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Short Communication

Formation of extractable organic nitrogen in an agricultural soil: A ^{15}N labeling study

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ABSTRACT

Few studies have investigated the extractable organic nitrogen (EON) formation mechanisms, and the sources of EON have long been debated. Using ^{15}N labeling, we performed a 120-day laboratory incubation experiment to explore the dynamic contributions of different types of added N (ammonium-N, ryegrass-N and their combination) to soil EON and the role that microorganisms play in N transformation into EON. We show that the ^{15}N abundances and recoveries in soil EON pool were relatively low during the incubation, except the first hours after ryegrass addition in ^{15}N-ryegrass addition treatments. In general, most of the EON during the incubation was soil derived, and both ammonium-N (80 mg kg^{-1}) and ryegrass-N (160 mg kg^{-1}) additions made minor contributions (3–4% and 8–13% during day 1–120) to the soil EON pool. Moreover, along with the decline in ^{15}N recoveries in microbial biomass nitrogen (MBN) pool, the lost $MB^{15}N$ did not enter into the $EO^{15}N$ pool. Our study demonstrates 1) that EON is a stable N pool in agricultural soil and is less affected by exogenous N addition and 2) that microbial N uptake and release processes contribute little to the soil EON pool.

Soil extractable organic nitrogen (EON) is defined as the organic forms of N that are extracted by water or salt solutions (e.g., K_2SO_4, KCl and $CaCl_2$) in soil and is considered to be the sum of the organic N dissolved in the soil solution (DON) and the extra organic N solubilized during extraction (Ros et al., 2009). Soil DON and EON is assumed to be an important N source to plants and microbes and play a pivotal role in soil N turnover (Murphy et al., 2000; Hodge et al., 2000; Jones et al., 2004; van Kessel et al., 2009), in particular in N-limited terrestrial ecosystems (Schimel and Bennett, 2004). In addition to NO_3^-, certain levels of DON (or EON) have also been detected in leachate, stream water and deep soils, implying that organic N is also an important form of N losses to ground and surface waters (Siemens and Kaupenjohann, 2002; Fang et al., 2009; Khalili and Nourbakhsh, 2012; Quan et al., 2014). Identifying the origin of EON is a key to evaluating its ecological and environmental function in soil (Liang et al., 2015), but the relative proportions of soil EON derived from exogenous N, as well as the related formation mechanisms, remain poorly understood due to the complexity of N transformation in soil, particularly in over-fertilized and intensively cultivated agricultural soil (Neff et al., 2003; Ros et al., 2009; Luce et al., 2014).

Here, we present the results of a 120-day incubation experiment with a silt loam soil to track the dynamics of ^{15}N tracers in six soil N pools (extractable NH_4^+, extractable NO_3^-, EON, microbial biomass N (MBN), mineral fixed NH_4^+ (MFN) and non-microbial organic N (NMON)) from ^{15}N-labeled ammonium sulfate (AS) and/or ^{15}N-labeled ryegrass. We have previously reported the fate of added $^{15}NH_4^+$ (Quan et al., 2016), and found that ryegrass incorporation provided a critical C source which enhanced microbial NH_4^+ use while reduced NO_3^- loss, leading to more retention of added $^{15}NH_4^+$ in the stable soil N pool first as MBN and then as NMON. Here we focus on the mechanisms of soil EON formation from the NH_4^+ and/or ryegrass we experimentally added and the role of microorganisms in EON formation. Our initial hypothesis was that exogenous N would be largely transformed to soil EON via microbial N uptake and release, thus microbial biomass N is an important source of soil EON pool (Appel et al., 1996; Perakis and Hedin, 2001; Ros et al., 2010).

The soil used for this study was collected from the surface layer (0–20 cm) of a greenhouse field planted with pepper in Damintun, Xinmin County, Liaoning Province, China (122°50′E, 41°59′N). Four N addition treatments were set up, namely, ^{15}N-labeled $(NH_4)_2SO_4$ alone (^{15}AS) or plus non-labeled ryegrass ($^{15}AS+R$), and ^{15}N-labeled ryegrass alone (^{15}R) or plus non-labeled $(NH_4)_2SO_4$ ($^{15}R+AS$). Ammonium

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Table 1
Selected physical and chemical properties of the soil and ryegrass.

Characteristic [a]	Soil	Non-labeled ryegrass	Labeled ryegrass
pH	6.41	- [b]	–
Clay (< 0.002 mm)	7%	–	–
Silt (0.002–0.05 mm)	59%	–	–
Sand (0.05–2 mm)	34%	–	–
Total organic C (TOC, g kg^{-1})	14.9	440	438
Total N (TN, g kg^{-1})	1.41	35.5	33.7 (46.8%) [c]
TOC/TN	10.5	12.4	13.0
Extractable organic C (EOC, g kg^{-1})	0.13	77.7	98.2
Extractable NO_3^--N (mg kg^{-1})	152	1917	2041 (54.9%)
Extractable NH_4^+-N (mg kg^{-1})	5	593	378 (44.5%)
Extractable organic N (EON, mg kg^{-1})	8	7760	6384 (44.8%)
(NO_3^--N + NH_4^+-N + EON)/TN (%)	11.7	28.9	26.1
EOC/EON	16.2	10.0	15.4

[a] Soil pH was determined in deionized water (w:v, 1:2.5) using a pH electrode (Model 868, Thermo Electron Corp., China). Soil texture was determined using a Bouyoucos hydrometer. Extractable C or N components were extracted with 2 M KCl (1:5 for soil and 1:20 for ryegrass, w:v). The TOC and EOC in the soil and ryegrass were measured on a TOC/TN analyzer (Multi N/C 3100, Analytik Jena, Germany). The TN in the soil and ryegrass was determined using the Kjeldahl method.

[b] "-" not determined.

[c] The percentages in brackets are the ^{15}N abundances in the related N pools in the labeled ryegrass.

sulfate and ryegrass (ground to < 0.5 mm powder) were added at 80 and 160 mg N kg^{-1} dry soil; rates similar to the local farmers' practices. The ^{15}N abundances of the labeled AS and ryegrass (total N) were 50.2 atm% and 46.8 atm%, respectively (Table 1).

Laboratory incubation, sampling and analysis methods were the same as described by Quan et al. (2016). Briefly, fresh soil samples after AS and/or ryegrass addition were incubated in centrifuge cups in an automatically controlled incubator (dark, 25 °C). Distilled water was added at regular intervals to maintain the soil moisture content to 60% water-filled pore spaces. After 0.1 (2.5 h), 1, 3, 7, 15, 30, 60 and 120 days of incubation, soil samples of each treatment were destructively sampled to analyze concentrations and abundances of the six soil N pools (See Fig. S1 in Supplementary Information for detailed experimental procedures). The ^{15}N recovery in each N pool (% of applied ^{15}N) was calculated based on the mass balance and mixing model in each pool (Quan et al., 2016).

The extractable NH_4^+ concentrations decreased by > 90% from > 80 mg N kg^{-1} to 2.7–5.6 mg N kg^{-1} within 3 days of AS addition (Fig. 1). Correspondingly, the NO_3^- concentrations increased by > 100 mg N kg^{-1} in all treatments during the incubation. Similar trends were also observed for the ^{15}N abundances and recoveries. These results suggest rapid mineralization (for ryegrass) and nitrification in the investigated soil. As a result, 70–92% of the added NH_4^+ and 56–58% of the added ryegrass-N were recovered as NO_3^- after the 120-day incubation (Fig. 1).

The ^{15}N recoveries in the EON pool were very low in all treatments (0.3–1.6%), except on day 0.1 (2.5 h after labeling) when 4.1–7.4% of the ^{15}N-labeled ryegrass we added was found as EON (Fig. 2). It seems likely that this EON pool was found within the ryegrass added. ^{15}N recoveries in the MBN pool peaked at day one and then gradually fell to a background level (3.3–7.1%) at the end of the 120-day incubation (Fig. 2) and were much higher in the ryegrass addition treatments

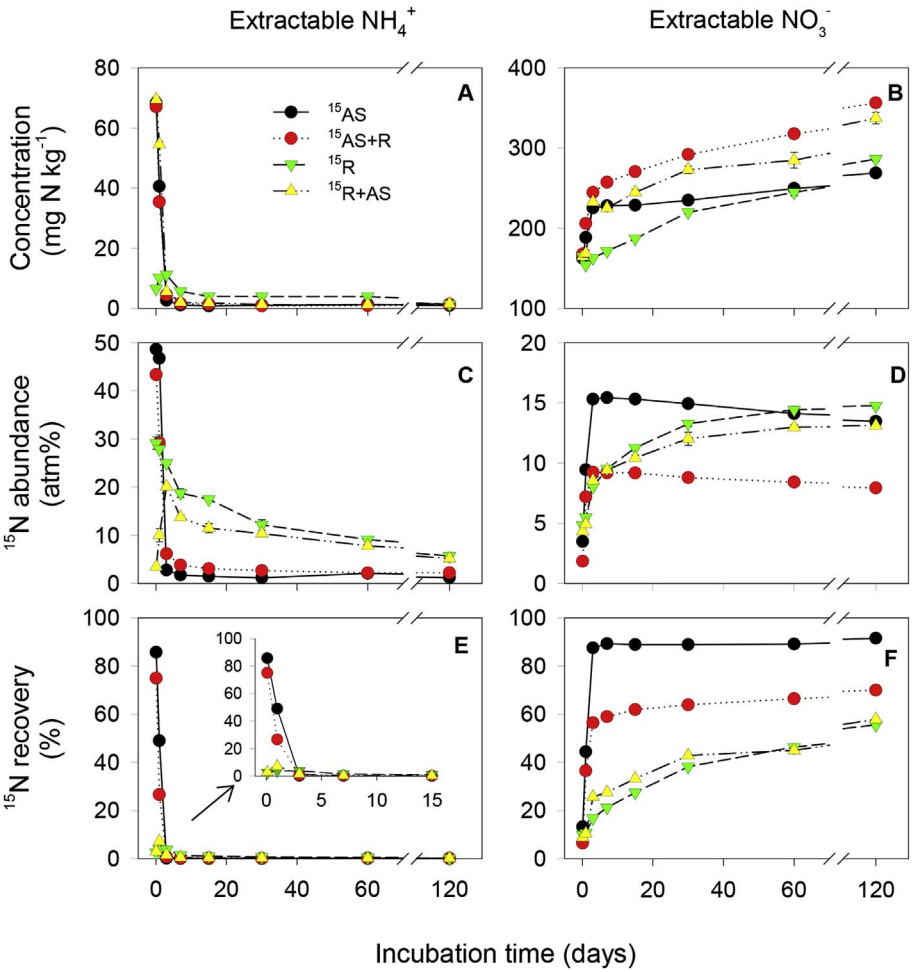

Fig. 1. Nitrogen concentrations, ^{15}N abundances and ^{15}N recoveries in the forms of extractable NH_4^+ and NO_3^- in soils with ammonium (AS, 80 mg N kg^{-1}) and/or ryegrass (R, 160 mg N kg^{-1}) addition. Bars represent the standard error (n = 4) and are smaller than the symbol when not visible.