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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

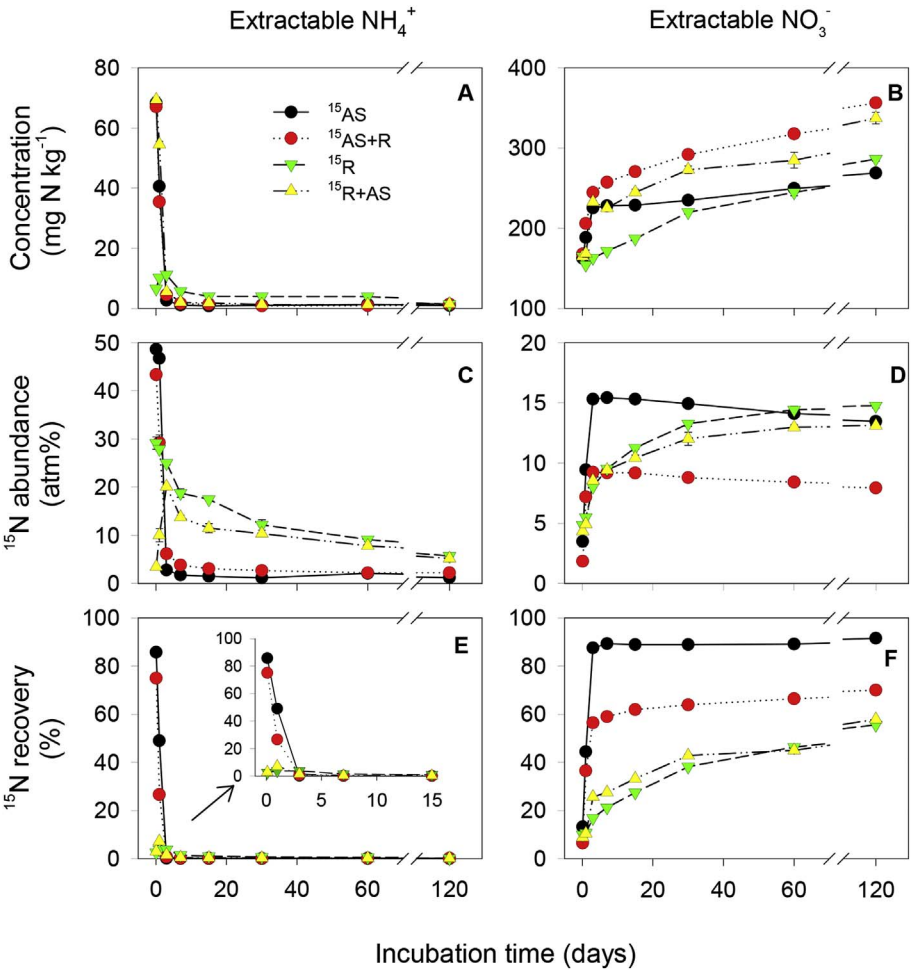

Fig. 1. Nitrogen concentrations, ^{15}N abundances and ^{15}N recoveries in the forms of extractable NH_4^+ and NO_3^- in soils with ammonium (AS, 80 mg N kg^{-1}) and/or ryegrass (R, 160 mg N kg^{-1}) addition. Bars represent the standard error (n = 4) and are smaller than the symbol when not visible.

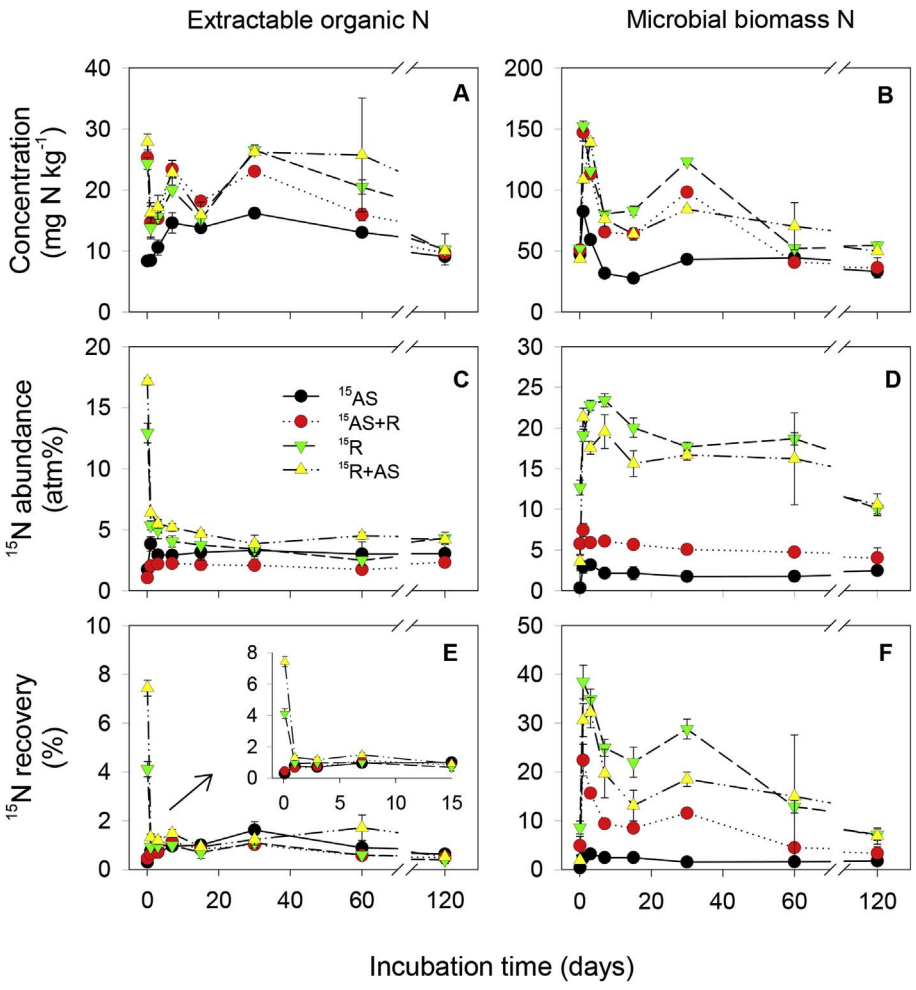

Fig. 2. Nitrogen concentrations, ^{15}N abundances and ^{15}N recoveries in the forms of EON and microbial biomass N (MBN) in soils with ammonium (AS, 80 mg N kg^{-1}) and/or ryegrass (R, 160 mg N kg^{-1}) addition. Bars represent the standard error (n = 4) and are smaller than the symbol when not visible.

(22.4–38.4%) than in the treatments without ryegrass addition (0.3–5.3%).

Soil EON and MBN (chloroform fumigation-released N) are separated by the microbial cytomembrane. The higher abundances and recoveries of ^{15}N in the MBN pool than in the EON pool during the entire experiment except first 2.5 h (Fig. 2) implied that the intracellular cytoplasm had greater affinity for added ^{15}N than extracellular extractable organic matter (EOM) did when available organic C was present (Luce et al., 2014). Meanwhile, the low ^{15}N abundances in the EON pool (1.7–6.4 atm% during day 1–120) indicated that most of the detected soil EON was not involved in the turnover of added ^{15}N due to their low bioavailability. A similar result was found in another study where more ^{13}C tracer entered into the microbial biomass carbon (MBC) than into the extractable organic carbon (EOC) after incorporating ^{13}C-labeled maize straw into one agricultural soil (De Troyer et al., 2011).

The mechanisms proposed for the generation of EON include a complex mixture of biotic and abiotic processes: extracellular enzyme degradation (Ros et al., 2010), abiotic reactions (Moritsuka et al., 2004; Toosi et al., 2012) and microbial uptake-release (Neff et al., 2003). We initially hypothesized that microbially assimilated N would be largely processed and released, and then transferred into EON pool. However, the results from the present study do not support our hypothesis. First, at the same time that ^{15}N recoveries in the MBN pool in the ryegrass addition treatments reduced, the ^{15}N recoveries in the EON pool did not increase (Fig. 2). Second, the abundances of ^{15}N-MBN (0.4–2.1 atm%) were lower than the abundances of ^{15}N-EON (1.7–3.8 atm%) in the ^{15}AS treatment during most of the incubation period (Fig. 2), indicating that abiotic (chemically and/or physically mediated association of mineral N with EOM) rather than biotic transformation plays an important role in EON formation (Dail et al., 2011; Toosi et al., 2012). The transformation from $^{15}NO_3^-$ to EO^{15}N is also considered to be low due to the large and stable gap in their abundances (Figs. 1 and 2); the abundance of EON would increase over time if some NO_3^--N was immobilized into the EON pool.

By ignoring the small compositional differences between labeled and non-labeled ryegrass (Table 1) and combining these two treatments with the addition of both AS and ryegrass (^{15}N cross labeled), we calculated the relative contributions of the three N sources (AS, ryegrass, and soil) to the EON and MBN (Fig. 3). The calculation showed that native soil N (most possibility, the soil intrinsic EON) was the main source of EON during the incubation from day 1 to day 120, while added AS-N and ryegrass-N contributed only 2.7–3.9% and 7.5–13% to the soil EON, respectively. In contrast, AS-N and ryegrass-N contributed much more to MBN production (8.6–16% and 24–49%) than to EON production. Thus, the results of the present study provide new and direct evidence to support the explanation that soil EON is a stable pool (long turnover or residence time, Fig. 4) mainly composed of natively derived inert components, and little affected by N addition (van Kessel et al., 2009).

The labile parts of EON, mostly with a low molecular weight (LMW-EON), might have decomposed during extraction and centrifugation process due to their rapid turnover. Considering the central role of LMW-EON in soil organic N turnover (Jones et al., 2004; Ros et al., 2009), we suggest that future studies should separate the labile and recalcitrant parts of soil EON according to the research objective, rather than considering them as a whole (Fig. 4). The labile components (e.g., free amino acids and polypeptides) in the soil EON pool are involved in

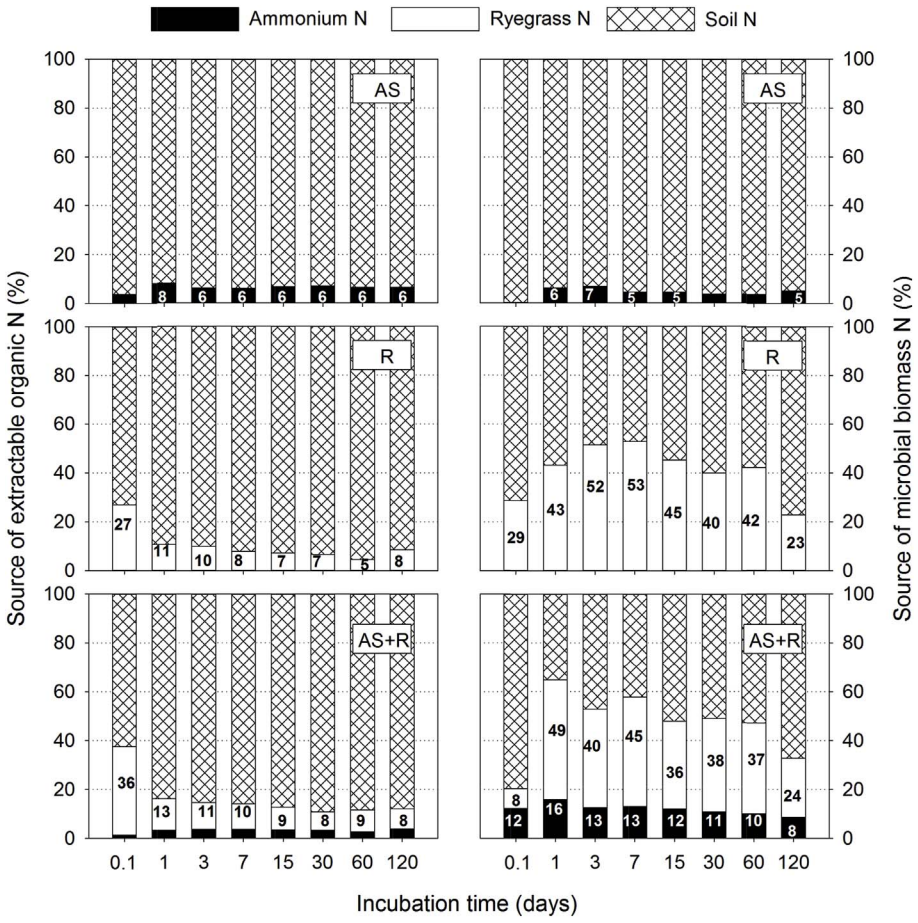

Fig. 3. Relative contributions of added ammonium-N, ryegrass-N and soil native N to the extractable organic nitrogen (EON) and microbial biomass N (MBN) in soil with ammonium (AS, 80 mg N kg^{-1}) and/or ryegrass (R, 160 mg N kg^{-1}) addition. Numbers on bars are contributions of related sources, which are not shown when < 5%.

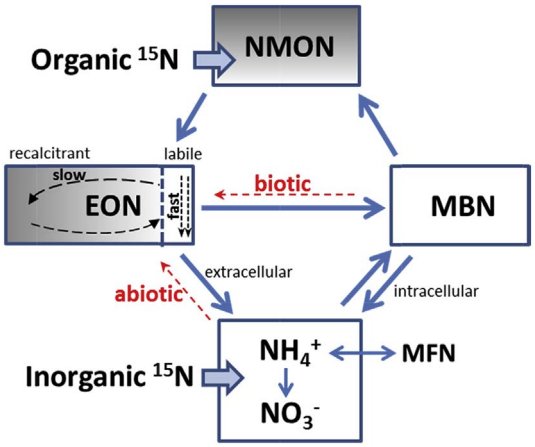

Fig. 4. Relationships among the transformations of extractable NH_4^+ and NO_3^-, extractable organic nitrogen (EON), microbial biomass N (MBN), mineral fixed NH_4^+ (MFN) and non-microbial organic N (NMON) after ^{15}N-ryegrass and/or $^{15}NH_4^+$ addition. The solid blue lines indicate the known pathways. The dotted red lines indicate the unclear pathways. See Fig. S2 in Supplementary Information for the dynamics of soil MFN and NMON during the incubation. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

many N-related processes, but their proportion is low due to their fast turnover (Neff et al., 2003; Luce et al., 2014), and the recalcitrant components (e.g., humic acids and aromatic substances) are subject to migration loss as DON when the appropriate conditions are satisfied, both due to their relatively high abundances and long residence times (Chen and Xu, 2008; van Kessel et al., 2009).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.soilbio.2017.12.015.

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