

EFFECTS OF LONG-TERM CONSERVATION TILLAGE ON SOIL NITROGEN CONTENT AND ORGANIC NITROGEN COMPONENTS IN A CHINESE MOLLISOL

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Abstract. The effect of different long-term (2001-2016) tillage practices on soil nitrogen forms in different soil layers were studied to provide a scientific basis for evaluating soil fertility and establishing rational fertilization measures. Soil contents of total nitrogen (TN), particulate organic nitrogen (PON), microbial biomass nitrogen (MBN), water soluble organic nitrogen (WSON), NO₃-N content, NH₄-N content ammonium nitrogen and organic acid nitrogen component contents were measured under no-tillage (NT) and mould plow (MP) treatments based on a long-term (16 year) conservation tillage experiment in Northeast China. The results showed that no-tillage increased soil total nitrogen, active nitrogen, inorganic nitrogen and organic nitrogen content, which varied with soil depth. Compared with MP, NT significantly ($p < 0.05$) increased total nitrogen and active nitrogen content in the surface soil (0-30 cm); TN, PON, MBN and WSON increased significantly ($p < 0.05$) by 28.32%, 23.07%, 15.13% and 25.21% in 0-5 cm and by 10.17%, 19.4%, 15.97%, and 31.33% in 5-10 cm, respectively. For soil inorganic nitrogen, the content of ammonium nitrate at 0-5 cm and at 5-10 cm under NT was significantly ($p < 0.05$) higher than that of MP by 28.1% and 32.13%, respectively, and the content of ammonium nitrogen in soil at 0-5 cm was significantly ($p < 0.05$) higher, by 12.86%, than that of MP. No-tillage significantly increased the content of total hydrolysable nitrogen, hydrolysable ammonia nitrogen, hydrolysable amino acid nitrogen and hydrolysable unidentified nitrogen in the 0-10 cm soil layer, but no significant effect was found on the content of hydrolysable amino sugar nitrogen. Soil nitrogen content was closely related to tillage practices. These results suggest that no-tillage was beneficial in augmenting soil nitrogen supply capacity by increasing soil total nitrogen, active nitrogen, inorganic nitrogen and organic nitrogen content.

Keywords: *no-tillage, mould plow, soil nitrogen forms, straw returning, Northeast China*

Introduction

Soil nitrogen (N) is one of the necessary nutrients for plant growth and development, as well as a major nutrient limiting factor for crop yield (Gao et al., 2008; Karra et al., 2018; Lenssen et al., 2007; Sainju et al., 2009). Soil organic and inorganic nitrogen are the main forms of the nitrogen pool. Soil organic nitrogen content of approximately 70%-90% in surface soil could not only maintain soil nitrogen fertility (Xu et al., 2003), but also has an important function for soil nitrogen supply capacity (Ju et al., 2004). Soil inorganic nitrogen pools with ammonium and nitrate, is the most effective nitrogen pool type for crop absorption (Mkhabela et al., 2008). Approximately 50% of the

accumulated nitrogen in crops comes from soil, especially for the black soil of Northeast China where more than 70% of the plant's accumulated nitrogen comes from the soil (Fuzzi, 1996; Ballb et al., 1999). Therefore, soil N mineralization from organic to inorganic N is the key process for crop absorbing and utilizing N.

Tillage practices have a great impact on soil N availability and nitrogen reserves. Intensive agricultural systems may deplete soil N pools by removal of the above ground portion of the plants, which may result in negative nutrient balance depleting soil fertility. Conservation tillage with returned straw could improve the soil aggregate structure, maintain soil moisture, reduce water runoff and evaporation, increase water infiltration, stabilize soil temperature changes, promote the reproduction of beneficial microorganisms and regulate nutrient metabolism, thus affecting the mineralization and transportation of soil N and plant absorption and utilization (Patra et al., 1993; Ram et al., 2003; Barajas-Guzmán et al., 2006). Therefore, it is critical to understand the dynamic and cycling of the soil N pools and their fractions under different agriculture management styles. Previous studies have shown that compared with traditional tillage, conservation tillage changed the soil microenvironment, thus affecting the mineralization and content of soil N, such as no-tillage significantly increasing the total N content in the 0-5 cm soil layer (Lou et al., 2012; Varvel et al., 2011; Sainju et al., 2009; Dikgwatlhe et al., 2014). However, the soil total N pool changes slowly responding to the management practice changes because of its large size, inherent spatial variability, and small fraction available to plants. Therefore, more attempts have been made to labile fractions as plant-available N (Wander, 2004; Haynes, 2005), which is the most active component of soil organic N. Particulate organic nitrogen (PON), microbial nitrogen (MBN) and water-soluble organic nitrogen (WEON) are the important active N pools, which play an essential role in the process of soil N cycle (Jones et al., 2004). No-tillage (NT) may temporarily increase N immobilization leading to reduced plant-available N, while long-term NT can also increase soil labile N pool to improve the availability of N to crops in the upper soil layers (Sun et al., 2015; Sainju et al., 2013).

The mollisol area in Northeast China is the main grain-producing area in the country. With the increment of reclamation years and predatory production, fertility degradation of the mollisol has become more and more prominent. It is now considered urgent to change the traditional farming methods and carry out conservation tillage as an effective means of preservation. Several previous studies have focused mainly on soil total nitrogen content change under conservation tillage, but focused less on soil active nitrogen, inorganic nitrogen and organic nitrogen components. Thus, in the current study, soil organic nitrogen contents between conservation tillage and conventional tillage were analyzed based on a long-term conservation experiment started in 2001. This involved analysis of soil nitrogen and organic nitrogen components under protective tillage and conventional tillage conditions, with a view to evaluating soil fertility, formulating reasonable fertilization measures and providing a scientific basis for sustainable development of agriculture.

Material and methods

Study site

The study was conducted on Dehui Experimental Station (44°12'N, 125°33'E) of the Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, in

Dehui County, Jilin Province, China. The climate is described as a temperate continental monsoon; based on data from the past 30 years, the annual mean air temperature is 4.4 °C, and the annual mean precipitation is 520.3 mm, with more than 70% of the rain occurring in June, July, and August. The winter (November–April) is long and cold. The soil is a clay loam (Typic Hapludoll) unusually covered with snow in winter. More details about the soil can be found in *Table 1* (Chen et al., 2018). Conventional management practices were used on the land to grow a monoculture of maize for more than 20 years before the establishment of this tillage experiment.

Experimental design

The present study was conducted using monoculture maize in a long-term (2001–2016) tillage field experiment. The tillage and rotation treatment are as follows. The tillage treatments consisted of no-tillage (NT), mould plow (MP) tillage and ridge tillage (RT), with two crop rotations (continuous maize (*Zea mays L.*), and maize-soybean (*Glycine max L.*). All the treatments were arranged in a randomized complete block design with four replicates at the whole plot level, and each plot was 5.2 m × 20 m. The NT treatment had no soil disturbance except for planting, usually in early May. The RT treatment included ridging in June and less than one-third row width smashing of maize stalks/roots in fall. The MP treatment included one approximately 15 cm in depth moldboard plowing after maize harvest, generally in early October, and one 7.5 to 10 cm in depth disking during the spring, as well as field cultivation (ridging in June). Herbicides were applied before and after seeding for all the tillage treatments to control weeds. Starter fertilizer (100 kg·ha⁻¹ N, 45.5 kg·ha⁻¹ P and 78 kg·ha⁻¹ K) and an additional 50 kg·ha⁻¹ N at the V-6 stage were applied for maize. Starter fertilizers (40 kg·ha⁻¹ N, 60 kg·ha⁻¹ P and 80 kg·ha⁻¹ K) were applied concurrently at the time of planting for soybean. The crop residues were returned onto the soil surface after harvest under NT and RT treatments with the exception of the plots under MP.

Table 1. Selected soil physical and chemical properties in 2001 prior to the tillage experiment (Chen et al., 2018)

Depth (cm)	pH	Clay (%) (<2 µm)	Silt (%) (2-20 µm)	Sand (%) (20-200 µm)	Bulk density (g/cm ³)	Soil organic carbon (g/kg)	Total soil nitrogen (g/kg)
0-5	6.48	36.03	24.00	39.97	1.24	16.48	1.42
5-10	6.45	35.83	23.78	40.39	1.38	16.29	1.39
10-20	6.51	35.68	24.35	39.98	1.36	16.08	1.37
20-30	7.03	36.56	25.00	38.72	1.38	14.22	1.16

Soil sampling and measurements

Soil samples (7 replicates per plot) were collected at depths of 0-5 cm, 5-10 cm, 10-20 cm and 20-30 cm with S-type sampling on 23 April, 2017. The collected samples were divided into two parts, one was stored at 4 °C as a fresh sample for determination of microbial biomass nitrogen (MBN) and water-soluble organic nitrogen (WSO), and the other was air-dried for soil total nitrogen (TN), organic nitrogen components, etc. Soil total nitrogen was determined by an elemental analyzer (Flash EA1112, Thermo Finnigan, Italy). Particle organic nitrogen (PON) was extracted first and then analyzed

by elemental analysis (Cambardella et al., 1992). Soil water-soluble organic nitrogen was determined by the TOC-VCPH automatic analyzer (Model TOC-VCPH, Shimadzu, Tokyo, Japan) (Gigliotti et al., 2002). Soil microbial nitrogen (MBN) was determined by a modified CHCL₃ fumigation-extraction method (Vance et al., 1987), and the extractions were analyzed by the automatic TOC-VCPH analyzer. Soil organic nitrogen components were determined according to Bremner's method (Bremner, 1965). Soil inorganic nitrogen was determined by a fully automatic continuous flow analyzer (SKALAR SAN ++, The Netherlands).

Data analysis

Statistical analyses were carried out using SPSS (Version 11.5, SPSS Inc., Chicago, IL, USA). Treatment means were compared using the least significant difference (LSD) and a significance level of $P < 0.05$.

Results

Total nitrogen content

Compared with MP, NT significantly ($p < 0.05$) increased the total nitrogen content by 28.32% and 10.17%, in the 0-5 cm and 5-10 cm soil depth intervals, respectively (Fig. 1). While the total nitrogen content of the 10-20 cm and 20-30 cm layers under NT treatment was slightly lower than that of MP, the difference was not significant. Under both NT and MP, soil total N decreased with increased soil depth.

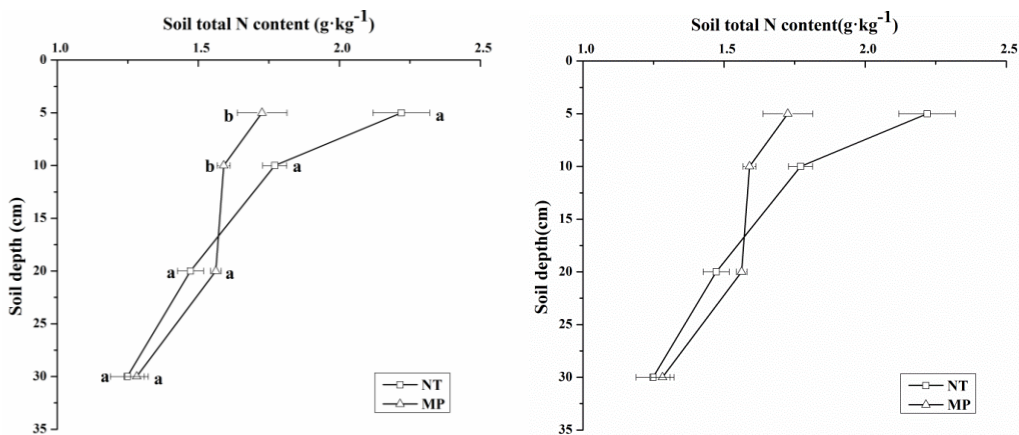


Figure 1. Soil total nitrogen at different soil depths under different tillage practices ($P < 0.05$). (NT: no tillage; MP: mould plow. Different letter indicates significant difference of soil total N content between different tillage practices at the same depth)

Active nitrogen

NT significantly ($p < 0.05$) increased soil active nitrogen in the 0-5 cm and 5-10 cm soil layers more than MP (Fig. 2). Particulate organic nitrogen increased by 23.07% and 19.4%, microbial biomass N increased by 15.13% and 15.97%, and soil soluble organic nitrogen increased by 25.21% and 31.33% in the 0-5 cm and 5-10 cm soil layers, respectively (Fig. 2). No significant differences were found between NT and MP for

PON, MBN, and WSON in the 10-30 cm soil layer. With the increment of soil depth, PON, MBN, and WSON showed a decreasing trend.

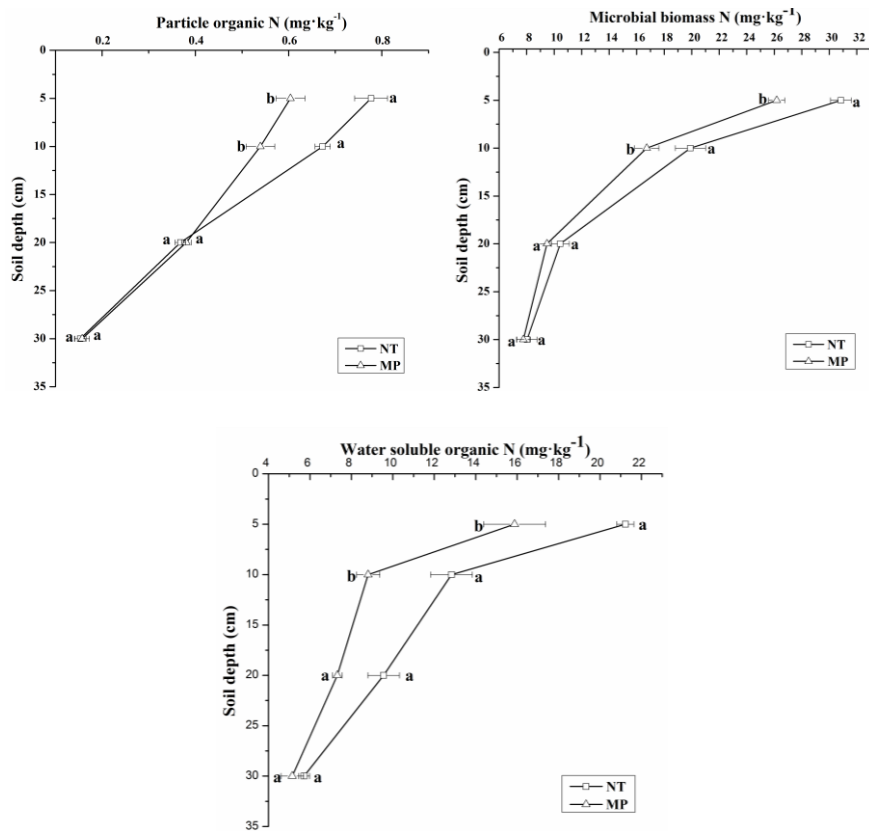


Figure 2. Depth distributions of particulate organic N, microbial biomass N and water soluble organic N under different tillage practices ($P < 0.05$). (NT: no tillage; MP: mould plow. Different letter indicates significant difference of particulate organic N, microbial biomass N and soluble water soluble organic N between different tillage practices at the same depth)

Soil inorganic nitrogen

The $\text{NO}_3^- \text{N}$ content in the 0-5 cm and 5-10 cm soil layers under NT were 28.10% and 32.13%, significantly ($p < 0.05$) higher than that of MP, respectively (Fig. 3).

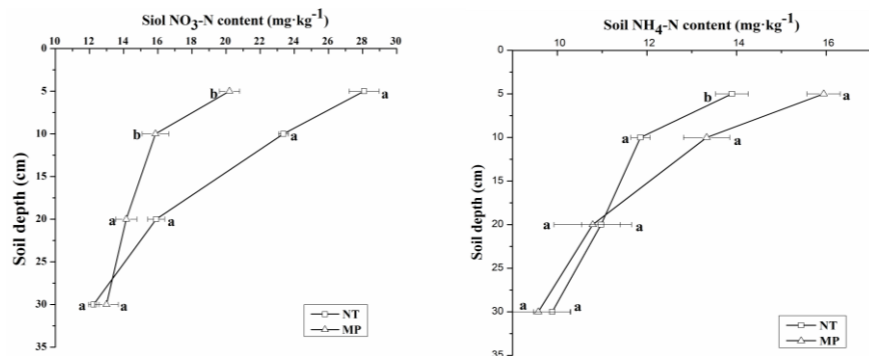


Figure 3. Depth distributions of soil $\text{NO}_3^- \text{N}$ and $\text{NH}_4^- \text{N}$ under different tillage practices ($P < 0.05$). (NT: no tillage; MP: mould plow. Different letter indicates significant depth difference of soil $\text{NO}_3^- \text{N}$ and $\text{NH}_4^- \text{N}$ between different tillage practices at the same depth)

The $\text{NH}_4\text{-N}$ content in the 0-5 cm soil layer under MP was 12.86% significantly ($p < 0.05$) higher than that of NT, the $\text{NH}_4\text{-N}$ content under MP in the 5-10 cm soil layer was also higher than NT although the difference was not significant. There was no significant difference between NT and MP for $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ in the 10-30 cm soil layer. With the increment of soil depth, the content of nitrate nitrogen and ammonium nitrogen decreased.

Soil organic nitrogen components

NT significantly ($p < 0.05$) increased total hydrolysable nitrogen content, hydrolysable ammonia nitrogen content, and hydrolysable amino acid nitrogen in topsoil (0-5 and 5-10 cm) more than MP (Table 2). Compared with MP, total hydrolysable nitrogen content increased by 22.03%, and 9.67%, hydrolysable ammonia nitrogen increased by 22.24% and 19.67%, and hydrolysable amino acid nitrogen increased by 21.48% and 22.95% in the 0-5 cm and 5-10 cm soil layers, respectively (Table 2). No significant difference was observed in the 10-30 cm soil layers for the hydrolysable nitrogen. Tillage practices had no significant effect on the content of hydrolysable amino sugar nitrogen. The content of hydrolysable amino sugar nitrogen in the 10-30 cm soil layer was slightly lower than MP, but the difference was not significant. The hydrolysable unidentified nitrogen content in the 0-5 cm soil layer of the NT treatment was 30.87% significantly ($p < 0.05$) higher than that in MP, while the differences between the two nitrogen types for NT and MP in the 5-30 cm depth interval were not significant. The differences of all types of nitrogen between NT and MP in the 10-30 cm soil layer were not significant.

Table 2. Soil organic nitrogen components under different tillage practices

Soil depth (cm)		0-5	5-10	10-20	20-30
Total hydrolysable nitrogen	NT	1438.76a	1249.49a	957.18a	812.79a
	MP	1121.87b	1038.37b	1015.78a	839.70a
Hydrolysable ammonia nitrogen	NT	587.23a	510.45a	412.49a	348.84a
	MP	484.31b	410.01b	437.46a	357.63a
Hydrolysable amino sugar nitrogen	NT	44.21a	35.54a	29.82a	24.29a
	MP	34.13a	31.72a	31.68a	24.87a
Hydrolysable amino acid nitrogen	NT	482.82a	450.74a	294.44a	250.21a
	MP	379.11b	347.28b	344.20a	281.50a
Hydrolysable unidentified nitrogen	NT	324.51a	252.76a	220.42a	189.46a
	MP	224.32b	249.36a	202.44a	175.70a

Same letter means no significant difference under different layers and tillage practices for a single soil nitrogen property. NT: no tillage; MP: mould plow

Discussion

Effect of tillage on soil total nitrogen content

After 16 years of conversion from traditional farming to conservation tillage, the TN content in the 0-10 cm surface layer increased under no-till practices. Our results were consistent with other research, such as that no-tillage had shown that total nitrogen at 0-5 cm and 0-30 cm layers was higher than that for other tillage (Power et al., 1998;

Purakayastha et al., 2009). As a result of the NT straw surface coverage, soil disturbance decreased, leading to a reduction of total nitrogen mineralization and eventually an increase in total nitrogen (Franzluebbers et al., 1999). No-tillage straw also increased the soil C/N ratio by adding organic matter to the soil, thus enhancing the solid holding capacity of nitrogen (Kushwaha et al. al., 2000; Hemwong et al., 2008; Zavalloni et al., 2011). The total nitrogen content both in NT and MP decreased with increases in soil depth. This was due to the accumulation of organic matter in the surface NT soil. In the 10-30 cm soil layer, total nitrogen content under MP treatment was higher than NT (*Fig. 1*). For this phenomenon, underground biomass may play an important role. In traditional farming, the stubble was turned into the deeper layer of soil. However, in NT, the root system showed a high density in the soil surface and a low density in the deep soil (Qin et al., 2006). Therefore, different subsurface biomass in NT and MP may cause the difference of total nitrogen in various soil layers between treatments. The results of the present study are consistent with other findings; no-tillage straw returning to the soil led to accumulation of soil nitrogen on the soil surface (Torbert et al., 1995; Thomas et al., 2005).

Effect of tillage on soil active nitrogen

Soil particulate organic nitrogen (PON) was considered as a stable part of soil organic nitrogen, as it could be converted into inorganic nitrogen, which could then be used to supply nitrogen sources for plant growth and crop yield, and maintain soil nitrogen balance (Rosell et al., 2000). PON was sensitive to farmland management practices such as soil disturbance and crop straw inputs; therefore, it responded quickly to management practice changes (Cambardella et al., 1992). These results showed that conservation tillage mainly increased the PON content in the 0-5 cm and 5-10 cm soil layers, which is consistent with other related studies (Fabrizzi et al., 2003; Bessam et al., 2003; Malhi et al., 2006; Sainju et al., 2012). There are three reasons for this. First, no-till straw returning could provide a good environment for soil aggregate formation, and a large amount of PON could be physically protected in soil aggregates. Second, cultivation such as autumn plowing could disturb the soil and destroy soil aggregates, thus accelerating PON mineralization (Wander, 2004). Third, the location of crop straw returning may affect the distribution of PON. In the NT treatment, the straw covered the soil surface. However, in the MP treatment, straws were buried in the soil stubble in more close contact with the soil. The variable distribution of straw returning to the field could explain that the PON content in NT treatment was significantly higher than MP in the 0-10 cm soil layer. To summarize, the results showed that no-tillage straw returned to the soil helps increase soil PON content.

Soil microbial biomass nitrogen was the source and reservoir of nitrogen nutrients and an important biological indicator of soil quality (Shukla et al., 2017). In the present study, conservation tillage significantly increases soil microbial biomass nitrogen. This may be due to the higher total nitrogen content in conservation tillage soils, which provided a higher material basis for MBN formation. The positive impact of NT on soil microbial communities could also be attributed to the reduction of soil disturbance, which led to the reduction of fungal mycelium damage, the conservation of microbial habitat, the improvement of soil moisture conditions and the reduction of extreme temperature conditions (Rhoton, 2000). In short, NT increased soil microbial and physic-chemical conditions, improved microbial habitat, disturbed soil less, and

provided relatively high levels of organic nitrogen, ultimately resulting in an increase in surface microbial biomass nitrogen.

Soil soluble organic nitrogen played an important role in the farmland nitrogen circulation system (Kranabetter et al., 2007). In this study, no-tillage straw surface coverage resulted in an increase in soil soluble organic nitrogen in the 0-10 cm soil layer. The disturbance of mould plow tillage soils improved the aeration of the soil and destroyed the soil aggregates, thus accelerating the mineralization of PON. Therefore, the soil soluble organic nitrogen tended to decrease, resulting in the decrease in the surface layer content. Meanwhile, no-tillage straw covering the surface could improve soil moisture and boost nitrogen fixation.

Effect of tillage on soil inorganic nitrogen

Soil inorganic nitrogen played an important role in the forms of nitrate nitrogen and ammonium nitrogen in the soil nitrogen cycle, and was the main form of crop absorption and utilization, although only a minor portion of the total nitrogen (Nguyen et al., 2017). Halvorson found that the $\text{NO}_3\text{-N}$ content in mould plow tillage in the northern plains of the United States was higher than that of no-tillage systems (Halvorson et al., 2000). In this study $\text{NO}_3\text{-N}$ content in 0-10 cm soil under NT layer was significantly higher than that of MP, which is not consistent with this finding, which may be related to different crop types, tillage years, soil types and planting systems. Our results showed that no-tillage with straw returned to the soil would increase the number and continuity of macropores in the topsoil and may cause a large amount of $\text{NO}_3\text{-N}$ to be stored in the surface soil (Li et al., 2007). At the same time, no-tillage could effectively store soil moisture and reduce $\text{NO}_3\text{-N}$ leaching to the lower soil. In addition, the straw covered the surface of no-tillage soil, which increased soil nitrogen fixation, thereby reducing the loss of $\text{NO}_3\text{-N}$. In contrast, conventional farming led to large disturbances of surface soil with high porosity with $\text{NO}_3\text{-N}$ tending to move downwards. Sainju et al. (2013) found that the $\text{NH}_4\text{-N}$ content in MP was higher than in NT, which is consistent with our findings. Under dry land conditions, Li et al. (2007) found that no-tillage increased the quantity of earthworms in farmland and the number and continuity of macropores in the soil, thus improving soil aeration. In well-aerated soil, $\text{NH}_4\text{-N}$ was more easily transferred into $\text{NO}_3\text{-N}$, resulting in soil $\text{NH}_4\text{-N}$ content decreasing under no-tillage.

Effect of tillage on soil organic activated nitrogen components

Soil organic nitrogen is the source of mineral nitrogen for crops, and as the main form of soil nitrogen, it plays a key role in the stability of farmland ecosystems (Schulten et al., 1997). In this study, no-tillage significantly affected the total hydrolysable nitrogen, hydrolysable ammonia nitrogen and hydrolysable amino acid nitrogen in the 0-10 cm soil layer, and hydrolysable unidentified nitrogen in the 0-5 cm soil layer. The reason may be that no-tillage increased the total nitrogen content of soil, and the increase in total nitrogen would inevitably lead to the increase in soil hydrolysable ammonia nitrogen (Follett et al., 1989). Hydrolysable ammonia nitrogen was composed of inorganic exchangeable ammonium and part of the fixed ammonium composition, the mineralization of two ammonium after soil disturbance accelerated under conventional farming, and the absorption and utilization of crops to ammonia, led to the average content of hydrolysable ammonia nitrogen decreasing obviously; hence,

hydrolysable ammonia nitrogen in the 0-10 cm layer of mould plow tillage was significantly lower than no-tillage (Jiang, 2005). The addition of organic material under no-tillage increased the soil organic matter content, which was continuously converted into humus under the action of microorganisms. Soil amino acids nitrogen and other compounds of N were fixed into humic acid during the humification (Porter et al., 1964). Under acidic conditions, the amino acid nitrogen released, which made the higher hydrolysable amino acid nitrogen content of the 0-10 cm soil layer than that of conventional tillage. Hydrolysable unidentified nitrogen is a difficult component to mineralization in soil organic nitrogen pools of acidolysis, and the mineralization rate of unknown nitrogen of acidolysis is very small, so it is easy to accumulate in soil (Stevenson, 1982). The increase in total nitrogen in the surface layer under no-tillage also leads to the increase in hydrolysable unidentified nitrogen. Tillage practices have no significant effect on hydrolysable amino sugar nitrogen. Since hydrolysable amino sugar nitrogen is mainly derived from residues of microbial cell walls, and hydrolysable amino sugar nitrogen reflects the accumulation of dead microorganisms rather than live microorganisms. Therefore, hydrolysable amino sugar nitrogen is a very small percentage of soil total nitrogen, and it is less affected by tillage practices (Stevenson, 1982). Liu's preliminary studies conducted in the same site reported that the soil organic nitrogen mineralization plays an important role in the nitrogen nutrition of crops. The total soil nitrogen mineralization rate in no-tillage has a significant stratification phenomenon. The contribution of no-till active organic nitrogen to total mineralization rate (75-89%) was significantly greater than that of conventional tillage (31-45%), indicating that tillage practices will affect the source of soil organic nitrogen mineralization. The degree of humification, humic acid content, and aromaticity of organic matter in long-term no-tillage mollisol were significantly lower than that of mould plow tillage, so the mineralization of active organic nitrogen pool contributed more to the total primary mineralization rate. This means that much more organic nitrogen in no-till agecan be converted into inorganic nitrogen and absorbed by plants (Liu et al., 2017).

Conclusion

The results of this study showed that the effects of conservation tillage on the soil nitrogen pool occurred in the surface layer by significantly increasing the contents of total nitrogen, particulate nitrogen, microbial biomass nitrogen, water-soluble organic nitrogen, nitrate nitrogen and acidolysis of organic nitrogen components. In sum, no-tillage was suitable to increase the accumulation of nitrogen in dry farming areas, which would provide a theoretical basis for optimizing mollisol management and sustainable development of agriculture with rational fertilizing in Northeast China.

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