



Dynamics of microbial necromass in response to reduced fertilizer application mediated by crop residue return

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ABSTRACT

Understanding the response of microbial-derived nitrogen (N) in the soil to N availability is essential for optimizing N fertilizer management. In this study, based on amino sugar biomarker assays in a conservation tillage agroecosystem, we evaluated the effects of 3-year fertilizer N reduction (reduced from 240 to 190, 135, 0 kg N ha⁻¹) and crop residue return on microbial necromass N dynamics. The stock of microbial necromass N declined with the decrease of N input, and the decline of bacterial necromass was greater than that of fungal necromass. However, the decrease of 7.3% in microbial necromass N following the cessation of fertilization indicated a dominant role of microbial necromass in soil N retention despite its compensation ability for N demand. Maize residue return alleviated N deficiency in the soil-crop system and favored the maintenance of the soil organic N pool by preferentially improving the net accumulation of fungal necromass.

Nitrogen (N) fertilization plays an essential role in guaranteeing food security. Unfortunately, low N use efficiency and N surpluses threaten soil quality and environmental health (Yu et al., 2019). Hence, reducing fertilizer application and crop residue return are advocated as national actions for agroecosystem sustainability, and the key to achieving this goal is to pay more attention to soil functions relating to N retention and supply.

Fertilizer reduction decreases N availability, and thus alters the transformation between organic and inorganic N constituents in soil (Müller et al., 2011). Microbial necromass, identified by amino sugars, not only contributes to long-term carbon (C) and N storage, but may also be involved in the mineralization process to balance C and N stoichiometry (Liu et al., 2016). Organic C inputs favor microbial necromass accumulation (Guggenberger et al., 1999; Plaza et al., 2013), but how microbial necromass dynamics respond to a decrease in N application is not understood. Heterogeneously, bacterial necromass is estimated by muramic acid (MurN) in bacterial cell walls, whereas fungal necromass is evaluated by chitin, a polymer of N-acetylglucosamine in fungal cell walls (Amelung, 2001). Considering the high C/N ratio of fungi and protection of chitin by melanin (Paul and Clark, 1989), the turnover rate

of fungal necromass was considered to be lower than that of bacterial necromass. However, field experimental data on the response of bacterial and fungal necromass to changing C and N status is lacking (He et al., 2011). We hypothesized that a net decline in soil microbial necromass occurs upon fertilizer reduction, and that this process is mainly regulated by bacterial necromass with low stability. Crop residue return favors microbial N immobilization, offsetting the degradation of microbial necromass and improving soil N stability by enhancing fungal necromass retention.

Our experiment was carried out in a no-tillage agricultural system in Northeast China. From 2008, the field was subjected to full maize residue mulching (ave. 7.5 Mg ha⁻¹) with a local N rate of 240 kg ha⁻¹y⁻¹. A fertilizer reduction experiment was conducted in 2016 with four replicates in a split-plot design. Urea was applied at 0 (N0), 135 (N135), 190 (N190), and 240 kg N ha⁻¹ (N240) either with maize residue removal (S0) or full return (S100). Soil samples were collected at a depth of 0–20 cm in October 2018. Fresh soil was extracted with KCl for ammonium (NH₄⁺-N) and nitrate (NO₃⁻-N) analysis. The N contents of soil and maize tissues were measured using an element analyzer (vario MACRO cube, Elementar Analysensysteme GmbH, Germany). Soil

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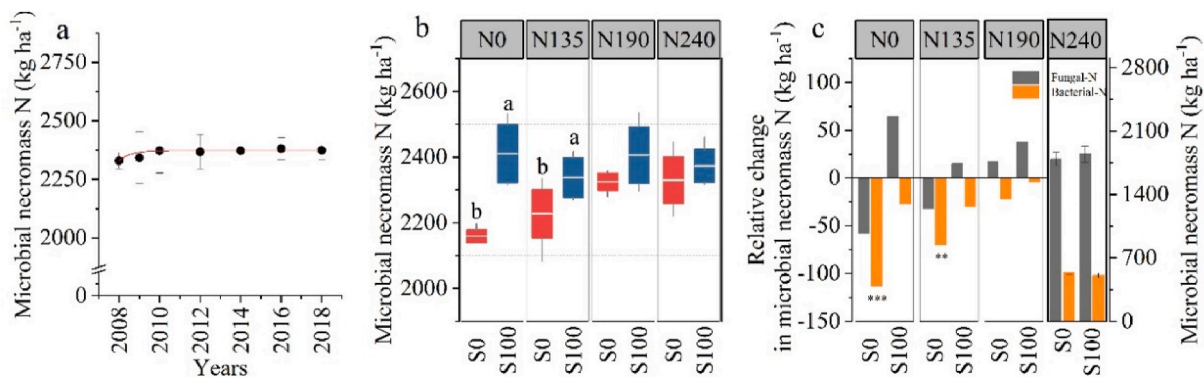


Fig. 1. The stock of microbial necromass N from 2008 to 2018 at a depth of 0–20 cm with full return of the harvested maize residue (a); microbial necromass N response to crop residue return under different N fertilizer rates (b); the relative changes of fungal and bacterial necromass N, and stocks of fungal and bacterial necromass N in N240 treatments (c). Asterisks denote significant differences from the values under the conventional fertilizer application rate (240 kg N ha⁻¹).

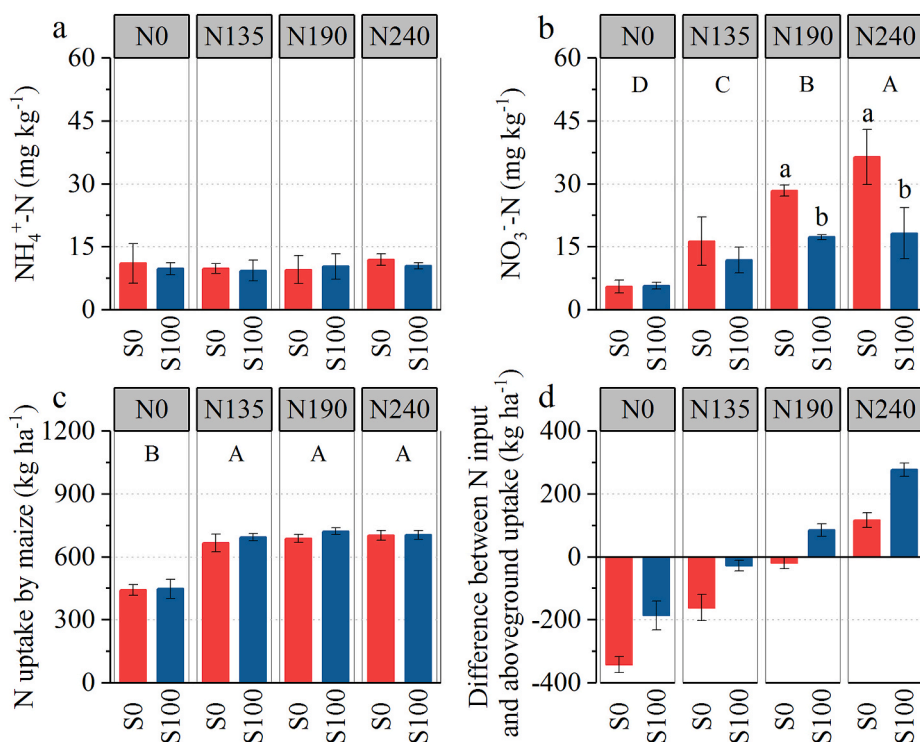


Fig. 2. Changes in NH₄⁺-N (a), NO₃⁻-N (b), and N uptake by maize (c), relative changes between cumulative input (stover N + fertilizer N + deposited N + asymbiotic N fixation) and aboveground uptake of N from 2016 to 2018 (d, data are shown in Table S2) under different fertilizer N application rates and crop residue return treatments. Error bars represent standard error (n = 4). Different uppercase letters indicate significant differences among fertilization treatments. Different lowercase letters indicate significant differences between crop residue treatments under each fertilizer N application rate.

amino sugars, i.e., glucosamine (GluN), galactosamine, and MurN, were analyzed according to the protocol of Zhang and Amelung (1996). Fungal and bacterial necromass were estimated based on fungal-derived GluN and MurN contents with conversion factors of 1.40 and 6.67, respectively (Liang et al., 2019), and the two were summed to provide microbial necromass (kg ha⁻¹). Two-way ANOVA with Duncan's post hoc test was performed to test the effects of fertilization and crop residue on changes in microbial necromass, soil mineral N, maize yield, and N uptake, followed by LSD test for simple effect when an interaction effect was detected by using IBM SPSS 20.0.

After 6 years of conservation tillage with full return of maize residue, the stock of microbial necromass N at a depth of 0–20 cm reached its maximum value, and this magnitude remained unchanged in the S100N240 treatment (Fig. 1a). In this case, the contribution of microbial necromass to the soil N pool (3900 kg ha⁻¹) was approximately 61% (Fig. S1a), confirming the prominent role of microbial remnants in N storage (Simpson et al., 2007; Liang et al., 2019). At the N application rate of 240 kg ha⁻¹ with maize residue removal (SON240), the uptake of

N by aboveground maize biomass accounted for approximately 74% of the total input and the NO₃⁻-N content (36.4 mg kg⁻¹, Fig. 2b) in soil was at a relatively high level compared with a local range of 6–40 mg kg⁻¹ (Su et al., 2021), suggesting a potential risk of N leaching. The reduction in N fertilizer application to 190 kg ha⁻¹ for 3 years did not influence maize yield, but the total N input was 688 kg ha⁻¹, slightly lower than crop uptake after maize residue removal (Figs. 2d and S2). Except for a slight decrease of NO₃⁻-N content (Fig. 2), the stock of microbial necromass N was not different from that in the SON240 treatment (Fig. 1b), implying that the N deficiency for crop demand might be counteracted without consuming stable microbial necromass.

When N application rate was reduced to 135 kg ha⁻¹ with maize residue removal, the deficiency of the input N for maintaining maize growth was estimated to be 161 kg ha⁻¹ (Fig. 2d), and the microbial necromass N decreased by 102 kg ha⁻¹ relative to the SON240 treatment (Fig. 1b). In the SON0 treatment, the N deficiency was up to 342 kg ha⁻¹, and the decline in microbial necromass N was 171 kg ha⁻¹, although maize yield decreased from the second year of treatment (Fig. S2).

Evidently, the decline in microbial necromass was positively correlated with the deficiency of N uptake by crops ($r = 0.58$, $p = 0.05$), being consistent with our hypothesis. However, the microbial necromass N stock decreased by only 4.4%–7.3%, and the declined amount was significantly lower than that of N deficiency, even if maize yield decreased sharply (Fig. S2c). Comprehensively, these findings not only confirmed the ability of microbial necromass to compensate for crop N demand via decomposition but also clarified the preferential role of microbial necromass in soil N retention. More importantly, sustainable crop growth cannot merely depend on the mineralization of the soil N pool; instead, the significant decrease in microbial necromass essentially denotes a decline in soil productivity.

In the plots subjected to maize residue mulching, the stock of microbial necromass N did not change significantly during the experiment even when no N fertilizer was applied (Fig. 1b). While the increased N input with maize residue return significantly alleviated N deficiency for crop demand, the decomposition of microbial necromass was balanced by its enhanced production. Compared with removal, maize residue mulching did not influence the NH_4^+ -N levels but decreased NO_3^- -N content in all the fertilized treatment (Fig. 2a and b), indicating that improved C availability competitively inhibited nitrification with enhanced microbial N immobilization (Li et al., 2020). Thus, maize residue return improved the N retention efficiency of soil and could reduce N loss potential.

Under maize residue removal, the decline in both bacterial ($p < 0.001$) and fungal ($p = 0.092$) necromass N over the 3 years of the experiment was positively correlated with fertilizer reduction, but with a lower decreased magnitude of fungal necromass N (Fig. 1c; S1b, c). Such a pattern might be partly attributed to the higher resistance of fungal necromass than bacterial necromass, as principally interpreted by Paul and Clark (1989). Compared with removal, maize residue return mitigated the decline in bacterial necromass N and simultaneously increased the accumulation of fungal counterparts to a greater extent in all the 3 fertilization reduction treatments (Fig. 1c, Table S1), which was mostly associated with the stronger competitiveness of fungi for recalcitrant substrate decomposition (Strickland and Rousk, 2010). Therefore, maize residue return enhanced N storage in microbial necromass and favored stabilization of microbial necromass pool under reduced N fertilization.

In summary, the dynamics of soil microbial necromass in response to changing C and N status indicate its critical roles in both N supply and maintenance, but with the latter function being dominant. Bacterial necromass N was apt to degrade under N deficiency in the soil-crop system, whereas fungal necromass N mainly contributed to the stability of the soil N pool. Essentially, it denoted a decline in soil productivity when the microbial necromass N stock declined when no N fertilizer was applied. Crop residue mulching alleviated the N deficiency for plant demand and counteracted the decline in microbial necromass N stock by preferentially increasing the net accumulation of fungal necromass, thus favoring N retention in microbial necromass and improving the stability of microbial necromass pool.

Declaration of competing interest

The authors declare that they have no known competing financial

interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.soilbio.2021.108512>.

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