# Effects of long-term fertilization and cropping regimes on total nitrogen and organic nitrogen forms in a Mollisol of Northeast China

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**ABSTRACT**

A 22-year (1990–2012) field experiment was conducted to assess the effects of different fertilization and cropping regimes on the quantitative changes of total N and organic N forms in the topsoil (0–20 cm) and subsoil (20–40 cm) of a Mollisol in Northeast China. This study included nine fertilizer treatments on maize monoculture [no fertilizer (CK), N, NP, NK, PK, NPK, NPK combined with maize straw (NPKS), NPK combined with pig manure (NPKM), and 1.5' the rate of NPKM (1.5NPKM)] and one fertilizer treatment with maize-maize-soybean rotation (NPKMR). Compared with the CK treatment, the application of mineral fertilizers alone or in combination with straw generally had no significant effect on the contents of total N and organic N forms, whereas the combined application of mineral fertilizers with manure significantly increased their contents. Manure levels and cropping regimes had no significant effect on the total N content in the topsoil. By contrast, the hydrolysable unknown N content significantly increased with the increasing manure levels, and the amino sugar N content was significantly lower in rotation than in monoculture treatment. Our results imply that manure application integrated with continuous maize cropping can be considered as an optimized strategy for improving soil fertility.

**Keywords**: acid hydrolysable nitrogen; long-term experiment; organic and mineral fertilization; continuous maize; maize-soybean rotation

Nitrogen (N) is a common limiting nutrient in crop production. The N content of soil has been used as a major soil fertility index (Xu et al. 2003). In most surface soils, more than 90% of total N occurs in organic forms. The contents of total N and organic N forms in soil are affected by fertilization and cropping practices (Ju et al. 2006, Jagadamma et al. 2008, Huang et al. 2009, Kaur and Singh 2014). However, previous studies have reported contradictory results in this respect.

Mollisol is an inherently fertile and productive soil. The Northeast China Plain is one of the four major Mollisol areas in the world. However, long-term intensive cultivation practices have resulted in a significant decrease of organic matter content in Mollisol (Liu et al. 2010). Adoption of appropriate fertilization and cropping regimes is vital for increasing or maintaining soil organic matter content. Studies in the past decades have focused primarily on the effects of long-term fertilization and cropping regimes on the organic carbon content in Mollisol (Zhu et al. 2007, Kou et al. 2012), but little is known about their effects on soil N content.

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This study aims at examining the effects of mineral (N, P, and K) and organic (manure and straw) fertilization on the total N content and the content and distribution of organic forms of N of soil in a long-term field experiment with continuous maize (*Zea mays* L.) cropping or maize-maize-soybean [*Glycine max* (L.) Merr.] rotation in a Mollisol of Northeast China.

## MATERIAL AND METHODS

**Experimental site, design and sampling**. The experimental site was established in 1990 at the Soil Fertility and Fertilizer Efficiency Long-Term Monitoring Base of Jilin Academy of Agricultural Sciences (43°30'N, 124°48'E), Jilin province, Northeast China. The mean annual temperature is 4–5°C, and the mean annual precipitation is 450–650 mm. Prior to this experiment, the field was homogenized by growing maize for 3 years without fertilization. The initial soil properties in the 0–20 cm layer at the start of the experiment were as follows: pH 7.60, organic C 13.2 g/kg, total N 1.40 g/kg, total P 1.39 g/kg, and total K 22.1 g/kg.

For this study, nine fertilizer treatments [i.e., no fertilizer (CK), N, NP, NK, PK, NPK, NPK combined with maize straw (NPKS), NPK combined with pig manure (NPKM), and 1.5’ the rate of NPKM (1.5NPKM)] on continuous maize monoculture, and one fertilizer treatment (i.e., NPKM) on maize-maize-soybean rotation (NPKMR) were examined. Each experimental plot covered 400 $m^2$ in a completely randomized design with three replicates. All mineral and organic fertilizers were applied based on equal total N (i.e., 165 kg N/ha), except for the PK and 1.5NPKM treatments (Table 1). The mineral N, P, and K fertilizers used were urea, calcium superphosphate, and potassium sulfate, respectively. The average compositions of pig manure and maize straw used were 46.9 g C/kg, 5.0 g N/kg, 1.7 g P/kg, 4.1 g K/kg and 239.5 g water/kg, and 435.4 g C/kg, 7.0 g N/kg, 0.7 g P/kg, 6.2 g K/kg and 81.2 g water/kg on a dry weight basis, respectively. All mineral P and K fertilizers and one-third of mineral N fertilizer were applied at seeding time, and the remaining mineral N fertilizer was top-dressed at a depth of 10 cm below the topsoil before jointing. The pig manure was applied as basal manure, and chopped (3–5 cm) maize straw was spread in furrows after top-dressing. Maize or soybean was sown in late April and harvested in early October. Aboveground crop residues were removed after harvesting, except for the added maize straw in the NPKS plot. The mean grain yield of maize during 1990–2012 under different fertilization treatments is listed in Table 1.

Soil samples were collected from the topsoil (0–20 cm) and subsoil (20–40 cm) after the maize harvest in 2012. In each replicate plot, soil samples from five locations were taken and then thoroughly mixed into a composite sample. The soil samples were air dried, milled, and sieved through a 2 mm sieve.

Table 1. Annual application rates of mineral and organic fertilizers (kg/ha) and mean grain yield of maize (kg/ha) during 1990–2012 in each treatment

| Treatment | N | P | K | Pig manure | Maize straw | Maize yield |
|---|---|---|---|---|---|---|
| CK | 0 | 0 | 0 | 0 | 0 | 3532 |
| N | 165 | 0 | 0 | 0 | 0 | 7002 |
| NP | 165 | 36.0 | 0 | 0 | 0 | 8614 |
| NK | 165 | 0 | 68.5 | 0 | 0 | 7952 |
| PK | 0 | 36.0 | 68.5 | 0 | 0 | 3882 |
| NPK | 165 | 36.0 | 68.5 | 0 | 0 | 8990 |
| NPKM | 50 | 36.0 | 68.5 | 23 000 | 0 | 9101 |
| NPKMR | 50 | 36.0 | 68.5 | 23 000 | 0 | 9237 |
| 1.5NPKM | 75 | 54.0 | 102.8 | 34 600 | 0 | 9713 |
| NPKS | 112 | 36.0 | 68.5 | 0 | 7500 | 9035 |

CK – no fertilization; R – maize/maize/soybean rotation; NPKM – NPK combined with pig manure; NPKMR – NPKM with maize-maize-soybean rotation; 1.5NPKM – 1.5' the rate of NPKM; NPKS – NPK combined with maize straw

**Analytical methods**. Total N was determined using the micro-Kjeldahl method. Organic forms of N were measured by the method described by Stevenson (1996). Treatment effects were analyzed using one-way ANOVA followed by *LSD* test at $P < 0.05$ (SAS 8.2, Cary, USA).

## RESULTS AND DISCUSSION

**Content of total nitrogen in soil**. The total N contents of soil under different fertilizer treatments are shown in Figure 1. The total N contents ranged from 1.34–2.65 g/kg in the topsoil and from 1.20–2.28 g/kg in the subsoil. Compared with the CK treatment, the application of mineral fertilizers alone or in combination with straw had no significant effect on the total N content in both topsoil and subsoil. There was also no significant difference between the NPK and other mineral fertilizer treatments. In contrast, the combined application of mineral fertilizers and manure significantly increased the total N content compared with the other fertilizer treatments. The results implied that the application of manure significantly increased the N supply potential of soil to the crop, which is in accordance with the findings of most previous studies (Ju et al. 2006, Sądej and Przekwas 2008, Huang et al. 2009, Hai et al. 2010, Tripathi et al. 2014). Meanwhile, the positive effect of animal manure in increasing the total soil N content was reported to exceed that of crop residues in other long-term experiments (Giacometti et al. 2013). However, previous studies have sometimes reported conflicting results. For example, some researchers found that the total N content was not significantly different between the treatments with mineral fertilizers alone and with mineral fertilizers combined with manure at two long-term experimental sites (Tong et al. 2009). Some other researchers indicated that the total N content was significantly higher in soil under mineral fertilizers alone than under the CK treatment (Sądej and Przekwas 2008, Tripathi et al. 2014). The conflicting results may be related to local soil qualities, fertilization patterns, and cropping systems.

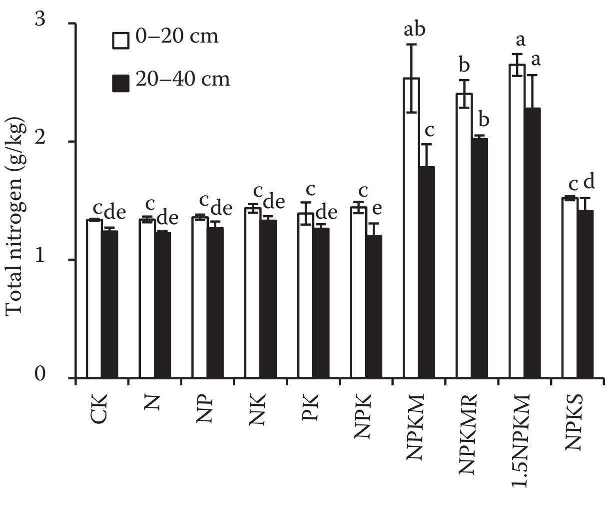


Figure 1. Total nitrogen contents of in the topsoil (0–20 cm) and subsoil (20–40 cm). Fertilizer treatments are described in Table 1. Vertical bars represent standard deviation of the mean ($n = 3$). Different small letters indicate significant differences at $P < 0.05$

Among the treatments with manure, the 1.5NPKM treatment exhibited the highest total N content. However, the significant differences between the 1.5NPKM and NPKM treatments and between the NPKMR and NPKM treatments were found in the subsoil rather than in the topsoil. These results suggested that manure levels and cropping regimes only significantly influenced the total N content in the subsoil.

**Content of organic nitrogen forms in soil**. The contents of different forms of organic N in soil under different fertilizer treatments are listed in Table 2. Compared with the CK treatment, the application of mineral fertilizers either alone or in combination with straw had no significant effect on the contents of most organic forms of N in both topsoil and subsoil. Only a few exceptions were observed. In the topsoil, the hydrolysable ammonium N under the NK treatment, the amino sugar N under the PK treatment, the hydrolysable unknown N under the NP treatment, and the total hydrolysable N under the NPKS treatment significantly increased compared with those under the CK treatment. In the subsoil, the hydrolysable ammonium N under the NP and NK treatments, the amino sugar N under the NK treatment, and the amino sugar N and amino acid N under the NPKS treatment were significantly higher than those under the CK treatment. In accordance with the present results, previous studies reported that long-term mineral fertilization generally had no significant impact on the content of different forms of organic N compared with the CK treatment (Huang et al. 2009).

For the mineral fertilizer treatments, the hydrolysable ammonium N was significantly lower and

Table 2. Contents of forms of organic nitrogen in soil (mg/kg)

| Treatment | Total hydrolysable N | $NH_3$ | Amino sugar | Amino acid | HUN fraction | Acid insoluble N |
|---|---|---|---|---|---|---|
| **Topsoil** (0–20 cm) | | | | | | |
| CK | 822[d] | 339[cd] | 56[e] | 242[b] | 185[e] | 516[b] |
| N | 882[cd] | 355[bcd] | 66[de] | 261[b] | 199[de] | 459[bc] |
| NP | 969[cd] | 374[bc] | 56[e] | 238[b] | 301[cd] | 389[c] |
| NK | 1012[cd] | 392[b] | 92[cde] | 312[b] | 216[de] | 423[bc] |
| PK | 1012[cd] | 317[d] | 125[bc] | 302[b] | 267[cde] | 380[c] |
| NPK | 960[cd] | 382[bc] | 68[de] | 298[b] | 212[de] | 483[bc] |
| NPKM | 1827[ab] | 555[a] | 159[ab] | 701[a] | 412[b] | 706[a] |
| NPKMR | 1705[b] | 555[a] | 102[cd] | 703[a] | 346[bc] | 697[a] |
| 1.5NPKM | 2005[a] | 602[a] | 171[a] | 711[a] | 521[a] | 643[a] |
| NPKS | 1085[c] | 368[bc] | 96[cde] | 363[b] | 257[cde] | 436[bc] |
| **Subsoil** (20–40 cm) | | | | | | |
| CK | 830[c] | 307[d] | 30[d] | 297[e] | 196[d] | 410[bc] |
| N | 870[c] | 318[cd] | 30[d] | 311[e] | 212[cd] | 357[c] |
| NP | 896[c] | 358[c] | 33[cd] | 288[e] | 216[cd] | 372[c] |
| NK | 950[c] | 356[c] | 62[ab] | 337[de] | 194[d] | 381[c] |
| PK | 886[c] | 321[cd] | 39[bcd] | 328[de] | 198[d] | 378[c] |
| NPK | 871[c] | 319[cd] | 35[cd] | 307[e] | 209[d] | 331[c] |
| NPKM | 1253[b] | 443[b] | 33[cd] | 470[c] | 306[ab] | 530[ab] |
| NPKMR | 1389[b] | 473[b] | 49[abcd] | 578[b] | 290[abc] | 631[a] |
| 1.5NPKM | 1702[a] | 584[a] | 65[a] | 788[a] | 265[bcd] | 578[a] |
| NPKS | 971[c] | 332[cd] | 55[abc] | 378[d] | 207[d] | 442[bc] |

Fertilizer treatments are described in Table 1. Different small letters indicate significantly differences at $P < 0.05$. HUN – hydrolysable unknown N

amino sugar N was significantly higher under the N-deficient (PK) treatment than under the NPK treatment in the topsoil. The amino sugar N under the P-deficient (NK) treatment was significantly higher than that under the NPK treatment in the subsoil. However, no significant difference was found between the K-deficient (N and NP) treatment and the NPK treatment.

In general, the application of mineral fertilizers combined with manure significantly increased the contents of various forms of organic N in soil compared with the application of mineral fertilizers alone, in agreement with previous results (Ju et al. 2006, Sądej and Przekwas 2008, Huang et al. 2009). However, Xu et al. (2003) found that the hydrolysable ammonium N content was lower in the treatments with than without manure application, which was due to the enhanced immobilization of mineral N by microorganisms during manure decomposition.

The contents of different organic forms of N were generally higher under the 1.5NPKM than under the NPKM treatment, and the significant differences were observed for the hydrolysable unknown N in the topsoil and for the total hydrolysable N, hydrolysable ammonium N, amino sugar N, and amino acid N in the subsoil. Therefore, the significantly higher total N content under the 1.5NPKM than under the NPKM treatment in the subsoil could be attributed to the translocation of labile organic N fractions into the deep soil layer during the long-term experiment.

The amino sugar N content was significantly lower in the topsoil under the NPKMR than under the NPKM treatment, which suggested that continuous maize monoculture was beneficial to the accumulation of microbial-derived organic matter in the topsoil. This could be explained by the higher maize residue carbon inputs in the treatment with maize monoculture than with maize-soybean rotation (Ding et al. 2011). On the other hand, the amino acid N content was significantly higher in the subsoil under the NPKMR than under the NPKM treatment, suggesting that the amino acid N was the main contributor of the total N content in the subsoil with rotation than with monoculture.

**Distribution of organic nitrogen forms in soil**. The proportion of the total hydrolysable N in total soil N ranged from 61.4–75.7%. In the total hydrolysable N, the hydrolysable ammonium N and amino acid N showed the highest proportion (21.9–28.2% and 17.5–34.8% of total N, respectively), followed by hydrolysable unknown N (11.6–22.2% of total N), and amino sugar N (1.94–9.08% of total N) (Figure 2). The proportion of the hydrolysable ammonium N was generally higher than that of the amino acid N after the application of mineral fertilizers alone, whereas the reverse was true after the combined application of mineral fertilizers and manure or straw. Compared with the CK treatment, the application of mineral fertilizers alone increased the proportion of the hydrolysable ammonium N, whereas the combined application of mineral fertilizers and manure or straw decreased its proportion. In general, the proportion of the amino sugar N was not different among the different fertilizer treatments. The proportion of the amino acid N increased after the application of mineral fertilizers alone compared with the CK treatment, and the application of mineral fertilizers in combination with manure or straw gave higher proportion than the application of mineral fertilizers alone. The proportion of the hydrolysable unknown N generally increased, whereas that of the acid insoluble N decreased in treatments with mineral fertilizers either alone or in combination with manure or straw, as compared to the CK treatment. Manure levels and cropping regimes had no effect on the proportion of different forms of organic N in the topsoil. However, the proportion of the amino acid N increased and that of the hydrolysable unknown N decreased with increasing manure levels in the subsoil. Moreover, the proportion of the hydrolysable ammonium N was lower in the subsoil with rotation than with monoculture. In previous studies, Ju et al. (2006) showed that the long-term application of mineral fertilizer or manure had no significant influence on the proportions of different organic N forms in the topsoil. However, Xu et al. (2003) indicated that the long-term application of combined manure and mineral fertilizers significantly decreased the percentage of the hydrolysable ammonium N, which was consistent with our result. However, they found that the percentage of the total hydrolysable N and hydrolysable unknown N decreased and that of the acid-insoluble N significantly increased, which was contrary to our result.

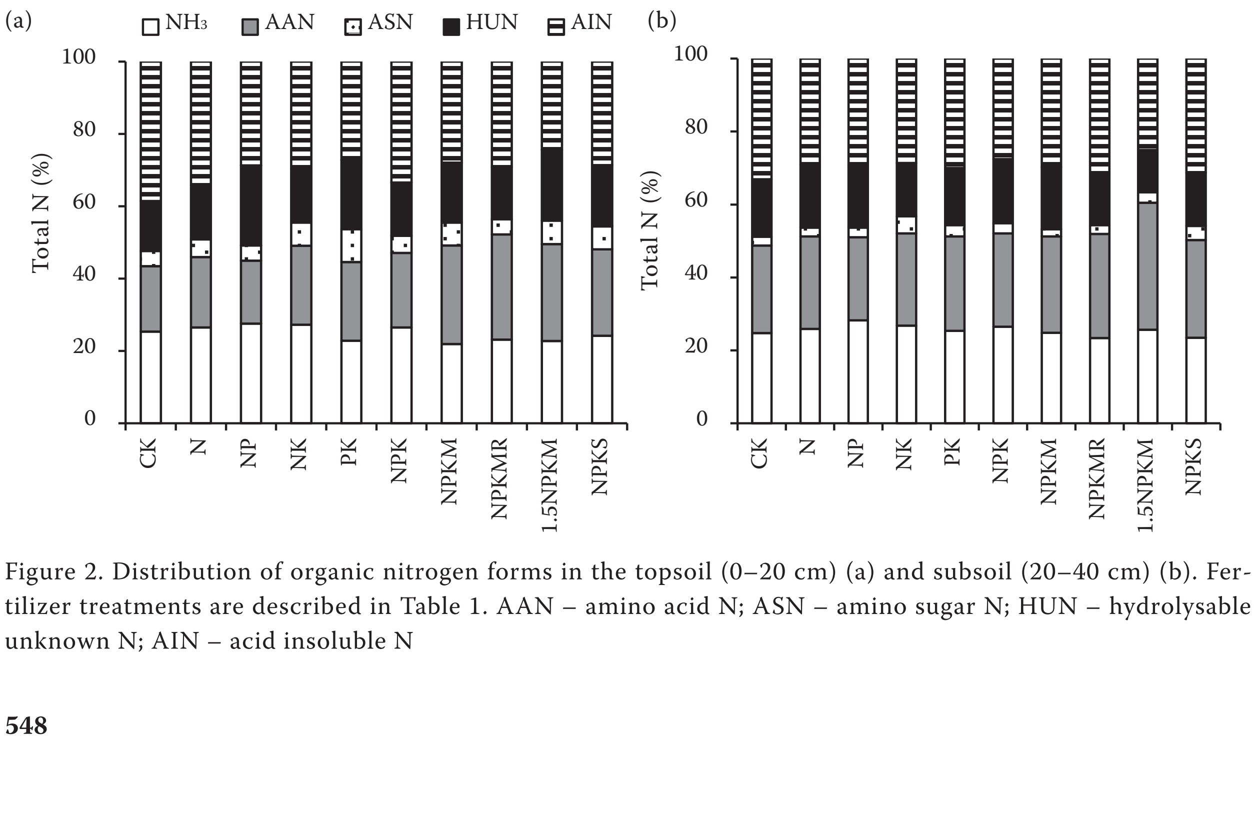


Figure 2. Distribution of organic nitrogen forms in the topsoil (0–20 cm) (a) and subsoil (20–40 cm) (b). Fertilizer treatments are described in Table 1. AAN – amino acid N; ASN – amino sugar N; HUN – hydrolysable unknown N; AIN – acid insoluble N

In conclusion, the long-term application of manure in combination with mineral fertilizers significantly increased the contents of total N and most organic N forms in the topsoil and subsoil. Meanwhile, maize monoculture provided a significantly higher amino sugar N content compared with maize-soybean rotation in the topsoil. Therefore, manure application integrated with continuous maize cropping can be considered as an optimized strategy for improving soil fertility.

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