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Relationships between N mineralization of soil organisms and soybean yield in conservation tillage systems

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Abstract

It is increasingly being recognized that conservation tillage systems favoring rich and abundant soil organisms can achieve optimal crop production by increasing nitrogen (N) mineralization. However, our understanding of the role of soil organisms in N mineralization promoting plant growth remains limited. In this study, the relationship between N mineralization of soil organisms and soybean (*Glycine max* Merr.) yield was investigated under a long-term (initiated in 2001) tillage trial, comprising conventional tillage (CT), ridge tillage (RT) and no tillage (NT). The amount of N released from soil organisms at 0-5 cm and 5-15 cm during the growing season of soybean was calculated using the monthly biomass data of soil microbes, nematodes, mites and collembolans, and the food web energetic model. The results showed that the soil food webs of RT and NT released more N than that of CT throughout the plow layer. Similar results were also observed for soybean yield which decreased in the order of NT > RT > CT. Multiple regression models revealed that soybean yield was significantly related to the mineralized N in RT and NT through fungal and plant channels in 0-5 cm and bacterial channel in 5-15 cm, demonstrating the role of spatial variability of soil organisms in linking N mineralization to plant growth. Furthermore, RT and NT significantly enhanced the N mineralization of trophic feeding guilds in these energy channels, which is beneficial in providing sufficient N to plants. Our results suggest that different soil organisms dominate at different depths in driving N mineralization and plant growth, and that the enhanced N mineralization of soil organisms is a cornerstone for conservation tillage systems to

achieve the optimal crop productivity.

Key words: conservation tillage, soil food web energetic approach, organism biomass, energy channels, soil N supply

1. Introduction

Nitrogen (N) is the most important growth-limiting nutrient for crops (Fageria et al., 2010). In order to achieve the maximum yield, N fertilizer is applied to crops all over the world; even legumes that fix N through symbiotic N-fixing microorganisms require additional chemical N application for maximum yield. However, globally, the N recovery rate by crops is only about 60% (Liu et al., 2010), which means that the rest of the fertilizer N is not available for the crop and is lost from the agroecosystems, resulting in undesirable environmental consequences. It is increasingly being recognized that exploiting the role of soil organisms in N mineralization is a promising approach to reduce the heavy dependence on N fertilizer without compromising the crop yield (Wall et al., 2015).

The process of N mineralization mediated by soil organisms is closely related to the predation in the food webs because soil organisms require carbon (C), N and other nutrients from the prey to support their metabolic activities (de Ruiter et al., 1993; Hunt et al., 1987). The N immobilized in the biomass of the lower trophic groups can be released by the predation of the higher trophic groups. Furthermore, the predators usually have a higher C:N ratio than their prey, which results in more N obtained than

73 their nutritional requirements, and the excess N is excreted into the soil (de Ruiter et
74 al., 1993; Hunt et al., 1987). It is estimated that the amount of N released by soil
75 organisms from predation accounts for 30%-80% of the annual N mineralization
76 under field conditions (de Ruiter et al., 1993; Holtkamp et al., 2011; Hunt et al., 1987;
77 Carrillo et al., 2016), and the value of this contribution varies with the biomass of soil
78 organism and the complexity of soil food webs (Carrillo et al., 2016; de Ruiter et al.,
79 1993; Holtkamp et al., 2011).

80 Conservation tillage, one of the most efficient practices to maintain optimal
81 productivity, has a prominent role in promoting the richness and abundance of soil
82 organisms (van Capelle et al., 2012). Several studies (Bender et al., 2015; Cole et al.,
83 2004; Thakur et al., 2014; Wagg et al., 2014) based on controlled (micro- or
84 meso-cosm) experiments found that the N mineralization of soil organisms increased
85 with the increase of soil biodiversity, which implies that a tillage system which forms
86 a complex soil food web is beneficial for releasing large amounts of N. However,
87 most of these cited studies have focused on the predation of microbial-feeding fauna
88 on microorganisms, and rarely consider the overall impact of all tropic levels of soil
89 organisms (bacteria, fungi, nematodes, mites and collembolans) on N mineralization.
90 As a result, our understanding of how the predation among soil organisms control the
91 N mineralization in the field is still limited.

92 Furthermore, relative to conventional tillage (CT), conservation tillage increases
93 the heterogeneity of soil organism distribution in the soil profile. For example,
94 bacteria and bacterivorous fauna dominate the whole plow layer of CT, while

conservation tillage is typically characterized by the fungi and fungivorous fauna near the surface and bacterial based communities at deeper soil depths (Hendrix et al., 1986; van Capelle et al., 2012). Moreover, conservation tillage also benefits by increasing the diversity of predaceous fauna since it reduces the tillage frequency. These changes in soil communities result in a more complex soil food web in conservation tillage, making it more difficult to understand the role of soil organisms in N mineralization promoting plant growth.

The objective of this study was to investigate the relationships between N mineralization of soil organisms and plant yield under contrasting tillage practices in a long-term (initiated in 2001) tillage trial. Soil food webs were composed of microbes, nematodes, mites and collembolans, and the amount of N released from soil organisms at each tropic feeding guild was quantified using the experimental data combined with the soil food web energetic model (de Ruiter et al., 1993). We hypothesized that (1) conservation tillage favors a greater release of N from soil organisms than CT, (2) soil organisms that play a key role in associating N mineralization and plant growth vary with soil depth in the conservation tillage system.

2. Material and methods

2.1 Experimental design and soil sampling

This study was conducted at the 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 station is located in a continental temperate monsoon zone. The soil is classified as Black soil (Typic Hapludoll, USDA Soil Taxonomy) with a clay loam texture. Tillage experiment was established in the fall of 2001 and included conventional tillage (CT), ridge tillage (RT) and no tillage (NT) with a two year maize (*Zea mays* L.) - soybean (*Glycine max* Merr.) rotation system. Each treatment had four replications. The soybean phase of the two-year maize-soybean rotation was sampled in 2015 in the present experiment.

Briefly, CT practice consisted of fall mouldboard plowing (20 cm) followed by the secondary seedbed preparation in the spring by disking (7.5-10 cm), harrowing and ridge-building. In RT, ridges were formed with a modified lister and scrubber and were maintained in June of each year with a cultivator. For the NT, no soil disturbance was practiced except for planting using a no-till planter. After harvest, the maize residue in the RT and NT plots was cut into about 30 cm pieces and left on the soil surface along with 30-35 cm standing stubble; soybean residue was directly returned to the soil surface. Residues in CT plots were removed prior to, and manually replaced on the soil surface after fall mouldboard plowing. Basal fertilizer was applied to the plots at rates of 40 kg N ha^{-1}, 60 kg P ha^{-1}, and 80 kg K ha^{-1}. The application rate of N is much lower than the local conventional application rate of 60 kg N ha^{-1}. Details of the experiment layout, tillage applications, crop rotations and fertilization were reported by Zhang et al. (2019).

Soil samples were taken at the end of each month from April to September during the soybean growing season when soil organisms are active. Seven soil cores

(2.5 cm in diameter) in each plot were randomly collected from a depth of 15 cm and each core was separated into 0-5 and 5-15 cm sections. Soil cores were combined to form a single composite sample for each plot and depth. Samples were immediately taken to the lab and stored at 4 °C. Soil bulk density for each plot was determined in the 0-5 and 5-15 cm depths using a slide-hammer probe with a 5 cm core diameter.

2.2 Soil mineral nitrogen and soybean yield

Soil mineral N was tested within 12 hours after soil samples were collected each month. Mineral N, including NO_3^- and NH_4^+, was extracted by 1 M KCl (soil : KCl = 1:2) and determined by a continuous flow analyzer (SAN++, Skalar, Netherlands).

Soybean yield was determined by hand-harvesting 3 m lengths of 6 interior rows from each plot after plants had reached the physiological maturity. Grain yield samples were dried to a constant weight at 75 °C in an oven, and then corrected to 13.5% grain moisture content.

2.3 Soil organism extraction

Soil organisms, including microbes, nematodes and microarthropods, were extracted from the soil taken from 0-5 cm and 5-15 cm depths within 2 weeks to obtain the reliable biomass data. All types of soil organisms were determined monthly except nematodes, which were only determined in April, June and August due to the limitation of labor. The nematode populations for non-sampled months were estimated by linear interpolation between adjacent sampling dates.

Microbial community was determined using the phospholipid fatty acid analysis (PLFA) as described by Bossio et al. (1998). Lipids were extracted from 8 g of freeze-dried soil with a Bligh and Dyer solution (chloroform: methanol: citrate buffer = 1: 2: 0.8 (v: v: v)). Polar lipids were separated from neutral lipids and glycolipids in a solid phase extraction column (Supelco Inc., Bellefonte, PA, USA) and transformed into fatty acid methyl esters with a mild alkaline methanolysis. Samples were then dissolved in hexane and analyzed in an Agilent 6850 series Gas Chromatograph with MIDI peak identification software (Version 4.5; MIDI Inc., Newark, DE, USA). Fatty acids were grouped as bacteria (14:0, i14:0, a14:0, 15:0, i15:0, a15:0, 15:1ω6c, 16:0, i16:0, a16:0, 16:1ω7c, 16:1ω9c, i17:0, a17:0, 17:1ω8c, 17:1ω9c,18:1ω7c, 18:0, 20:0), saprophytic fungi (18:1ω9c and 18:2ω6c) and arbuscular mycorrhizal fungi (AMF) (16:1ω5c) (Bach et al., 2010; Dempsey et al., 2013). Microbial biomass was estimated using the following conversion factors of fatty acid concentrations (nmol): bacterial biomass, 363.6 nmol = 1 mg C; saprophytic fungal biomass, 11.8 nmol = 1 mg C; and AMF biomass, 1.047 nmol = 1 μg C (Tsiafouli et al., 2015).

Nematodes were extracted from a 50 g soil sample (fresh weight) using a modified cotton-wool filter method (Liang et al., 2009). At least 100 nematode specimens from each sample were selected randomly and identified to genus level (see Table S1 for the list of identified taxa) using an Olympus BX51 microscope (OLYMPUS, Tokyo, Japan) according to Bongers (1994). Nematodes were assigned into four trophic groups: bacterivores, fungivores, plant-parasites and omnivores-predators (Ferris, 2010). Body length and maximum body diameter of

183 nematodes were measured using an ocular micrometer to calculate the nematode fresh
184 body mass (μg) (Andrássy, 1956). Nematode biomass was estimated by assuming that
185 the dry weight of a nematode is 20% of the fresh weight, and the C in the body is 52%
186 of the dry weight (Ferris, 2010).

187 Microarthropods were extracted from 200 mL fresh soil using modified
188 high-gradient Tullgren funnels (Crossley and Blair, 1991) for 120 h at room
189 temperature. Individuals were collected and stored in vials containing 95% ethanol for
190 identification. Mites and collembolans were identified to species or morphospecies
191 level (see Table S2 for the list of identified taxa) according to Christiansen and
192 Bellinger (1980-1981), Balogh and Balogh (1992), Bellinger et al. (2019), Pomorski
193 (1998) and Niedbala (2002). Soil microarthropods were allocated into four different
194 functional groups: fungivorous (oribatid) mites, predaceous mites, fungivorous
195 collembolans and omnivorous collembolans. Individual body length and width were
196 measured to estimate the dry weight based on regression equations from the literature
197 (Douce,1976; Hódar, 1996). Mite and collembolan biomass were estimated by
198 assuming the C in the body as 50% of the dry weight (Berg, 2001).

199 The unit of soil organism biomass was converted to mg C m^{-2} using soil bulk
200 density data. Taking into account the changes in abundance of soil organisms over
201 time, the biomass of soil organisms during the soybean growing season was estimated
202 by summing the monthly biomass.

203

204 **2.4 Modelling N mineralization of soil organisms**

Trophic feeding guild is defined as a group of species that exploit the same trophic resources (Burns, 1989). Before calculating the N mineralization of soil organisms, the identified soil organisms were first assigned into six functional feeding guilds: bacteria, fungi, herbivorous feeders, bacterivorous feeders, fungivorous feeders, and predaceous feeders to construct the structure of soil food webs (Fig. S1). Omnivorous-predaceous nematodes were assumed to feed on all other nematode groups (Yeates et al., 1993). Omnivorous collembolans, which mainly feed on bacteria, fungi, plant and microfauna (Barnes et al., 2014; de Vries et al., 2013), were proportionately assigned to bacterivorous, fungivorous, herbivorous and predaceous collembolans according to the assumption that their diet consists of 25% bacteria, 25% fungi, 25% plant and 25% other microfauna. The N mineralization of soil organisms was calculated with the food web energetic model (de Ruiter et al.,1993).

The calculation of N mineralization delivered by soil organisms is based on the assumption that the energy flowing into the biomass of a group is equal to the energy flowing out through natural death and predation. Following equations were used to calculate the N mineralization of soil organisms according to de Ruiter et al. (1993):

$$F_{ij} = \frac{w_{ij} B_i}{\sum_{k=1}^{n} w_{kj} B_k} \quad (1)$$

$$F = \frac{d_j B_j + P_j}{e_{ass} \times e_{prod}} \times F_{ij} \quad (2)$$

$$N_{min} = e_{ass} \times \left(\frac{1}{C:N_i} - \frac{e_{prod}}{C:N_j}\right) \times F \quad (3)$$

where, in equation 1, F_{ij} is the feeding preference of predator (j) on prey (i), which

was calculated based on the density independent feeding preference of j on i (w_{ij}, dimensionless; listed in Table S3), n is the total number of potential prey types (k = 1, 2, 3… n), and B is the biomass of prey (mg C m^{-2}). In equation 2, F is the feeding rate of predator on prey (mg C m^{-2} yr^{-1}); d_j is the natural death rate of j (yr^{-1}); B_j is the biomass of j (mg C m^{-2}); P_j is the energy loss of j due to the predation (mg C m^{-2} yr^{-1}); e_{ass} and e_{prod} is the assimilation efficiency and production efficiency of j, respectively. In equation 3, N_{min} is the N mineralization mediated by the predation of j on i (mg N m^{-2} yr^{-1}); $C:N_i$ and $C:N_j$ is the body C:N ratio of prey (i) and predator (j), respectively. The parameters of d, e_{ass}, e_{prod}, C:N of soil organisms are presented in Table S4.

The calculation of the N mineralization was started with the top predators, which are considered to have no energy loss from the predation, and then proceeded to the lower trophic groups. Based on the specific primary actors that drive energy flow from the basal resource to the soil food webs, the energy channels of the soil food webs can be divided into fungal channel (i.e. energy flux driven by fungi and then flow to fungivores and their predators), bacterial channel (i.e. energy flux driven by bacteria and then flow to bacterivores and their predators) and plant channel (i.e. energy flux driven by herbivores and then flow to their predators). The N mineralization of each channel was the sum of N mineralization of all functional feeding guilds within the channel.

2.5 Statistical analyses

Data were ln(x + 1) transformed to increase normality prior to statistical analysis.

Two-way analysis of variance (ANOVA) was performed to test the effect of tillage, soil depth and their interaction on the biomass of each feeding guild, and the N mineralization of soil food webs. When their interaction was significant, multiple comparisons were performed based on post hoc test to determine if tillage effects were significant in each soil depth. Tukey's honestly significant difference test was used for means comparisons and a difference at the $P < 0.05$ level was considered statistically significant.

Forward stepwise multiple linear regression (MLR) was used to identify the main channel that most accurately affects the crop yield at each soil depth. In stepwise regression, only one independent variable is considered at a time and another variable is added to the model at each step until no significant (*P*-value was set at 0.05) improvement in the percentage of explained variance is obtained. Prior to MLR, all parameters were min-max normalized to accurately preserve all relationships of data value and prevent potential bias from the domination of large numeric ranges over those with small numeric ranges. Min-max normalization subtracted the minimum value of an attribute from each value of the attribute and then divided the difference by the range of the attribute. The normalized value lay in the range [0, 1] (Jayalakshmi and Santhakumaran, 2011). All statistical analyses were performed using the R software (R 3.4.0, R Development Core Team 2017) using the car package for ANOVAs and the stats package for MLR analyses.

3. Results

3.1 Soil mineral N and soybean yield

Tillage effect on the soil mineral N varied with soil depths. At 0-5 cm, the amount of soil mineral N was higher ($P < 0.05$) in RT and NT than in CT, while the entire plow layer (0-15 cm) and the deep layer (5-15 cm) showed an opposite trend decreasing in the order of CT > RT > NT. There was no statistical significance for soybean yield among tillage treatments (Table 1); however, the yield of RT and NT increased by 6.6% and 26.5%, respectively, in comparison with CT.

3.2 Soil organism biomass

For soil microbes, a higher ($P < 0.05$) biomass of bacteria and fungi was observed under RT and NT than that under CT at both soil depths (Table 2). The similar trend was also found for the bacterivores and predators with a significant ($P < 0.05$) increase in biomass under RT and NT at both soil depths. For herbivores, a higher ($P < 0.05$) biomass was found under NT than that under CT, while for fungivores, RT significantly ($P < 0.05$) increased the biomass at both soil depths (Table 2).

3.3 Mineralization N of soil food webs

A greater ($P < 0.05$) amount of mineralized N of the whole soil food web was found under RT and NT than CT throughout the plow layer (Table 3); however, these positive effects varied with the energy channels. Compared to CT, RT and NT significantly ($P < 0.05$) increased the amount of mineralized N delivered by bacterial

and fungal channels at both soil depths. The components within these channels exhibited similar trends. For the components in the bacterial channel, the amount of mineralized N from the basal resource to the bacteria, and then from the bacteria to the bacterivores was greater ($P < 0.05$) under RT and NT than that under CT at both soil depths. However, RT and NT significantly ($P < 0.05$) increased the mineralized N from the bacterivores to the predators only at 5-15 cm. For the components in the fungal channel, the amount of mineralized N from the basal resource to fungi was significantly ($P < 0.05$) increased under RT and NT at both soil depths, while the amount of mineralized N from the fungi to the fungivores was only significantly ($P < 0.05$) increased under NT at 0-5 cm. For the plant channel, a greater ($P < 0.05$) quantity of mineralized N was released from RT and NT than from CT at 0-5 cm (Table 4). A similar result was also observed in the amount of N mineralized from basal resource to herbivores in RT and NT at the same soil depth.

3.4 Relationship between soil organisms and soybean yield

At 0-5 cm, 83.6% of the variation of the soybean yield was explained by the combined influence of fungal and plant channels (Table 4). Their relative contributions to the soybean yield decreased in the order of fungal channel (0.557) > plant channel (0.550), which means that when the min-max normalized fungal channel and plant channel increases by one unit, the min-max normalized soybean production would correspondingly increase by 0.557 and 0.550 times respectively. At 5-15 cm, only the bacterial channel significantly affected soybean yield and accounted

for 37.3% of the yield variance. The yield of soybean would increase by 0.656 times when the bacterial channel is increased by one unit.

4. Discussion

4.1 Performance of modelling N mineralization of soil organisms

The calculation of N mineralization of soil organisms was based on the predation relationship of soil food web structure (de Ruiter et al., 1993; Hunt et al., 1987), which highly depends on the assignment of species into functional feeding guilds. In this study, one of the weaknesses is that omnivorous collembolans were assumed to be divided in equal proportions among bacterivores, fungivores, herbivores and predators. To test how this assumption might affect the calculation of N mineralization, a sensitivity analyses was performed by re-assigning omnivorous collembolans into fungivores and herbivores (50% each) according to Barnes et al. (2014). This resulted in a very small deviation between these two models and an overall decrease of up to 0.24% among the tillage systems (Table S5), suggesting that the presented approach in this study is robust to estimate the mineralized N in the food webs.

The physiological parameters, such as assimilation efficiency, production efficiency and death rate, of trophic groups required for the calculation of N mineralization, are very difficult and impractical to determine under the field conditions because soil organisms have high spatiotemporal heterogeneity. Therefore, these physiological parameters are often cited from the literature (de Ruiter et al., 1993; de Vries et al., 2013; Hunt et al., 1987), and kept the same in all treatments to

facilitate the calculation of C and N mineralization of soil organisms (Holtkamp et al., 2011). Although this may lead to a certain deviation (maximum 30%) between the simulated and observed values (Carrillo et al., 2016; de Ruiter et al., 1993), a series of studies across natural and agricultural systems (Barnes et al., 2014; Carrillo et al., 2016; de Ruiter et al., 1993; Holtkamp et al., 2011; Schwarz et al., 2017) demonstrated that this approach is very useful in simulating C and N mineralization in soil organisms and can effectively reflect the changing trend of mineralization among treatments.

The biomass of organisms can be used to predict the potential of mineralized N because the biomass is the predominant factor in the calculation of N mineralization (Carrillo et al., 2016; de Ruiter et al., 1993; Holtkamp et al., 2011). In this study, the biomass of trophic feeding guilds under RT and NT increased significantly relative to CT, leading to the corresponding increase in N mineralization of the food webs. For example, higher biomass of bacterivorous feeders in RT and NT resulted in higher N released from bacteria at both soil depths. But, this predictable relationship between biomass and N mineralization of soil organisms is not consistent for the higher trophic level groups, i.e. predaceous feeders. The biomass of predaceous feeders was significantly increased under RT and NT soils throughout the plow layer, while the corresponding N mineralization increase occurred only from bacterivores to predators at the lower soil depth (5-15 cm). This may be mainly due to the existence of more than one prey resource for predators, and consequently, it is difficult to predict which prey has the greatest contribution to changes in N mineralization. Overall, modelling

N mineralization of soil organisms can effectively integrate soil organism communities and their functions related to N process, which may provide mechanistic predictions of the response of soil organisms to different tillage systems.

4.2 Relationships between N mineralization of soil organisms and soybean yield

Soybean is a legume and can obtain some N through the colonization of rhizobia in the root system, but the N provided by rhizobia cannot meet its requirement (Thilakarathna and Raizada, 2017). Therefore, soil N supply is an important determinant of achieving the maximum yield of soybean. Soil N supply is highly dependent on the level of mineral N and mineralizable N regulated by soil organisms (Whalen et al., 2013). In this study, the content of mineral N in the plow layer (0-15 cm) decreased in the order of CT > RT > NT over the whole growing season of soybean. This is counter intuitive as the soybean yield followed the reverse order, NT > RT > CT. At the critical growth stage, due to the strong demand for N by the crops, the soil mineral N content may decline (Fageria et al., 2010). However, this decline is short-lived and does not last the entire growing season.

Mineralization N delivered by soil organisms, which is another important source of soil N supply, was prominently improved in RT and NT soils. The multiple linear regression analysis further showed that there was a positive correlation between the N mineralization of soil organisms and soybean yield. These results suggest that the mineralized N from soil organisms produced over the growing season plays a key role in meeting the requirements of plant growth in RT and NT soils; it could also explain

379 the apparent inconsistency of higher soybean yield but lower decline in soil mineral N
380 over the growing season in RT and NT soils than in CT. Our result is consistent with
381 the reports of Carrillo et al. (2016) and Evans et al. (2011) that were also conducted in
382 field conditions and suggests that farming practices favoring a rich and abundant soil
383 organisms can improve crop yield by increasing N availability to plants. Although the
384 amount of mineralized N in RT and NT soil was increased, it does not mean that all
385 mineralized N may be taken up by the plant. For example, at the upper soil layer (0-5
386 cm), only the trophic feeding guilds within fungal and plant channels strongly linked
387 N mineralization with plant yield. This implies that the N released from other soil
388 organisms in the corresponding soil layer might be re-utilized by organisms or
389 leached from the soil, reducing the N availability to plants (Bender et al., 2015;
390 Thakur et al., 2014).

391 Numerous studies (Hunt et al., 1987; Thakur et al., 2014; Wagg et al., 2014;
392 Whalen et al., 2013) have demonstrated that the presence of predators that feed on
393 microbes can promote the N mineralization and the absorption of N by crops. This is
394 consistent with our results, which found that the association between N mineralization
395 in fungal and bacterial channels and soybean yield was enhanced in RT and NT soils.
396 However, there was a spatial difference in the distribution of fungal channel and
397 bacterial channel in the plow layer, in which the fungal channel at 0-5 cm and the
398 bacterial channel at 5-15 cm were the driving factors in mediating N mineralization.
399 This difference may largely result from the location of residues in RT and NT soils,
400 which were placed on the surface of the soil instead of being mixed with the soil.

Unlike bacteria, fungi are less dependent on nutrient spatial distribution in soils because they can transfer nutrients from surface residues to mineral soil via the hyphal growth (Frey et al., 2003). Additionally, the residue layer can serve as a habitat for many microarthropod groups, such as collembolans, which prefer to feed on fungi (Schwarz et al., 2017). These soil communities favored by the surface residues may account for why fungal channel plays a dominant role in mediating the N supply in the upper layer (0-5 cm) of RT and NT soils.

Fungal channel and bacterial channel are the main regulatory channels for N mineralization but they differ in turnover rate for processing N (de Vries et al., 2013; Wardle et al., 2004). In contrast to the "slower" fungal channel, which favors N retention in the soil (de Vries et al., 2011), the bacterial channel supports a faster N turnover rate and provides more mineralized N for crop production (de Vries et al., 2013; Whalen et al., 2013). This suggests that the dominant bacterial channel at 5-15 cm in RT and NT soils promotes the supply of N to plants. Furthermore, along this bacterial channel, the N mineralization from the bottom bacteria to the intermediate bacterial feeders, and then to the top predaceous feeders was greatly enhanced in RT and NT soils. There is general agreement with other researches (Carrillo et al., 2016; Wagg et al., 2014) that the tight interlinkage within trophic levels in the food web stimulates the release of N from soil organisms. The enhanced N mineralization of bacterial-channel may partially explain why the severe shortage of soil mineral N at 5-15 cm in RT and NT soils during the growing season did not result in a compromise of soybean yield.

Plant channel has been considered to have a very minor effect on N mineralization (Holtkamp et al., 2011). In this study, the amount of N mineralization in the plant channel was indeed the least among the different channels across tillage systems. However, to our surprise, a positive association between plant channel and soybean yield at 0-5 cm was evident in RT and NT soils. This may primarily due to the significant increase of mineralized N delivered by herbivores in plant channel under RT and NT soils, indicating that herbivores play a non-negligible role in the process of associating N mineralization with plant growth. Verschoor (2002) reported that the N mineralization of herbivores accounted for 10% of total N mineralization in a grassland system, and attributed these beneficial effects of herbivores to the activity of soil microbes that was stimulated by the increase in root exudates after infection by herbivores. In our study, most groups classified into herbivores are the facultative feeders. For example, herbivorous collembolans can switch their diet from plant roots to decaying litter (Endlweber et al., 2009). Therefore, we propose that the positive role of herbivores at 0-5 cm in RT and NT soil may partly be due to their manipulation on surface residues by fragmenting and mixing. Therefore, the surface area of litter in contact with soil microbes would be increased, which is beneficial for N mineralization (Soong et al., 2016).

In this study, the N mineralization of soil organisms was quantified using the experimental data and the food web energetic model based on the steady-state assumption. This method yields relatively static data that cannot reflect the dynamics nutrient flow of the soil food webs. However, it can filter some useful information

from the complex food web to help us better understand which soil organisms play a key role in N mineralization promoting crop growth. This forms background information for further study on the dynamics of the soil food web in N mineralization using ^{15}N tracer technology.

5. Conclusion

Our results showed that, during the whole growing season, almost all soil organisms in the food webs of RT and NT released more N than CT throughout the plow layer. However, the ability of soil organisms to supply N for soybean growth varied with energy channels and soil depths. Soil organisms in the fungal and plant channels at 0-5 cm and in the bacterial channel at 5-15 cm were the main drivers in associating N mineralization with crop yield. In conclusion, the long-term application of conservation tillage systems has promoted the N mineralization of soil organisms, which is favorable for achieving the optimal crop yield.

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625 **Table 1** Effects of tillage systems on the crop yield and the soil total N and
626 cumulative mineral N concentrations (means (SE)).

627

	CT	RT	NT
Yield (kg ha^{-1})	1242 (96) a	1324 (189) a	1570 (221) a
Mineral N (g m^{-2})			
0-5 cm	15.27 (1.44) b	20.09 (2.90) a	17.90 (1.46) ab
5-15 cm	28.10 (1.05) a	21.33 (1.79) b	20.06 (2.14) b
0-15 cm	21.68 (0.65) a	20.71 (1.86) ab	18.98 (0.67) b

628 CT, conventional tillage; RT, ridge tillage; NT, no tillage. Same lowercase letter in the same row
629 indicates no significant difference among tillage systems ($P > 0.05$).

Table 2 Cumulative soil biotic biomass (expressed as mg C m^{-2}) under different tillage practices (means (SE)).

	0-5 cm			5-15 cm			ANOVA		
	CT	RT	NT	CT	RT	NT	Tillage (T)	Depth (D)	T × D
Bacteria	6077 (499)	7367 (363)	8452 (1408)	9000 (1362)	11393 (1324)	12780 (733)	< 0.001	< 0.001	ns
Fungi	16386 (1309)	22375 (1639)	26646 (7661)	18558 (2409)	23938 (3622)	26168 (1769)	< 0.001	ns	ns
Herbivorous feeders	67 (5)	90 (23)	95 (13)	73 (4)	87 (25)	110 (18)	0.017	ns	ns
Bacterivorous feeders	78 b (15)	168 a (17)	128 a (30)	56 b (14)	93 a (13)	112 a (11)	< 0.001	< 0.001	0.045
Fungivorous feeders	58 (15)	98 (12)	99 (19)	34 (12)	57 (16)	55 (24)	0.023	0.002	ns
Predaceous feeders	60 (14)	88 (14)	78 (8)	96 (15)	123 (22)	176 (49)	0.002	< 0.001	ns

CT, conventional tillage; RT, ridge tillage; NT, no tillage; ns indicate no significant difference ($P > 0.05$). Means for the different tillage systems at the same depth and followed by the same lowercase letter are not significantly different ($P > 0.05$).

635 **Table 3** The amount of mineral N delivered by soil food webs (expressed as mg N m^{-2} $year^{-1}$) under different tillage practices (means (SE)).

636

Channel	Feeding guild	0-5 cm			5-15 cm			ANOVA		
		CT	RT	NT	CT	RT	NT	Tillage (T)	Depth (D)	T × D
Plant channel	Total Nmin	52.55 c (2.80)	63.30 b (3.28)	75.95 a (4.76)	140.50 a (27.74)	118.40 a (7.21)	159.77 a (23.46)	< 0.001	< 0.001	0.020
	Nmin (resource→herbivores)	41.69 c (3.25)	53.16 b (2.69)	61.65 a (3.19)	72.29 c (7.72)	77.35 b (4.38)	86.09 a (12.00)	< 0.001	< 0.001	ns
	Nmin (herbivores→predators)	10.85 ab (2.40)	10.15 b (0.78)	14.30 a (2.22)	68.21 ab (20.22)	41.04 b (8.95)	73.68 a (13.67)	0.003	< 0.001	ns
Bacterial channel	Total Nmin	4517.74 b (353.44)	5855.59 a (307.55)	6425.15 a (916.86)	6550.21 b (970.00)	8830.57 a (145.38)	9565.72 a (438.29)	< 0.001	< 0.001	ns
	Nmin (resource→bacteria)	4271.71 b (349.71)	5205.55 a (257.29)	5951.09 a (822.55)	6314.26 b (954.98)	8457.56 a (103.94)	8979.14 a (512.34)	< 0.001	< 0.001	ns
	Nmin (bacteria→bacterivores)	225.41 b (30.23)	622.04 a (46.52)	449.51 a (133.83)	186.04 c (40.46)	296.38 b (36.76)	428.84 a (47.20)	< 0.001	< 0.001	0.002
	Nmin (bacterivores→predators)	20.62 a (3.70)	28.00 a (10.27)	24.54 a (2.52)	49.91 b (11.11)	76.64 ab (20.63)	157.71 a (74.42)	0.002	< 0.001	0.013
Fungal channel	Total Nmin	5447.57 b (436.59)	7434.05 a (551.69)	7646.12 a (794.07)	6537.00 b (302.66)	7949.78 a (990.23)	8468.86 a (313.37)	< 0.001	0.007	ns
	Nmin (resource→fungi)	5421.75 b (433.21)	7402.99 a (542.76)	7613.55 a (798.64)	6509.06 b (299.44)	7919.58 a (521.04)	8414.91 a (325.48)	< 0.001	0.007	ns

Nmin (fungi→fungivores)	20.09 b (4.24)	25.49 ab (6.92)	26.74 a (4.11)	17.32 b (1.00)	21.53 ab (4.57)	29.44 a (4.25)	0.003	ns	ns
Nmin (fungivores→predators)	5.72 ab (2.13)	5.57 b (2.58)	5.83 a (2.13)	10.61 ab (3.99)	8.68 b (3.83)	24.52 a (10.89)	0.034	< 0.001	ns
Mineral N of the whole soil food web	10017.85 b (789.55)	13352.94 a (687.93)	14147.22 a (1549.39)	13227.71 b (1065.70)	16898.76 a (1177.10)	18194.35 a (568.77)	< 0.001	< 0.001	ns

Resource is a collective resource of residues and plant roots; residues and plant roots supply energy to microbial channel and plant channel, respectively.

CT, conventional tillage; RT, ridge tillage; NT, no tillage; Nmin(i→j) indicates the mineral N delivered by the predation of j on i; ns indicates no significant difference ($P > 0.05$); Same lowercase letter in the same row and same depth indicates no significant difference among tillage systems ($P > 0.05$).

640 **Table 4** Relationships between N mineralization of different energy channels and
641 soybean yield based on multiple linear regression. Data were min-max normalized
642 and are dimensionless.

Soil depth (cm)	Variable	Beta standardized coefficient	T value	Adjusted R^2	F value of the regression
0–5	Fungal channel	0.557	2.886*	0.836	19.737**
	Plant channel	0.550	2.437*		
5–15	Bacterial channel	0.656	2.745*	0.373	7.555*

643 * and ** indicate significant at 0.05 and 0.01, respectively.