4 presents the obvious difference of SWC that occurred between late June and early July as well as the whole of August. The peak fractions of SWC curves for both two treatments were concentrated during middle July to end July. The highest SWCs occurred on 29th July, with 42.9% and 42.8% for NT and SRT, respectively. The lowest SWCs were 29.5% and 30% for NT and SRT, which were measured on 16th September. The average SWCs were 36.33% and 35.39% for NT and SRT respectively. In terms of SWC during the growing stages, significant differences (p<0.05) between NT and SRT were observed

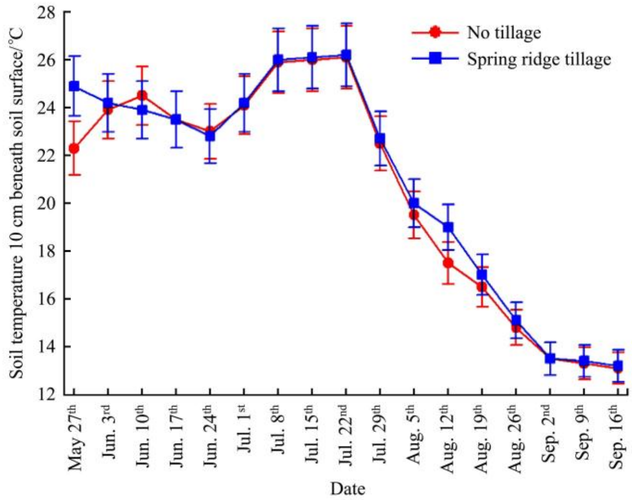
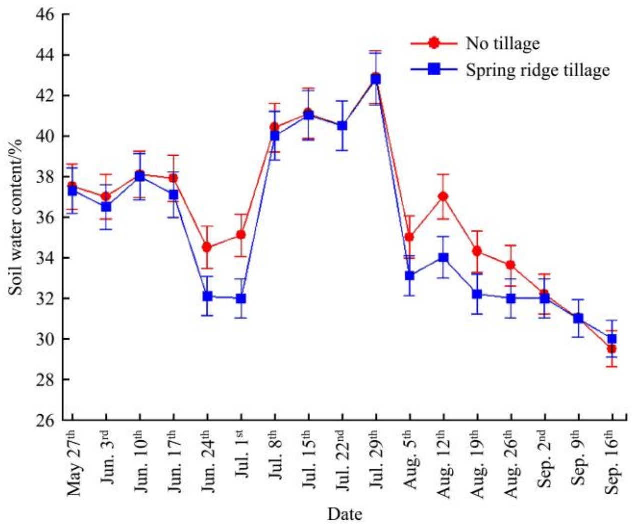


Figure 3 Soil temperature of 10 cm beneath soil surface. Error bars indicated the standard deviations



Note: Error bars indicated the standard deviations.

Figure 4 Soil water content of 10 cm beneath soil surface

3.4 Soil porosity

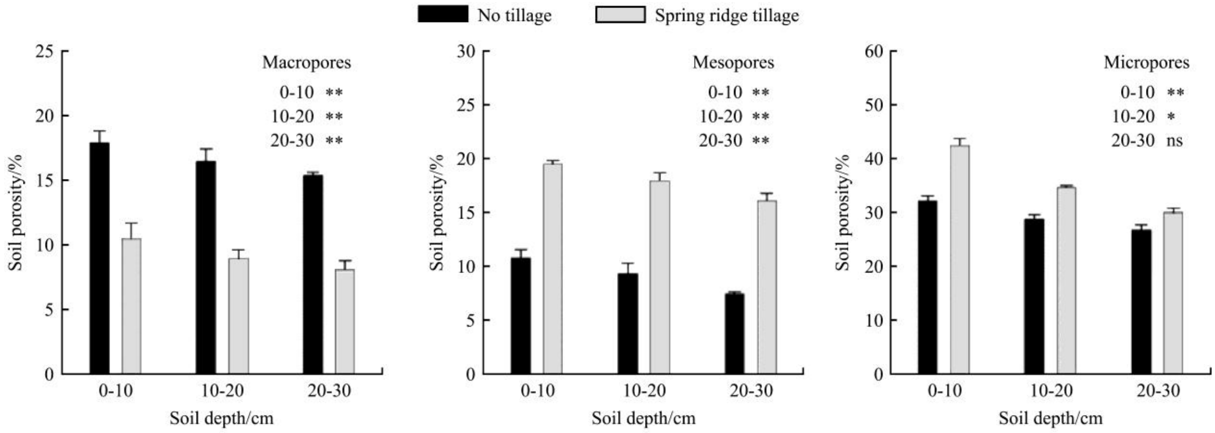
Soil porosity decreased along with the increase of soil depth (Figure 5). Micropores were dominant in both NT and SRT. NT has higher macro-porosity but lower meso-porosity and micro-porosity compared with SRT. The average macro-porosities for top 30 cm were 16.5℃ and 9.2% for NT and SRT, and the means of mesopores and micropores were 9.2℃ and 17.8% for NT, they were 29.2% and 35.7% for SRT.

3.5 Soil microbial biomass

The abundance of soil microbes decreased in both treatments in the descending order of bacteria, actinomycetes, fungi (Figure 6). In the NT treatment, total PLFA, bacteria and fungi biomass were 9.9%, 13.6% and 32.6% higher compared with SRT. In the SRT treatment, actinomycetes biomass was 34.2% higher than in NT.

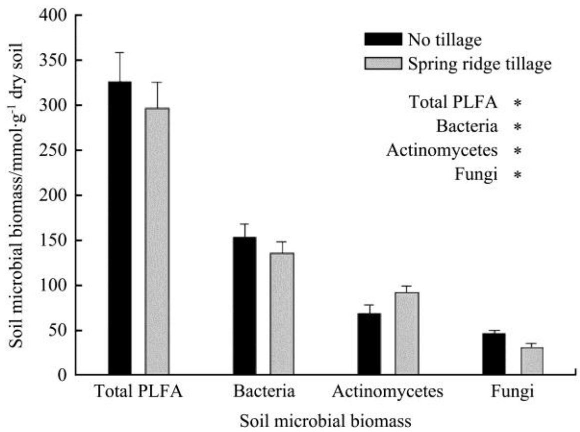
3.6 Soil enzymatic activity

Figure 7 demonstrates a minor difference in soil enzymatic activity for the two treatments. There was a consistent trend for NT to have stronger enzymatic activity, the activities of urease, sucrase and catalase of the NT were 8%, 2.1% and 4.8% higher than those of SRT, but these differences were not significant (p>0.05).



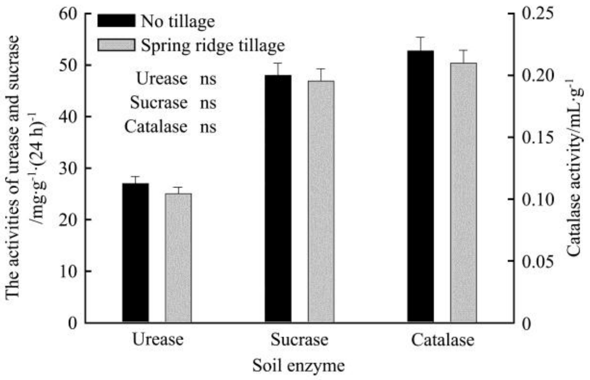
Note: Error bars indicated the standard deviations. Significance levels: **, $p< 0.01$; *, $p < 0.05$; ns, not significant.

Figure 5 Soil porosity distribution for the no tillage and spring ridge tillage systems



Note: Error bars indicated the standard deviations. Significance levels: *, $p <$ 0.05.

Figure 6 Soil microbial biomass for no tillage and spring ridge tillage



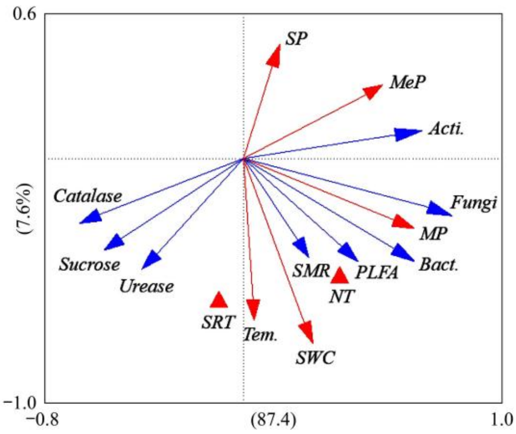
Note: Error bars indicated the standard deviations, ns, not significant at significance levels of 0.05.

Figure 7 Soil enzymatic activities for no tillage and spring ridge tillage

3.7 Correlation analyses

Pearson correlation analysis was performed to determine the correlation between soil enzymatic activities and SMB, with the correlation coefficients shown in Supplementary material 1. It demonstrates that SMB and enzymatic activities were not significantly correlated (p>0.05).

Figure 8 presents the results of the RDA. The constrained variance occupied the proportion of 96.1%, whilst the proportion of unconstrained variance was 0.39%. The horizontal RDA axis explained 87.4% of the response variables, and the vertical RDA axis explained 7.6% of the response variables. The RDA showed NT and SRT to be strongly correlated with soil temperature and SWC (p<0.05). Furthermore, NT was strongly correlated with soil macro-porosity (p=2.13), while there was no profound correlation between the two treatments and soil enzymes (p=4.44), meanwhile, the soil enzymes did not show significant positive correlations with the other factors, either. SMR was more dependent on total PLFA (p=13.32), and positively correlated with soil temperature (p<0.05), SWC (p<0.05) and macropores (p<0.05). SMB was extremely positive correlated (p<0.01) with macropores and positively correlated (p<0.05) with SWC. Bacteria (p<0.01) and fungi (p<0.01) were positively correlated with macropores, while actinomycetes (p<0.05) were positively correlated with mesopores. Micropores did not show any significant correlation with any index within this study.



Note: *SRT* means spring ridge tillage; *Tem.* means soil temperature; *SWC* means soil water content; *SMR* means soil microbial respiration; *NT* means no tillage; *Bact.* means bacteria; *MP* means macropores; *Acti.* means actinomycetes; *MeP* means mesopores; *SP* means micropores.

Figure 8 Redundancy analyses (RDA)

4 Discussion

4.1 Relationships among soil microbial respiration, soil temperature, soil water content and soil porosity

The relationship between SMR and soil temperature has been reported by other scholars, for example, Luo et al.[47] had concluded the SMR increased along with the increase of soil temperature. In this experiment, the SMR rate for both two treatments started to decline after mid-July (Figure 2), which corresponds to the decrease in soil temperature for both treatments (Figure 3). Taking the two treatments as a whole, this study concurs with the conclusion that SMR declines with soil temperature reduction[48], Owing to the ridge transect (Figure 1) creating greater soil surface in SRT, the soil temperature in SRT was slightly higher compared with NT (Figure 3) from beginning to end, which was presumably a result of higher net irradiation[49]. It is no doubt the higher soil temperature, the more benefit for plant growth[14]. Especially in Northeast China which has a relative shorter plant growth period. If the soil temperature could be improved by choosing an appropriate tillage method, farmers can choose more late maturing varieties, and the crop grain can also be fuller.

Although soil temperature in SRT was slightly higher than in NT (Figure 3), and it is already known that SMR has positive correlation with soil temperature, but SMR in NT was slightly higher than in SRT from beginning to end (Figure 2), which indicates besides soil temperature, there also other factors that affected the SMR. We have to mention that SMR has a close relationship with global warming, which is a serious environmental problem. During the growing season, which generates a lot of CO_2 emissions, SRT is more beneficial in environmental protection. Jia et al.[50] had conducted a similar research with different maize stalk retention in the same location, their research demonstrated the average CO_2 emissions were 14.02 mmol/m^2·h and 16.55 mmol/m^2·h for shattered maize stalk retention and standing maize stalk retention during the growing season, these numbers equal to 168.2 and 198.6 mg·C/m^2·h, respectively. Compared with our study, their values were a little higher than our peak values, which may illustrate crop residue cover is more dominant than tillage.

In this study, the SWC of NT was higher compared with SRT (Figure 4), especially during June and August[51]. Curiel et al.[52] confirmed that SWC enhances the efficiency of the soil carbon substrate, especially for the living organic fractions, and the soil carbon substrate is an import source for heterotrophic respiration, in other words, a greater SWC would result in increased SMR. If the other controlling factors were neglected, the higher SWC would be sufficient to validate the higher SMR in the NT system, but the soil porosity is also very critical for CO_2 diffusion. Plants need more water during the growing season than the other seasons, thus within an appropriate range, the higher of SWC, the more favorable for crop growth, especially for arid or semi-arid regions. It is the water retention reason, NT has been adopted more and more widely[7].

Guo et al.[53] reported that CO_2 diffusion takes place mostly in macropores. A higher SWC would reduce effective soil pores, which would limit SMR by hindering CO_2 diffusion[8,54]. If higher SWC will reduce effective soil pores hold true, the threshold of field saturated water holding capacity should be taken into consideration. Chen et al.[55] found arable soils in Northeast China to have a saturated water holding capacity of ca. 50% under conventional tillage, and Liu et al.[56] reported that conservation tillage improved the saturated water holding capacity significantly. NT and SRT belong to conservation tillage treatments, and the maximum SWC was below 45% in NT (Figure 4), so the SWC in this study was far from the saturated water capacity. To sum up, the higher SWC in NT did not reduce the effective soil porosity. O_2 fluxes are taken place mostly in macropores, which is essential for crop metabolism so as to grow[16]. Since NT leads to more macropores compared with SRT, NT may be more favorable for crop growth from the viewpoint of root O_2 supplement.

On the one side, the soil pore is a requirement for gas diffusion, one the other side, the soil pores provide the habitats for microorganisms, and Badin et al.[57] pointed out that soil porosity distribution could lead to changes in microbial community structure. More specifically, Sun et al.[58] concluded that macropores had positive correlation with fungi. Figure 8 shows that fungi and bacteria were significantly positive correlated with macropores. Owing to NT had higher percentages of macropores (Figure 5), the abundance of fungi in NT was more than that in SRT (Figure 6).

4.2 Influence of tillage on soil microbial biomass

This study observed NT resulted in more soil microbes (Figure 6) than SRT, and soil microbes are the major contributors to decompose organic matters, so as to release crop essential nutrients[19]. So if there is enough soil organic matter, NT maybe more favorable for crop growth from the viewpoint of nutrient supplement.

It was observed that the SRT resulted in fewer macropores and more mesopores than NT, which is in line with results that compaction and tillage practices disrupt soil pore continuity as well as mechanically breakdown of soil structures[59]. NT causes less soil disturbance; this favors formation of continuous biological macropores, which are the microhabitats of soil microbes. Besides that, Young et al.[60] insisted that tillage would damage the hypha, which may hinder nutrient transfer. Shukla et al. and So et al.[61,62] argued that NT favors soil aggregates formation, promoting hyphal growth[63]. In conclusion, both the physical and biological advantages of NT should increase SMR.

Guo et al.[9] found that soil bacteria and actinomycetes were positively correlated with soil moisture but negatively correlated with soil temperature in 0 to 50 cm soil layer. Fungi were positively correlated with soil temperature but negatively correlated with soil moisture in the same soil depth layer. But this study result showed that fungal abundance in soils under NT was higher than that of SRT (Figure 6), which is contradictory with Guo et al.[9]. Figure 8 indicates that the macropores were significantly correlated (p<0.01) with SMB and SWC was less significantly correlated (p<0.05) with SMB. In spite of this, RDA showed that the macropores affect SMB more strongly than SWC.

4.3 Soil microbial respiration and soil microbial biomass

Many studies report that increased SMB results in higher SMR[21,64,65]. Figure 6 shows that NT had more SMB than SRT, so we assume that NT had higher SMR compared with SRT in this study. As shown in Figure 8, SMR was more dependent on total PLFA, thus this study concludes that SMB is a major control of SMR. At the same time, Birge et al.[22] pointed out that available soil organic matter, rather than a lack of microbial biomass limits soil respiration. But under conservation tillage, substantial crop residues were returned into the field in this study, so there was sufficient soil organic matter to supply SMR. Under the assumption of no substrate limitation, SMB and SMR should be correlated.

4.4 Soil enzymatic activity

Both soil microbes and plant roots can generate soil enzymes. As root trenching was utilized, the soil enzyme should merely generate from soil microbes[66]. NT led to higher SMB, so NT should have higher soil enzyme content, but this study did not observe this. In addition, as Supplementary material 1 shows there was no significant correlation between soil enzymatic activity and SMB. In fact, biochemical reactions do not require a high enzyme content[25], so even there is enough SMB and soil organic matter, it does not mean the higher of soil enzyme can benefit crop production. On the other side, when soil enzymes meet the demand for soil microbial metabolism, soil enzyme secretion should be limited. Weintraub and Schimel[67] observed the accumulation of soil extractable enzyme in late summer in Arctic soils, they concluded that it was the result of enzyme activity in excess of enzyme demand, which indicated that inducible enzyme production will stop once meeting the requirement. Schimel and Weintraub[68] conducted a decomposition modelling study, their study incorporated enzyme as the agents for organic matter breakdown, at last, they concluded that microbes would allocate a minimum of 2% of assimilate carbon to enzyme production to sustain biomass. This study returned maize stalks to fields after harvesting, provided enough carbon substrate to sustain SMB[69]. We speculate that soil enzyme in both two treatments meet the demand, explaining the lack of a correlation of soil enzyme content and SMR, as shown in Figure 8.

Both NT and SRT are widely used in China, NT is more cost-effective for agricultural production, at the same time, NT is more favorable for SWC retention, soil macroporosity and SMB, these parameters may contribute to crop production. But SRT improves soil temperature slightly, which may also do some contribution to crop growth. From the perspective of crop yield, farm managers should evaluate NT and SRT carefully. More importantly, SRT provides better conditions for less SMR. Therefore, SRT should be adopted preferentially if from the perspective of environmental issues.

5 Conclusions

This work provided a novel insight into the relationship between tillage treatments and soil microbial respiration (SMR). The hypothesis was supported by observing variations in soil physical and biochemical factors of two treatments. No tillage (NT) has unique advantages in forming macropores and sustaining soil water. These advantages lead to suitable microhabitats for increasing SMB. Owing to the correlation of SMB and SMR, the NT system generated higher SMR during growing seasons compared to spring ridge tillage (SRT).

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