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Soil fauna increase nitrogen loss in tilled soil with legume but reduce nitrogen loss in non-tilled soil without legume

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ABSTRACT

We conducted a factorial microcosm experiment to determine whether the effects of soil fauna on N dynamics differ in tilled vs. non-tilled soil with and without a legume. Soil monoliths were taken from two neighboring fields that had been continually tilled or non-tilled for the past 15 years; the soil was defaunated, and meso- and macrofauna were added back to half the monoliths. In addition, half of the monoliths were planted with a legume (*Lotus corniculatus*), and the other half were maintained without vegetation. Nitrogen losses by leaching from soil were periodically determined. After 3 months, the plants were harvested, and the soil and plants in each treatment were analyzed for, plant biomass, microbial respiration and biomass, PLFA. Fauna significantly increased N loss only in the tilled soil with legume and significantly decreased N loss in the non-tilled soil without legume. When fauna were absent, legume biomass was higher in the non-tilled soil than in the tilled soil, but when fauna were present, legume biomass did not differ between tilled and non-tilled soil. Our results show that non-tilled soil with fauna conserves soil N and enhances the efficient use of N by plants.

1. Introduction

Nitrogen (N) is a limiting nutrient in terrestrial ecosystems (Vitousek and Howarth, 1991) and is also essential for crop production in agroecosystems. In agroecosystems, N fertilizers are typically added to soil, and substantial quantities of nitrate often leach into the groundwater (Velthof et al., 2009). The leaching of nitrates and other nutrients of soil is higher in tilled than in non-tilled system because tillage increases the rate of organic matter decomposition (Hendrix et al., 1986; Arshad et al., 1999; Six et al., 2000). By conserving N in the soil–plant system, non-tilled can reduce the ecological cost of agriculture (Six et al., 2000).

Substantial research has demonstrated that tillage influences the composition and abundance of soil microbes (e.g., Frey et al., 1999; Pankhurst et al., 2002) and also soil invertebrates (Nuutinen, 1992; Fraser et al., 1996; Peigne et al., 2009; Umiker et al., 2009; Postma-Blaauw et al., 2010). Although the relationship between tillage intensity and the soil decomposer community is well documented, there are few data about the effects of soil fauna on nutrient cycling under various tillage regimes or systems with various tillage history (Liiri et al., 2012). Soil invertebrates affect primary decomposition by directly altering microbial communities through grazing (Hedlund and Augustsson, 1995; Byzov et al., 1998) or indirectly by modifying the microbial environment (Visser, 1985; Tiunov and Dobrovolskaya, 2002) and by modifying organic matter availability for microorganisms (Lavelle et al., 1997). These effects of soil fauna change depending on carbon (C) availability (Tiunov and Scheu, 2004). Because crop residues tend to accumulate on the soil surface in non-tilled systems but are translocated into deeper soil layers in tilled systems, the two kinds of systems differ in the timing of C supply (Six et al., 1999, 2000). These studies also indicate that non-tilled practices reduce decomposability of organic matter by enhancing soil aggregation and thereby enhancing C sequestration (Lal et al., 2003).

In addition to being reduced in non-tilled systems, N and C losses from soil may also be reduced in long-term legume cropping systems (Drinkwater et al., 1998). Previous studies from grassland manipulation experiments show that interaction of legumes and soil fauna, namely earthworms may substantially affect dynamics or decomposition process (Milcu et al., 2008; Birkhofer et al., 2011) and the change in legume incidence have major effect on functioning of decomposer food web (Milcu et al., 2008). How legumes

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(which form N-fixing symbiotic relationships with root bacteria) alter the effects of soil fauna on N mineralization, however, is poorly understood.

In this study, we tested the two hypotheses. First, we hypothesized that soil fauna affect N dynamics differently in tilled vs. non-tilled systems; we predicted that soil fauna would accelerate N leaching with tillage but decrease N leaching with non-tilled. Second, we hypothesized that the effects of soil fauna on N leaching would be altered by a legume crop. To test these hypotheses, we conducted a factorial laboratory experiment using soil monoliths from experimental field plots that had been subjected to tilled or non-tilled treatments for 15 years.

2. Materials and methods

2.1. Source of soil monoliths

Soil monoliths were collected from a Praha-Ruzyně in the Czech Republic (50°05′N 14°20′E) where an experiment comparing tilled and non-tilled treatments has been ongoing since 1995. The mean annual precipitation in this area is 472 mm, and the mean annual temperature is 8.4 °C. The soil is a clay loam cambisol with a pH(H_2O) of 7.0, C content was 1.41%, 1.84% in tillage and no tillage soil, respectively. Two adjacent fields with similar properties at this site have either been tilled to 22 cm depth or not tilled. In both fields, the crops were alfalfa in 1995 and 1996; wheat in 1997, 1998, 2000, 2002, 2004, 2006, and 2008; barley in 2003, oil seed rape in 2005 and 2009; and peas in 1999, 2001, and 2007, yields were comparable on both treatments. Pesticide application consist mainly from fungicides in both treatment and herbicide in no tillage, no pesticide was applied at least one month before soil cores sampling.

2.2. Experimental design

We collected intact soil cores (10 cm diameter and 8 cm deep) from the tilled and non-tilled areas of the long-term field experiment in November 2009. Cores were taken before autumnal tillage, but the stubble was already incorporated by stubble-tillage in tillage treatment. As consequence both treatments have the same amount of post harvest residues which were on soil surface in no tillage and incorporated in tillage treatment. Cores were placed in plastic containers that were 10 cm in diameter and 10 cm high. The bottom of the container contained a 1-cm layer of coarse sand (2-mm grain size); a rubber tube that was inserted through the container wall and into this sand layer was used to collect leachate. A nylon mesh (0.1-mm openings) cylinder (35 cm high and sealed at the top) that prevented fauna from moving into or out of containers was applied to every container.

In the laboratory, we established a factorial microcosm experiment with all combinations of tillage (T), no tillage (NT), legume (*Lotus corniculatus*) present (LP) and absent (LA), and soil fauna present (FP) and absent (FA). Each of the eight treatment combinations was replicated eight times. All soil cores were defaunated before the experiment by two freezing cycles of 24 h at −40 °C. Soil mesofauna and macrofauna were added to FP treatments as follows. For the addition of mesofauna, soil cores of the same volume as used in the microcosms were placed in Tullgren funnels and wrapped in mesh that allowed mesofauna but not macrofauna to escape into a FP microcosm that was positioned below the funnel. The open space between the core and the microcosm was wrapped in plastic film to reduce the loss of soil water, and the mesofauna were allowed to move from the upper core to the microcosm for 48 h. Microcosms without fauna addition were processed in the same way under an empty Tullgren funnel to expose all microcosms to the same temperature fluctuation. For addition of macrofauna, two *Aporrectodea calliginosa* earthworms, one *Lumbricus rubellus* earthworm, and two *Oniscus asellus* isopods were added to each microcosm. Added macrofauna consists from species common in agroecosystem, species selection was based on previous soil fauna survey in given area (Frouz et al., 2008). The legume *L. corniculatus* was pre-cultivated in sand, and three seedlings (2 cm high) were planted in each LP microcosms on day 0, immediately after fauna were introduced. The microcosms were maintained for 90 days in a chamber at 25 °C with a 14-h light: 10-h dark cycle. Soil water content in the microcosms was maintained at 50% of field capacity by adding de-ionized water every 24 h. Microcosms also received water when leachate was measured (see next section).

2.3. Samples and analysis

To estimate temporal variation in potential N loss by leaching, we obtained leachates from the microcosms on day 15, 45, and 75 by adding 50 ml of de-ionized water per microcosm. Leachate water that had drained through each microcosm was collected in a bottle and its volume was recorded. For analysis of NO_3^- and NH_4^+ content, leachates were centrifuged at 4000 *g*, and the supernatant was passed through a glass fiber filter. Concentrations of NH_4^+ and NO_3^- in the filtrate were determined with a colorimetric method (Zbíral et al., 1997) and a spectrophotometer (Genesys™ 6, Thermo Spectronic, USA).

The experiment was terminated on day 90. Biomass and total C and N content of aboveground plant parts were determined. After plant parts were oven-dried at 40 °C for 72 h and weighed, they were crushed and analyzed for C and N content with a Carlo–Erba CHN analyzer.

Four microcosms per treatment were used for soil analysis. After coarse roots and plant litter were removed from a microcosm, about 50 g of soil was dried at 40 °C for 72 h, homogenized by passing through a 2-mm sieve, crushed, and analyzed for total soil C and N by the same method as described above for plant C and N analysis. About 5 g of fresh soil was immediately used for PLFA analysis, and the remaining soil was stored at 4 °C and then analyzed within 5 days. For determination of inorganic soil N, a fresh subsample (10 g dry weight equivalent) of each soil sample was shaken (160 rpm) in 50 ml of 2 M KCl for 2 h, passed through filter paper, and stored at −30 °C. Ammonium and nitrate N contents of the extract were determined, and the totals were considered to represent the inorganic N content. For estimation of the net N mineralization rate, the water content of a subsample (10 g dry weight equivalent) was adjusted to 60% of its water-holding capacity, and the soil was incubated at 25 °C for 28 days. After incubation, inorganic N was extracted and determined as described above. All measurements of NH_4^+ and NO_3^- were performed with a colorimetric method and spectrophotometer as described above. Net N mineralization was calculated as the difference in inorganic N content at the start and at the end of the incubation. The net N mineralization rate was estimated by dividing the net increase in inorganic N during the incubation by the number of incubation days.

Microbial biomass was quantified by the chloroform fumigation–extraction method (Jenkinson and Powlson, 1976). Microbial respiration was measured as CO_2 production, which was measured by the trapping of CO_2 with NaOH in an airtight vial (for 1 week in 20 °C) and the subsequent titration of NaOH by HCl after BaCl addition.

Soil samples for phospholipid fatty acid (PLFA) analysis were extracted with a mixture of chloroform–methanol–phosphate buffer (1:2:0.8) (Šnajdr et al., 2008). Phospholipids were separated using solid-phase extraction cartridges (LiChrolut Si 60,