Plant Soil
https://doi.org/10.1007/s11104-024-06764-x

RESEARCH ARTICLE

Leguminous green manure promotes N accrual in labile and persistent soil organic matter pools

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Received: 23 October 2023 / Accepted: 20 May 2024

Abstract

Background and Aims Soil organic nitrogen (SON) provides an important source of crop N uptake. Our study aimed to better understand N incorporation in soils and the importance of various labile and persistent SOM pools in supplying plant-available N.

Methods In this 80-day greenhouse study, we used ^{15}N isotope to trace the fate of N from green manure (Austrian winter pea) vs. ammonium sulfate among uptake by wheat plants, accrual to various soil pools, and losses. We compared new N retention and changes in existing SON reserves between two soils with long-term histories of receiving leguminous green manure or solely inorganic fertilizer (F_i) inputs.

Results The green manure had a greater capacity to build SON pools, with 51% of the green manure N retained in soil compared to 31% of the ammonium-N recovered from soil. Soil with historical green manure inputs showed a 19% greater SON content and an 18% greater plant N uptake than soil with a history of solely F_i input. Microbial assimilation of the newly added N was positively correlated with N incorporation in the mineral-associated organic matter (MAOM) and the occluded particulate organic matter (oPOM) pools, which were negatively associated with N loss. Moreover, plant N uptake was positively correlated with initial oPOM-N content and the corresponding decline in oPOM-N as plants grew.

Conclusions Enhancing microbial N assimilation and its subsequent incorporation into soils, particularly the oPOM pool, can be an effective strategy to reduce N loss and to replenish SON for long-term soil fertility.

Keywords Soil N accrual · POM · MAOM · ^{15}N isotope · Plant N acquisition

Abbreviations

C	Carbon
ENM	Ecological Nutrient Management
F_i	Inorganic fertilizer
F_{org}	Organic fertilizer
fPOM	free Particulate Organic Matter
N	Nitrogen
MAOM	Mineral-Associated Organic Matter
Mbio	Microbial biomass
N	Nitrogen
oPOM	occluded Particulate Organic Matter

Responsible Editor: Elizabeth M Baggs.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11104-024-06764-x.

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Published online: 10 June 2024

SOM Soil Organic Matter
SON Soil Organic Nitrogen

Introduction

Nitrogen (N) loss from croplands is a persistent problem in modern food production systems that contributes to greenhouse gas emissions and water pollution (Howarth et al. 2002; Venterea et al. 2012). Conventional N management has primarily focused on improving fertilizer use efficiency by enhancing crop uptake of the added fertilizers and/or inhibiting fertilizer loss pathways (Dimkpa et al. 2020). Such management strategies, e.g., 4Rs (application of the Right nutrient sources at the Right amount, at the Right time, and in the Right place), can maintain crop productivity while providing some improvements in N use efficiency, largely by reducing the magnitude of surplus N additions (Johnston and Bruulsema 2014). However, cropping systems with sole reliance on inorganic fertilizer (F_i) may deplete endogenous soil N reserves over time (Mulvaney et al. 2009; Ladha et al. 2011), which contributes to the "fertilizer treadmill" that intensifies F_i dependency and the associated environmental risks (Drinkwater and Snapp 2007; Houser and Stuart 2020).

Soil organic N (SON) is a major source of plant available N. Based on meta-analyses of ^{15}N fertilizer tracer experiments conducted in annual grain cropping systems, over half of the crop N uptake is derived from existing soil N pools rather than the newly added fertilizers even when adequate N fertilizer is added (Gardner and Drinkwater 2009; Yan et al. 2020). Despite many efforts to improve crop assimilation of added fertilizers, on average only 43% of the applied fertilizer N is taken up by the intended crops in annual grain systems (Yan et al. 2020). The remaining fertilizer N is either incorporated into SON, which can be available for crop uptake in subsequent seasons or lost to the environment. As such, increasing N retention in soil is important to reduce off-farm N loss and to replenish soil N reservoirs for long-term soil fertility (Xu et al. 2021).

Ecological nutrient management (ENM) is a broad framework that aims to reduce N losses by increasing N flows to soil organic matter (SOM) reserves while maintaining crop production (Drinkwater and Snapp 2007, 2022). Recognizing the importance of the internal cycling capacity of soil food webs to retain N in soil and to support plant N acquisition from SOM, ENM emphasizes managing soil carbon (C) abundance in conjunction with N additions to fuel soil communities. ENM practices that re-couple soil C and N cycling, such as the use of legume cover crops and the co-amendment of organic C with F_i, have shown success in improving SOM conditions and crop productivity (Wei et al. 2016; Abdalla et al. 2019; Zhao et al. 2022). Differences in F_i and organic fertilizer (F_{org}) inputs can further alter SOM composition and soil microbiome (Li et al. 2019); these management-induced effects tend to persist and are commonly referred to as "management history" or "legacy" effects (Cuddington 2011). For instance, soils with historical F_{org} inputs support a greater plant N uptake and a greater abundance of microbial genes involved in the nitrification and denitrification processes as compared to soils with F_i input histories (Schmidt et al. 2020). A better mechanistic understanding of how SOM quantity and composition affect N partitioning among plant uptake, soil retention, and losses will help to develop effective ENM strategies for agronomic and environmental benefits.

The distribution of N in various labile and persistent SOM pools is important in affecting their turnover rates in soil and subsequent accessibility for plant acquisition. Conventional understanding of the soil N cycle highlights microbial de-polymerization and mineralization of labile particulate organic matter (POM), and assumes that plant roots are passive players in this process and would only take up "residual" ammonium and nitrate after the N demand of soil microbes has been met (Schimel and Bennett 2004). However, there is growing evidence that plant roots actively acquire N from SOM pools by excreting carbohydrates in the rhizosphere to simulate microbial mineralization of SON (e.g., priming effect) (Jackson et al. 2008; Meier et al. 2017). Such root priming can occur in the POM surfaces or in the mineral-associated organic matter (MAOM) pool (Jilling et al. 2018). The relative importance of POM vs. MAOM in supplying bioavailable N may depend on the ratio of incoming supply of POM-N to mineral sorption potential and the specific nature of microbial communities and plant-soil interactions (Daly et al. 2021). The degree of root priming of POM vs MAOM has important implications for the persistence of SOM (Cotrufo et al. 2019). However, direct experimental testing of plant N acquisitions from POM and MAOM pools remains rare due to the difficulty of tracking changes in various SON pools.

Our study aimed to better understand how SOM quantity and composition and newly added N sources would affect N partitioning among crop uptake, N losses, and N accrual to soil pools. We explicitly targeted the knowledge gaps regarding the accrual of newly added N in various SOM pools and the importance of different SON pools for plant N acquisition. To provide a thorough and accurate tracking of SOM pools and fluxes, we conducted a pot study in the greenhouse to minimize potential confounding effects from soil heterogeneity and other variability under field conditions. We compared ammonium vs. leguminous green manure as N sources in two soils from fields with distinct management histories. The high SON (HON thereafter) soil was collected from a certified organic farm where symbiotic N from leguminous cover crops is the primary N source with small additions of composted animal manures. The low SON (LON thereafter) soil was collected from a conventional farm where synthetic N fertilizers were the sole N source. These soils have similar inherent properties but differ in SOM quantity/composition, N cycling and microbial metabolic function (Schipanski and Drinkwater 2012; Berthrong et al. 2013; Han et al. 2017). We hypothesized that N source would be the main factor driving new N retention vs. loss. We predicted that (1) new N accrual to SOM pools would be greater from green manure compared to ammonium, and (2) greater N retention in soil would correspond with reduced environmental losses of newly added N. We also expected that plant N uptake would be largely from existing SON reserves rather than newly added N and hypothesized that (3) the HON soil would have larger oPOM and MAOM pools and support greater plant N uptake compared with the LON soil.

Materials and methods

Soil collection and soil characterization

We collected soils from two neighboring commercial grain fields near Penn Yan, New York, USA (42° 40.3′N, 77° 2.4′W). The two fields are located on the same soil series of Honeoye (fine-loamy, mixed, semiactive, mesic Glossic Hapludalfs, USDA Soil Taxonomy 2014; haplic ochric Luvisol, World Reference Base for Soil Resource, 2014) and have been under distinct management regimes for over 20 years at the time of the study. The certified organic field relies primarily on symbiotic N fixation and uses a diverse rotation of soybean (*Glycine max* (L.) Merr.)-spelt (*Triticum spelta* L.)-red clover (*Trifolium pratense* L.)-maize (*Zea mays* L.) with small, periodic additions of poultry manure (2–4 Mg ha^{-1}). The adjacent conventional field had been in continuous maize production fertilized with Haber–Bosch N and inorganic P and K fertilizers. Both fields were tilled in preparation for planting or weed control (the organic field). We collected soil on May 1st, 2017, when the conventional field had corn residues visible on the bare soil surface, and the organic field had been planted with spelt the previous fall. The spelt was still small and was 10–20 cm in height. In each field, we delineated four 6 m×6 m replicate plots along a 24 m transect, with the two transects being ~ 30 m apart and parallel to each other on similar slopes. Soil was collected using 7.5 cm (diameter)×15 cm (depth) soil cores and combined from 30–40 cores within each plot, resulting in four soil sources per field. A detailed sampling layout in the field was provided in Fig. S1 (Online Resource 1). Soil was stored in plastic buckets with lids on at 4 °C for ~ 1.5 months before use in the experiment. One week before the greenhouse experiment, soil was sieved through 9-mm sieves to remove large rocks and plant residues while avoiding the disruption of large soil aggregates. A subset of the sieved soils was set aside to quantify baseline soil C and N pools and other soil characteristics at the start of the greenhouse experiment (Sect. "Fractionation of soil N and C pools").

Experiment design and pot set up in the greenhouse

To prepare the leguminous green manure, Austrian winter pea (*Pisum sativum* subs. arvense (L.) Poiret), a common winter cover crop in the Northeastern USA, was grown in a growth chamber and enriched with ^{15}N by adding enriched ammonium sulfate (99% atom percentage of ^{15}N, Sigma-Aldrich, St. Louis, MO, USA) periodically during pea growth. Pea shoots were harvested after 4 weeks of growth. The resulting green manure had a N concentration of 19.6 mg g^{-1} and a C concentration of 418 mg g^{-1}, with a enrichment level of 37% ^{15}N atom percent. The ^{15}N-encirched ammonium sulfate was mixed with the natural abundance ammonium sulfate to create a similar 37% ^{15}N enrichment level before use in the greenhouse experiment. Details of pea shoot generation and isotope enrichment are provided in Text S1 (Online Resource 2).

We grew wheat plants in pots (12 cm × 12 cm × 30 cm depth) filled with field-collected soils. Wheat seeds (*Triticum aestivum* L. cv. Glenn) were kindly provided by Dr. Mark Sorrells (Plant Breeding, Cornell University, Ithaca, NY). To allow for two sampling dates, one at the anthesis stage (T1) and another at the grain stage (T2), we established 32 pots consisting of two soils × two N fertilizers × two sampling dates × four replicates per treatment. We also established four additional pots (two per soil type) which received no N fertilizers to obtain the baseline level of ^{15}N natural abundance of native soil N acquired by the wheat plants. Anion resin beads enclosed in finely woven silk cloth bags were placed at the bottom of each pot to collect NO_3- leaching throughout the experiment. To encourage the acquisition of SON reserves by wheat plants, we used a low rate for newly added N sources. The ^{15}N-encirched ammonium sulfate or the pea shoots, which were first cut into small pieces of 1–2 cm in length, were mixed with 3 kg dry weight equivalent of soil per pot to achieve a rate equivalent to 22 kg N ha^{-1} (or 11 μg N g^{-1} soil). Fertilizers were manually mixed with soil in plastic bins on the day of sowing before being transferred to pots. We further added perlite (30% v:v, around 120 g per pot) to the fertilizer/soil mix to help with drainage in the pot environment. Five wheat seeds were planted in each pot and thinned to three plants one week after germination.

We arranged the pots in a randomized block design, with blocks composed of one pot from each of the 4 soil/fertilizer treatments. Soils from the numbered field plots were randomly arranged within the same block (e.g., soils from plot #1 in each field were all in block 1, etc.). The greenhouse received supplemental light to maintain 16:8 h of light (26 °C): dark (22 °C) cycles. Nitrogen-free Hoagland solutions were added to the pots to provide nutrients other than N and control for differences in extractable P and K between the two soils. We watered the plants with distilled water two to three times a week which simulated ~ 4 cm of rain per week throughout the experiment and did not observe any pest problems.

Plant harvest

Plants were destructively sampled at the anthesis stage (**T1**, Day 40 after germination) and at the grain stage (**T2**, Day 80 after germination). Shoots were cut at the soil surface and placed in separate paper bags for oven drying. We carefully emptied the soil from each pot into a large plastic bin and the root system of each plant was separated by gently removing soil while keeping most of the roots still attached to the crown. The root system was washed clean of soil under running tap water and further rinsed with deionized water. The crown was then clipped and combined with the shoots from the same pots. Grains from T2 were manually removed from the plants and placed in paper bags. The separated shoots and roots (T1 and T2) and grains (T2) were oven-dried at 65 °C for at least 48 h to obtain dry weights.

Fractionation of soil N and C pools

Soil OM fractions and inorganic N pools were quantified for soil samples collected from the pot experiments (T0, T1, and T2) following the procedures in Fig. 1. First, intact and partially decomposed litter was extracted using the wet sieving method (Elliott and Cambardella 1991). Litter retained on the 2-mm sieve (macro-OM > 2 mm) and the 0.5-mm sieve (macro-OM 0.5–2 mm) were collected. Particulate organic matter (POM) was extracted using the size/density fractionation method described in detail by Marriot and Wander (2006). The density separation step used a sodium polytungstate solution (SPT, $Na_6H_2W_{12}O_{40}$, density = 1.78 g cm^{-3}) to separate the free POM (fPOM) from the heavier POM occluded in soil aggregates (oPOM, > 1.78 g cm^{-3}) (Marriott and Wander 2006). To separate oPOM (≥ 53 μm) from MAOM which is associated with silt and clay particles (< 53 μm), the heavy fraction was dispersed with sodium hexametaphosphate before wet sieving. Particulate OM and sand remaining on the sieve were separated by decanting. Both fractions (fPOM and oPOM) were then analyzed for C and N. Whole soil samples (air dry, sieved < 2 mm) was used to quantify total C and N in the soil. The dissolvable C and N pool and microbial biomass were extracted from moist soils (sieved < 2 mm) using the chloroform fumigation method with 0.05 M K_2SO_4 as the extraction solution (T0 and T2 soils only) (Brookes et al. 1985; Bruulsema and Duxbury 1996). Inorganic N pools (NH_4^+, NO_3-, T0 soil only) were extracted in a 2 M KCl solution. We did not directly quantify the mineral-associated organic matter (MAOM) pool, which was calculated by subtracting POM, dissolvable pool, microbial biomass

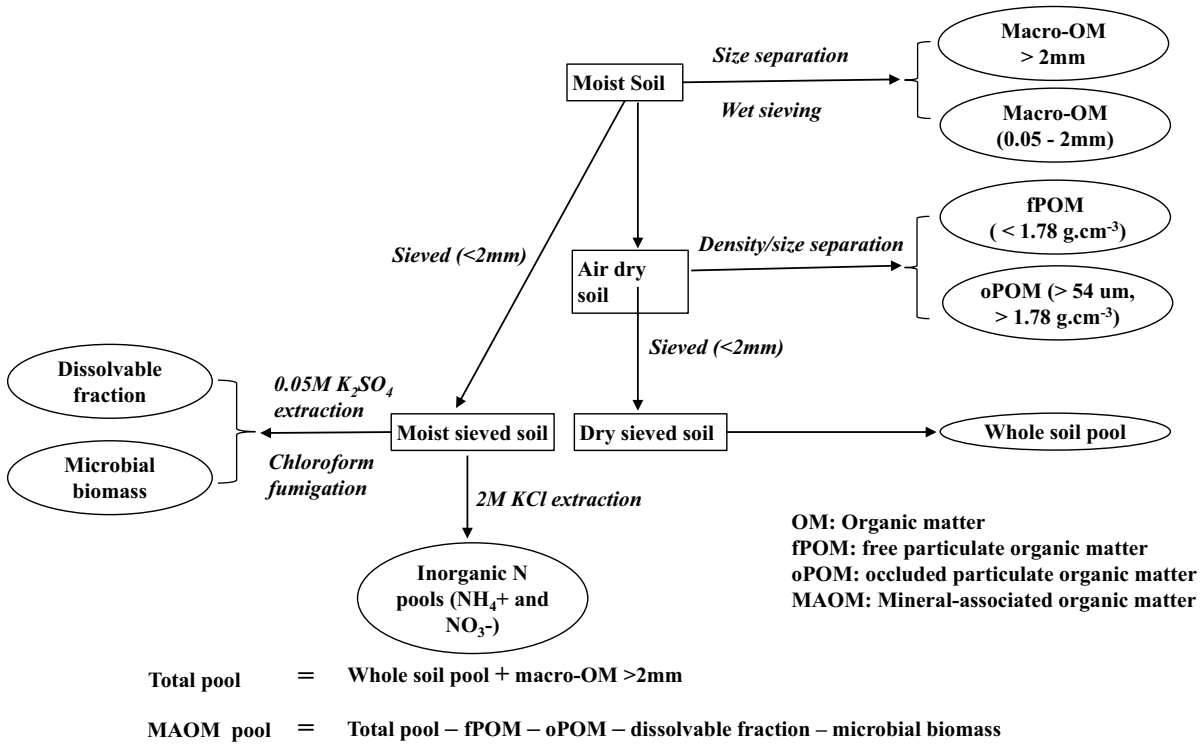

Fig. 1 Various SOM pools quantified in this study. Each pool's C and N content and their ^{15}N composition were measured. Method details are provided in Online Resource 2

from the total pool (whole soil + macro-OM > 2 mm) in the pots. Details of extraction methods for different C and N pools were provided in Online Resource 2. In addition, soil pH, cation exchange capacity, and extractable nutrients (Mehlich-3 extractant) of T0 soil samples were measured by the Penn State Agricultural Analytical Services Lab (College Park, PA, USA).

Stable isotope analysis

Nitrogen isotopic composition of plant samples and various soil pools were analyzed by the UC Davis Stable Isotope Facility (Davis, CA, USA). The harvested shoots, roots, and grains were finely ground using a Wiley mill (Thomas Scientific Inc., Swedesboro, NJ, USA) followed by a ball mill (Retsch mm200, Verder Scientific Inc., Newtown, PA, USA). Phosphorous and other macro- and micronutrient concentrations of the grain were analyzed by the Cornell Nutrient Analysis Lab at Cornell University (Ithaca, NY, USA).

Whole soils (<2 mm) and the extracted materials (macro-OM > 2 mm, macro-OM 0.5-2 mm, fPOM, oPOM) were oven dried at 65 °C for 48 h. The dissolvable pool and microbial biomass that were extracted in salt solutions were lyophilized to obtain their dry weights. All samples were finely ground and packed in tin capsules for isotopic analyses. Leachate NO_3- collected by the anion resin bags was first recovered with a 2 M KCl solution and then reduced to NH_3 using Devarda's alloy following the procedure described in Goerges and Dittert (1998). The resulting NH_3 was collected by filter papers infused with $KHSO_4$ solution and the filter papers were packed in tin capsules for ^{15}N analyses. The ^{15}N isotopic signatures of the samples were reported as the δ values as well as the atom percentage (atom%, the percentage of the heavier isotope from the total isotopes).

Calculation of new N in various pools

The contribution of new N (f_{new}, i.e., the N fraction derived from the newly added N) and existing soil N (f_{old}, i.e., the N fraction derived from existing soil N pool) in plant uptake and various soil pools was calculated using two-source mixing models:

$$f_{new} = (\delta_{new} - \delta_{old})/(\delta_{harvest} - \delta_{old}) \quad (1)$$

$$f_{old} = 1 - f_{new} \quad (2)$$

whereas δ_{new} is the $\delta^{15}N$ of the newly added fertilizer, δ_{old} is the natural abundance $\delta^{15}N$ of existing soil pools measured at T0 (or $\delta^{15}N$ of plant tissues from the no-fertilizer reference pots), and $\delta_{harvest}$ is the enriched $\delta^{15}N$ of various soil pools or plant samples harvested at T1 or T2.

The proportion of the newly added N (*NewN*, %) distributed in plants and various soil pools was then calculated as:

$$NewN(\%) = (N\ amount\ in\ plants\ or\ soil\ pools\ x f_{new})/total\ new\ N\ added\ x\ 100\% \quad (3)$$

The amount of newly added N that was not recovered in plants, soils, or leachate was considered lost through gaseous pathways.

Statistical analyses

The difference in initial soil characteristics between the LON and the HON soils was evaluated using students' t-tests. To evaluate the effects of soil legacy (LON vs. HON) and N source (ammonium vs. green manure) on plant productivity and plant nutrient uptake, we used linear mixed models with the two-way factors and their interactions as fixed factors and block as a random factor. Sampling date (T1 vs T2) was also included as a fixed factor in the models when measurements from both sample dates were available. Similar linear mixed models were used to assess the effects of soil legacy and N source on the fates of the newly added N, as well as changes in various soil pools after the greenhouse experiment. Net changes in various soil N pools were calculated by subtracting N pools at harvest (T2) from initial N pools at the beginning of the study (T0 soil N + newly added N). Negative values would indicate a decline in specific soil pools after the 80-day experiment. To understand the drivers underlying the distribution of the newly added N, correlation analyses were used to explore the relationships among plant uptake, soil incorporation, vs. losses of the newly added N. For any significant pairwise correlations, we also added N source as a covariant to examine if the relationships remain after controlling for difference between the two N input types. In addition, correlations between plant N uptake and initial soil N pools as well as their net changes were evaluated to identify which soil N pool is most accessible for plant uptake. For all linear mixed models, data were log or square root transformed where necessary to meet the requirement of normal distribution and homogeneity of variance, which were verified by examining normal probability plots and residuals vs. fitted values. ANOVA was performed for each linear model to assess the significant effect of fixed factors. We used R software version 2.15 for all data analyses (R Core Team 2023) with the following packages: *lme4* (Bates et al. 2015) for linear mixed modeling and *emmeans* for post hoc comparisons of least-square means (Lenth 2016).

Results

Initial soil characteristics at the start of the greenhouse experiment

Both soils are similar in texture (49% sand and 18% clay), pH (pH = 7.4), and cation exchange capacity (12.0 cmol kg^{-1}). However, soil N (sieved < 2 mm) was 19% greater in the HON soil than the LON soil (1701 vs. 1435 µg g-1 respectively, Table 1), and this pattern was consistent in the MAOM pool, the dissolvable fraction, and the nitrate–N pool ($P < 0.01$). Occluded POM-N was 27% higher in the HON than in the LON soil ($P = 0.09$) and had a greater within-field variability than other N pools. In contrast, C and N stocks in the macro-OM (> 2 mm and 0.5-2 mm) and fPOM pools were greater in LON than the HON soil, probably due to corn stover litter in the soil profile and on the surface at the time of collection. In addition, C:N ratio was wider in the LON soil than in the HON soil for fPOM, oPOM, and the dissolvable fraction (Table 1). Overall, a majority (~ 87%) of the soil N was distributed in MAOM, with ~ 9% residing in the POM pools, ~ 3% in the microbial biomass, and < 1% in the immediately available inorganic ammonium and nitrate forms. Lastly, LON soil had a greater extractable P and K resulting from residual inorganic fertilizer in the LON field.

Table 1 Soil N and C pools and other soil characteristics at the start of the greenhouse experiment (T0). Data are reported as means averaged from four field plots for each soil, with standard error reported in brackets. *P* values from the Student's t-test between the two soils are also included. Bold values are statistically higher than those from the other soil at $P < 0.05$

Initial soil properties	N (nutrient) conc., μg g^{-1} soil			C conc., mg g^{-1} soil			C:N ratio		
	LON soil	HON soil	P val	LON soil	HON soil	P val	LON soil	HON soil	P val
SOM pools									
Total	1455 (27)	**1706 (32)**	*< 0.001*	16.5 (0.77)	17.8 (0.47)	*0.14*	11.3 (0.4)	10.4 (0.22)	*0.09*
Soil (< 2 mm)	1435 (26)	**1701 (31)**	*< 0.001*	16 (0.78)	17.7 (0.47)	*0.08*	11.1 (0.42)	10.4 (0.23)	*0.14*
MAOM	1259 (25)	**1487 (16)**	*< 0.001*	13.4 (0.82)	15.1 (0.3)	*0.10*	10.7 (0.52)	10.1 (0.24)	*0.35*
Macro-OM > 2 mm	**20.6 (2.8)**	5.4 (0.7)	*0.006*	**0.5 (0.06)**	0.14 (0.02)	*0.002*	24.5 (0.99)	25.8 (1.68)	*0.50*
Macro-OM 0.5—2 mm	**27.8 (2.2)**	19.4 (2.7)	*0.03*	**0.51 (0.04)**	0.34 (0.05)	*0.03*	18.1 (0.38)	17.5 (0.25)	*0.20*
fPOM	**47.8 (6)**	31.3 (3.1)	*0.04*	**1.17 (0.16)**	0.67 (0.07)	*0.03*	**24.3 (0.91)**	21.5 (0.42)	*0.03*
oPOM	95 (6.5)	121(12.6)	*0.09*	1.57 (0.09)	1.68 (0.2)	*0.57*	**16.5 (0.2)**	14 (0.14)	*< 0.001*
Dissolvable fraction	13.9 (0.5)	**19.8 (1.2)**	*0.005*	0.11 (0.003)	0.11 (0.009)	*0.80*	**7.7 (0.21)**	5.3 (0.45)	*0.004*
Microbial biomass	39.7 (1.9)	47.1 (5.1)	*0.19*	0.19 (0.03)	0.283 (0.042)	*0.09*	4.7 (0.57)	6 (0.83)	*0.19*
Inorganic nutrient pools									
Ammonium-N (KCl extraction)	1.82 (0.06)	1.73 (0.08)	0.39						
Nitrate–N (KCl extraction)	6.5 (0.33)	**13.9 (1.04)**	*0.001*						
Extractable P (Mehlich 3)	**93.5 (6.6)**	19.8 (1.9)	*< 0.001*						
Extractable K (Mehlich 3)	**189.8 (8.7)**	71.3 (11.9)	*< 0.001*						

The fate of the newly added N

Consistent with our first prediction, N accrual to various soil N pools was consistently greater from green manure compared to the ammonium fertilizer (Table 2, N source, $P < 0.01$ for all fractions), and such effects were similar in both soils (N source x Soil, $P > 0.05$ for all fractions except for $P = 0.033$ with fPOM) and in both sampling dates (N source x Date, $P > 0.05$ for all fractions) (Table 2). Total N retention in soil was not affected by soil legacy (Soil legacy, $P = 0.106$) and declined from the anthesis stage to grain harvest (Sampling date, $P = 0.004$) (Table 2). At grain harvest, about half of the N from green manure was retained in soil (55% and 47% for LON and HON soils, respectively) as compared to about 30% of the ammonium N was recovered in soil (34% and 27% for LON and HOM soils, respectively) (Fig. 2). The majority of organic N retained in soil had been processed by decomposers and resided in MAOM, oPOM and microbial biomass regardless of soil legacy (Fig. 2). A small fraction (7 to 8%) of organic N derived from the added green manure was recovered in the fPOM pool which may contain original green manure fragments or newly senesced wheat roots enriched with ^{15}N derived from mineralization.

The amount of new N taken up by plants differed by N treatments (Table 2, N source, $P = 0.003$) and was not impacted by soil legacy ($P = 0.224$) or sampling date ($P = 0.32$) (Table 2). The ammonium fertilizer was more accessible for plant uptake than green manure, with 30% of the ammonium N vs. 25% of the green manure N taken up by wheat plants at grain harvest (Fig. 2, averaged from both soils).

Loss of the newly added N (leaching + not recovered) was affected by N source ($P < 0.001$) and soil legacy ($P = 0.048$) but did not differ between sampling dates ($P = 0.07$) (Table 2). The proportion of new N lost was higher with the ammonium treatments than in the green manure (43% vs 30% in the HON soil, and 36% vs.18% for the LON soil) (Fig. 2) and was higher in the HON soil than the LON soil. For both N sources, a proportion (17 to 38%) of the newly added N was not recovered while leaching (0.7 to 4.9%) was the minor loss pathway (Fig. 2). Soil legacy interacted with N source in affecting leaching loss (Table 2, Soil legacy x N source, $P = 0.005$), in that leaching from

Table 2 The amount of newly added N (μg g^{-1} soil) recovered in soils, plants, or lost out the pot. Each pot received 11 μg g^{-1} soil (or 33 mg per pot) of new N from ammonium or green manure at the beginning of the experiment. Data are means (±S.E.) for each soil/fertilizer treatment. *P* values associated with treatment effects from linear mixed models of three factors (Soil legacy, N source, and Sampling date) are also provided. When there are significant Soil x N source effects, lowercase letters indicate differences between treatments within the same sampling date from *post-hoc* Tukey comparisons at $P < 0.05$. Mbio: microbial biomass

Soil legacy	N source	Retention of new N in various soil pools						Plant uptake of new N	Loss of new N		
		fPOM	oPOM	Dissolvable	Mbio	MAOM	Total		Leaching	Not recovered	Total loss
Anthesis stage (day 40 after germination)											
LON	Ammonium	0.63 (0.03) bc	0.45 (0.03)	na	na	na	3.9 (0.5)	3.7 (0.1)	0.11 (0.02) b	3.5 (0.5)	3.6 (0.5)
	Green manure	0.95 (0.03) a	0.94 (0.07)	na	na	na	7.4 (0.2)	2.2 (0.3)	0.02 (0.01) c	1.4 (0.3)	1.4 (0.3)
HON	Ammonium	0.38 (0.08) c	0.33 (0.06)	na	na	na	4.0 (0.6)	2.8 (0.5)	0.46 (0.11) a	3.8 (1.0)	4.3 (1.0)
	Green manure	0.92 (0.11) ab	0.92 (0.09)	na	na	na	6.4 (0.6)	2.5 (0.3)	0.12 (0.04) b	2.0 (0.47)	2.1 (0.5)
Grain harvest (day 80 after germination)											
LON	Ammonium	0.57 (0.09) bc	0.36 (0.02)	0.13 (0.01)	0.55 (0.04)	2.23 (0.54)	3.8 (0.5)	3.3 (0.3)	0.15 (0.04) b	3.9 (0.5)	4.0 (0.4)
	Green manure	0.86 (0.1) a	0.68 (0.07)	0.21 (0.02)	0.79 (0.09)	3.46 (0.48)	6.0 (0.5)	3.0 (0.3)	0.08 (0.02) b	1.9 (0.2)	2.0 (0.2)
HON	Ammonium	0.42 (0.05) c	0.3 (0.04)	0.10 (0.01)	0.41 (0.03)	1.76 (0.07)	3.0 (0.1)	3.3 (0.3)	0.54 (0.04) a	4.3 (0.4)	4.8 (0.4)
	Green manure	0.83 (0.08) ab	0.65 (0.06)	0.18 (0.02)	0.66 (0.09)	2.89 (0.14)	5.2 (0.1)	2.5 (0.2)	0.12 (0.04) b	3.2 (0.3)	3.3 (0.2)
P values											
Soil legacy		0.153	0.066	0.050	0.088	0.26	0.106	0.224	**< 0.001**	0.062	**0.048**
N source		**< 0.001**	**< 0.001**	**< 0.001**	**0.005**	**0.002**	**< 0.001**	**0.003**	**< 0.001**	**< 0.001**	**< 0.001**
Sampling Date		0.326	**0.001**	na	na	na	**0.004**	0.32	0.100	**0.036**	0.07
Soil x N source		**0.033**	0.215	0.926	0.796	0.98	0.340	0.55	**0.005**	0.240	0.730
Soil x Date		0.420	0.683	na	na	na	0.477	0.898	0.561	0.611	0.640
N source x Date		0.375	0.050	na	na	na	0.198	0.414	0.925	0.400	0.540
Soil x N source x Date		0.461	0.615	na	na	na	0.331	0.069	0.376	0.795	0.700

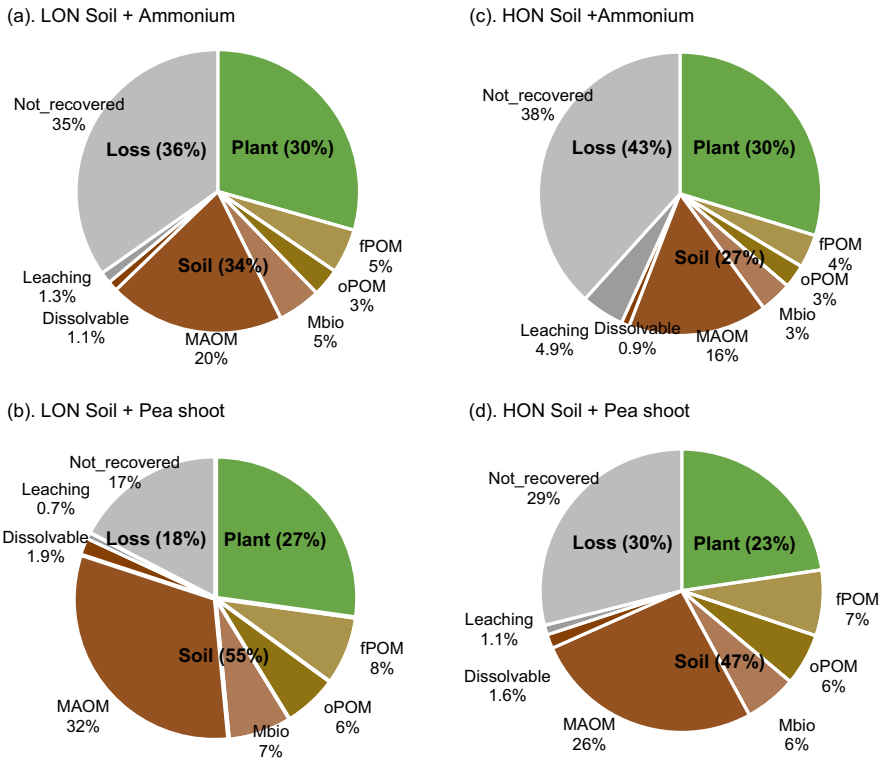

Fig. 2 Proportions of the newly added N recovered from various soil pools, plants, or lost out of the pots at grain harvest. Data (mean ± S.E.) of the absolute amount of new N are provided in Table 2. Plant: total N uptake in roots, shoots, and grains; Soil: N retention in fPOM, oPOM, microbial biomass (Mbio), MAOM, and the dissolvable pools; Loss: N losses in leachate and the not-recovered pool

the HON soil with ammonium fertilizer (Fig. 2c, 4.9%) was almost five times higher than leaching from the other three treatments (Fig. 2a, b &d, 0.7 to 1.3%). However, total new N loss was comparable between the conventional (LON soil with ammonium) and the organic (HON soil with green manure) treatments. Furthermore, most of the leaching and gaseous loss occurred primarily during the first 40 days (Table 2), accounting for an average of 78% of total N lost over the course of this 80-day experiment.

Consistent with our second prediction, loss of the newly added N was negatively associated with the retention of new N in various soil fractions (Fig. S2, $r = -0.62$ to -0.82, $P < 0.05$ for all soil fractions including fPOM, oPOM, dissolvable fraction, microbial biomass, and MAOM). The most significant relationship was between new N loss and microbial N assimilation ($r = -0.82$, $P < 0.001$, Fig. 3a), and the negative correlation remains significant ($P = 0.01$) after controlling for confounding effects from different N sources. In contrast, there was no relationship between new N loss and plant uptake of the newly added N (Online Resource 1, Fig. S3). In addition, new N retention in various soil fractions was positively correlated with microbial assimilation of the newly added N (Fig. S2, $r = 0.60$ to 0.74, $P < 0.05$). The most significant relationship was between new N retention in microbial biomass and MAOM ($r = 0.74$, $P < 0.001$, Fig. 3b), and such positive correlations remained marginally significant after including N source as a random factor ($P = 0.051$).

Effects of soil legacy and N source on plant nutrient uptake and plant productivity

Total N uptake did not differ among treatments at the anthesis stage (Fig. 4a) but was 18% greater in the HON soil than in the LON soil at grain harvest (Fig. 4b); the magnitude of this difference was similar to the initial difference in total N reserves between the two soils. Newly added N accounted for 5 to 8% of total plant N uptake (Fig. 4a), and uptake of both sources of the new N plateaued at anthesis (Table 2, Sampling date, $P = 0.32$). In contrast, plant acquisition of soil N continued after anthesis, reaching > 95% of total N uptake at grain harvest (Fig. 4b). For the most part, other nutrient content in the grain was not significantly different across treatments. Grain P

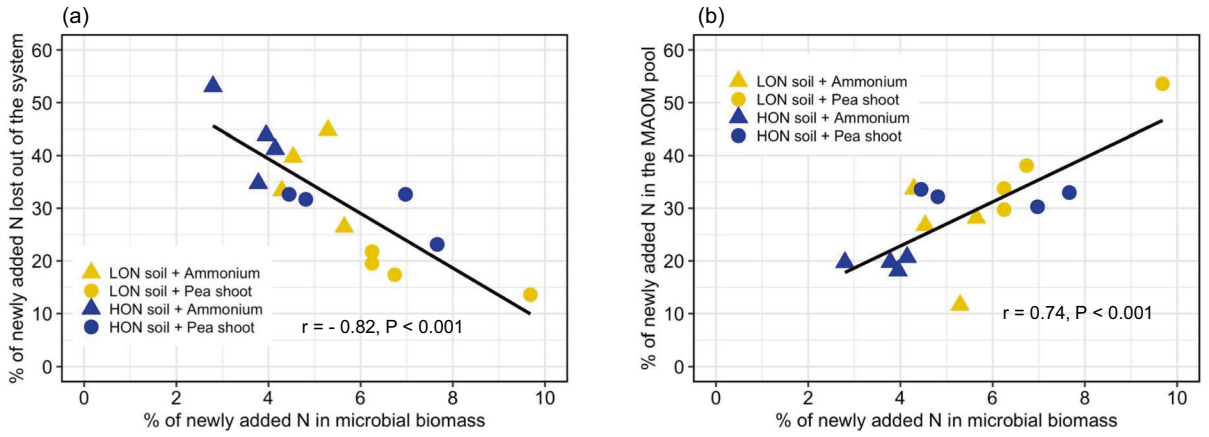

Fig. 3 Correlations between the proportion of new N incorporated in microbial biomass and that lost out of the pots (**a**) or recovered in the MAOM pool (**b**) at grain harvest

concentrations were similar among treatments (Online Resource 3, Table S2), suggesting that the addition of P and other nutrients in the N-free Hoagland solution compensated for the initial difference in P availability between the two soils. Grain K, Mg, Zn, Fe, and other nutrient concentrations were similar among treatments, with the exception of greater grain Ca and Cu concentrations in wheat grown in the HON soil compared to the LON soil (Table S2). Grain S concentration was higher with the ammonium sulfate fertilizer treatment.

Plant biomass at the anthesis stage was affected by an interactive effect of soil legacy and N source (Soil legacy x N source, $P=0.02$, Fig. 5a). Total biomass was 22% greater in the LON soil than in the HON soil (Soil legacy, $P=0.008$); within the LON soils, plants produced 11% more biomass when receiving ammonium fertilizer than those receiving green manure. The overall lower biomass in the HON soil was associated with a 35% lower shoot P concentration but a 33% greater shoot N concentration than in the LON soil (Online Resource 3, Table S1), suggesting that early plant growth in the HON soil was primarily limited by soil P availability. Such P limitation in the HON soil during early plant growth was further demonstrated

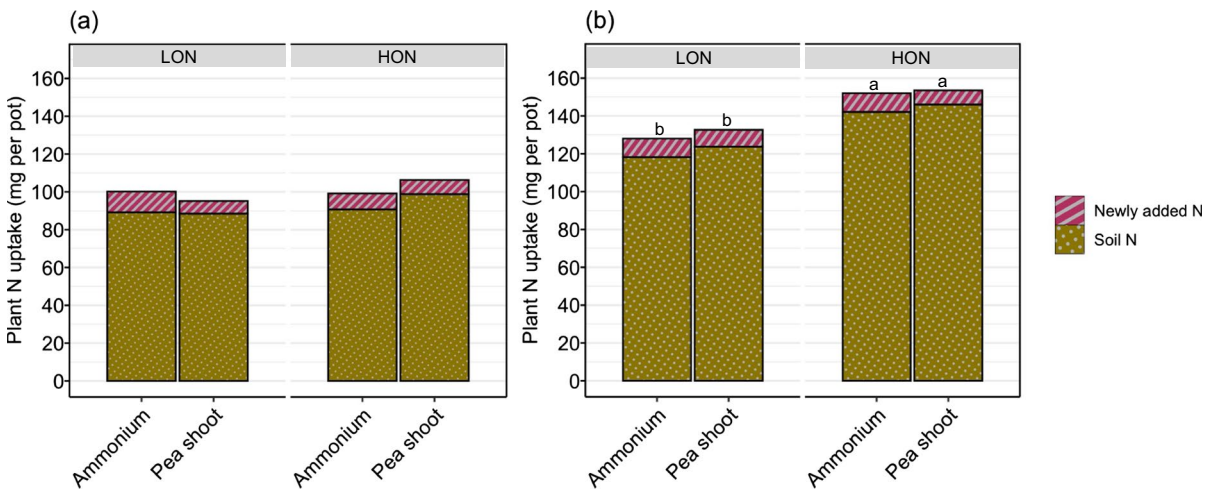

Fig. 4 The contribution of soil reserves and newly added N to total plant N uptake at the anthesis stage (**a**) and at grain harvest (**b**). Lowercase letters in (**b**) indicate differences in total N uptake among treatments from *post-hoc* Tukey comparisons at $P<0.05$

by the 30% greater P uptake from green manure than ammonium in the HON soil, probably due to the extra P introduced from the green manure (Table S1).

Grain yield was affected primarily by N source (Fig. 5b). In both soils, plants receiving green manure produced 6.7% more shoot biomass and 11.7% more grain compared with those receiving ammonium (N source, $P<0.05$, Table S2). We observed that spike numbers tended to be higher in the green manure treatment than the ammonium treatment (8.5 ± 0.71 vs 7.6 ± 0.51, mean $\pm$ S.D. of panicles per pot; $P=0.10$, Table S2), and there was a slight positive association between panicle numbers and grain yield ($r=0.49$, $P=0.056$). The higher N uptake in the HON soil (Fig. 4b) did not increase grain yield (Fig. 5b) but resulted in a 10% greater grain N density compared to the LON soil (Table S2). Low N availability in the LON soil promoted resource allocation belowground for N acquisition, as evidenced by the greater root biomass and vegetative biomass, and the lower harvest index in the LON soil (Table S2).

Changes in existing soil N pools and plant N uptake from various soil reserves

By the end of the experiment, N accrual from the newly added N (+3 to 6 μg N g^{-1} soil, Table 2) was small compared to the decline in total N in soil (−92 μg N g^{-1} soil, averaged across all treatments, Table 3, or an average reduction of 6% compared to the T0 total soil N pool). MAOM-N exhibited the greatest mass loss (−82 μg N g^{-1} soil, averaged across all treatments), followed by oPOM-N (−28 μg N g^{-1} soil, averaged across all treatments, Table 3). However, the percent oPOM-N lost from the original T0 pool size was substantial, reflecting the higher turnover rate of oPOM compared to MAOM (−25% vs −6% for oPOM-N vs. MAOM-N, averaged across all treatments, Table S3). In addition, the amount of oPOM-N decline was greater in the HON soil than in the LON soil (Table 3), although the relative changes were similar between the two soils (Table S2) due to the initial lower oPOM-N in the LON soil (Table 1). N and C content in the dissolvable fraction and the microbial biomass did not show a consistent response; there was a net increase in the dissolvable N in the LON soil whereas there were net decreases in microbial biomass C and N in the HON soil (Table 3). In contrast, fPOM-N and C pools showed net increases probably from the senesced plant roots (Table 3).

The reduction in existing soil N pools was partly due to plant uptake, which ranged from 39.4 to 48.7 μg N g^{-1} soil (Table 3) and accounted for 39% to 59% of the reduction in existing soil N pools. There was a reduction of 27.4 to 65.6 μg N g^{-1} soil that was not accounted for and was assumed lost to gaseous pathways, and this non-recovered pool was highly

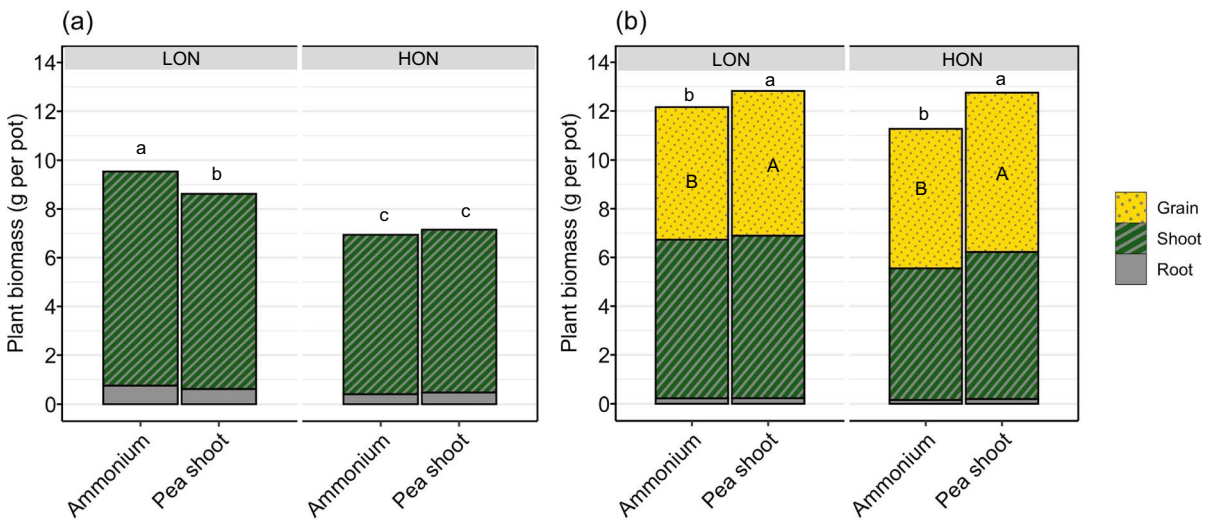

Fig. 5 Plant biomass at the anthesis stage (**a**) and at grain harvest (**b**). Lowercase letters indicate differences in total biomass among treatments from *post-hoc* Tukey comparisons at $P<0.05$, whereas uppercase letters in (b) indicate differences in grain yield among treatments. Detailed statistics for shoots and roots are provided in Table S1 and Table S2

Table 3 Changes in the concentrations of N, C, and C:N ratio of existing SOM pools after grain harvest. Fates of existing soil N fluxes are also provided. Net changes in soil N (or C) are calculated as: (soil N (or C) at grain harvest – new N (or C) incorporation) – initial soil N pools. Net changes in C:N ratio are calculated as: soil C:N ratio at grain harvest – initial soil C:N ratio. Lowercase letters indicate differences between treatments from significant soil legacy effects at $P < 0.05$. Relative changes (%), calculated as proportions of net changes relative to T0 pool size, are provided in Table S3. Mbio: microbial biomass

Soil legacy	N source	Changes in existing SOM pools						Fates of existing soil N		
		fPOM	oPOM	Dissolvable	Mbio	MAOM	Total	Plant uptake	Leaching	Not recovered
Net change in N, μg N g^{-1} soil										
LON	Ammonium	12.4 (4.3)	-25.6 (4.6) a	2.6 (1.2) a	-6.1 (1.3)	-87 (43.4)	-97.6 (41.8)	39.4 (1.5) a	2.2 (0.3) a	63.8 (43.9)
	Green manure	18.8 (4.8)	-18.5 (5.1) a	2.1 (1.2) a	1.5 (4.2)	-109 (46.8)	-106 (45.1)	41.2 (1.9) a	2.8 (0.6) a	65.6 (51.4)
HON	Ammonium	20 (2.6)	-34.8 (8.8) b	-1 (2.2) b	-9.2 (5.4)	-66 (26.1)	-81.9 (22.5)	47.4 (3.7) b	7.8 (1.7) b	32.8 (23.8)
	Green manure	20.9 (3.5)	-34.2 (2.6) b	-2.9 (1.4) b	-10.1 (3.2)	-67.6 (13.2)	-83.8 (12.7)	48.7 (3.9) b	7.3 (1.6) b	27.4 (15.1)
Net change in C, mg C g^{-1} soil										
LON	Ammonium	0.047 (0.13)	-0.446 (0.08)	-0.003 (0.006)	-0.048 (0.035) a	-1.07 (0.4)	-1.48 (0.38)			
	Green manure	0.305 (0.24)	-0.353 (0.06)	-0.003 (0.009)	0.005 (0.040) a	-2.03 (0.46)	-2.08 (0.48)			
HON	Ammonium	0.22 (0.05)	-0.496 (0.12)	0.005 (0.005)	-0.089 (0.054) b	-0.87 (0.31)	-1.14 (0.21)			
	Green manure	0.246 (0.06)	-0.492 (0.05)	0.01 (0.006)	-0.099 (0.036) b	-1.14 (0.41)	-1.37 (0.36)			
Net change in C:N ratio										
LON	Ammonium	-4.34 (1.49)	-0.45 (0.16) a	-1.43 (0.5) a	-0.5 (0.96)	-0.18 (0.52)	-0.32 (0.43)			
	Green manure	-3.24 (1.27)	-0.67 (0.25) a	-1.13 (0.6) a	0.11 (1.11)	-0.77 (0.16)	-0.67 (0.11)			
HON	Ammonium	-4.14 (0.65)	-0.18 (0.11) b	0.77 (0.46) b	-0.94 (0.85)	-0.16 (0.06)	-0.19 (0.04)			
	Green manure	-3.87 (0.73)	-0.2 (0.09) b	1.58 (0.26) b	-1.08 (0.82)	-0.33 (0.23)	-0.32 (0.2)			

variable and did not statistically differ among treatments (Table 3). N leaching from existing soil pools was higher in HON soil than in LON soil (Table 3). We noted that root biomass tended to be higher in the LON soil than the HON soil (Table S2, $P=0.057$), and a significant proportion of root biomass (~25%) in the LON was in the resin bags placed at the bottom of the pots while only 7% of root biomass was recovered from the resin bags in the HON soil, which may have contributed to the lower amount of NO_3 leaching captured in the resin beads in the LON soil (Table 3).

The significant reductions in oPOM-N and MAOM-N suggest that these SON pools serve as major sources for plant uptake but also may account for soil N losses. We examined the correlations between plant N uptake and initial SOM pools and found that initial oPOM-N was the best predictor of plant N uptake from soil ($r=0.93$, $P<0.001$, Fig. 6a, and Fig S4). This correlation remained statistically significant after controlling the difference between soils by including soil as a random factor in the linear mixed model. Second, we examined the net changes in soil N pools and their relationships with plant N acquisition. Plant N uptake from soil was positively correlated with the decline in the oPOM-N pool ($r=0.57$, $P=0.02$, Fig. 6b). This correlation was not entirely due to the difference between the two soils or the N sources, as there was substantial variability within each group. The positive trend remained when using N source as a random factor ($P=0.02$) but disappeared when soil was included as a random factor ($P=0.11$). In comparison, there were no significant relationships between plant N uptake from soil and the decline in MAOM-N pool (Fig. S5). The net changes in oPOM-N and MAOM-N did not show any significant correlations with N leaching (Fig. S5). In contrast, the net decline in MAOM-N pool and the non-recovered pool was highly correlated ($r=0.98$, $P<0.001$, Fig. S5), probably due to the fact that these two fluxes were both indirectly calculated based on changes in total soil N pool.

Discussion

Our study underscores the importance of microbially-mediated processes in determining the fate of the newly added N sources and points to the central role of oPOM and MAOM as both sources and sinks in short-term N cycling. Compared to inorganic N fertilizer, organic N has a greater capacity to build oPOM-N and MAOM-N pools, and this greater retention leads to reduced N loss to the environment. Higher SON reserves, presumably due to the long-term history of leguminous cover crops and organic residue inputs (Schipanski and Drinkwater 2012; Berthrong et al. 2013), support a greater soil N acquisition in wheat plants, confirming the importance of building SON for improving long-term soil fertility.

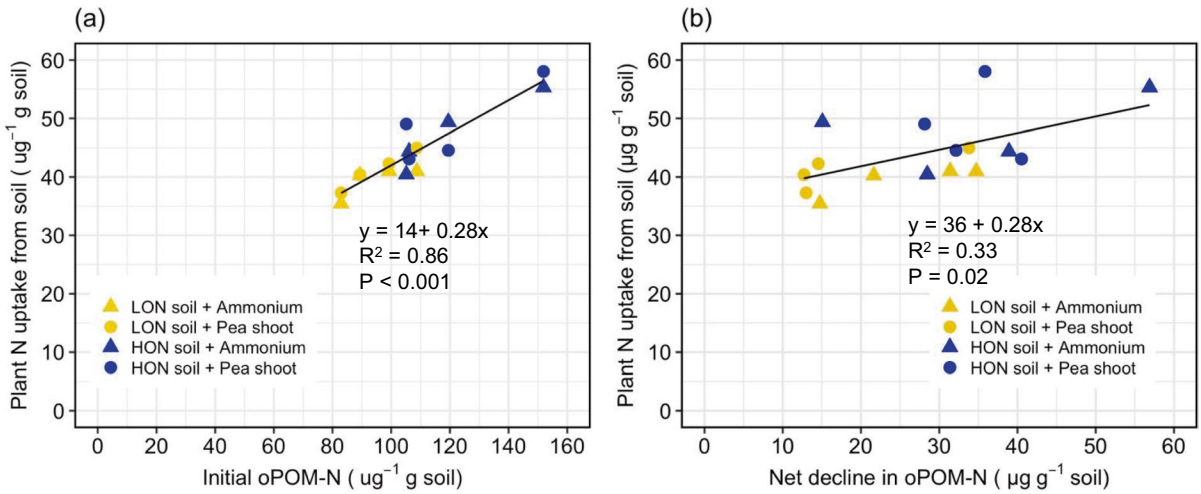

Fig. 6 Plant N uptake at grain harvest was positively correlated with the initial oPOM-N pool (**a**) and the net decline in the oPOM N pool (**b**)

The distribution of newly added N among crop uptake, losses, and accrual to soil pools

The fate of added N is primarily affected by the source of the N input and the resulting difference in plant and microbial utilization of the newly added N. Compared to N derived from legume residues, N from the ammonium fertilizer is slightly more accessible for plant uptake, however, it is less likely to accumulate in SON reserves and therefore more vulnerable to loss pathways. Earlier ^{15}N tracer studies have revealed higher N retention in soil from F_{org} than F_i sources (Azam et al 1985; Ladd and Amato 1986). While F_i application may inhibit microbial activity and reduce microbial biomass (Ramirez et al 2010; Treseder 2008), the addition of organic C associated with F_{org} inputs usually increases microbial biomass (Fauci and Dick 1994) and thus can enhance the assimilation of the F_{org}-N in microbial biomass. In our study, the amount of new N in microbial biomass was consistently higher with the green manure than with the ammonium fertilizer input (Table 2). Moreover, our study demonstrates microbial N assimilation and necromass deposition as a major driver of SON accrual, as evidenced by the positive correlations between N recovery in the living microbial biomass and accrual of new N in the MAOM (Fig. 3b) and the oPOM pool (Fig. S2). It has been shown that microbial growth during the decomposition of crop residues can lead to aggregate formation with POM occlusion and organo-mineral associations (Witzgall et al.2021). One recent study further revealed that microbial necromass contributes 14 to 57% of the oPOM-C pool and 14 to 68% of the MAOM-C pool, although microbial contribution to the N pools in these fractions was not quantified (Angst et al. 2024).

While the contribution of microbial necromass to soil organic C has become increasingly recognized (Cotrufo et al. 2013; Kallenbach et al. 2016; Liang et al. 2017; Wang et al. 2021), less is known about the persistence of microbial-derived N compounds in soils (Ma et al. 2022). Recent ^{15}N tracing studies showed rapid utilization of microbial necromass N in building new microbial biomass (Buckeridge et al. 2022), and necromass N retention in MAOM was linked to microbial turnover rate (Wang et al. 2020). Newly added N that was initially immobilized through microbial assimilation can be released back to the soil solution through two main pathways: (1) predation of soil microbes by protozoa, nematodes, microarthropods, and other soil fauna, which excrete excessive mineral N in the process known as "the microbial loop" (Coleman 1994); (2) production of exoenzymes and lysis of microbial cells after cell death or by phage virus (Braga et al. 2020), releasing proteins and other N-rich compounds (e.g., peptidoglycan in microbial cell walls) that may be further mineralized or interact with organic compounds/mineral surfaces that are physically occluded from further microbial attack (Buckeridge et al. 2020). In our study, new N retention in the oPOM and total SON pool declined by 10–20% from day 40 to day 80 (Table 2). The release of this initially immobilized N was associated with an increase in new N loss, as there was little plant uptake of new N after the anthesis stage. Thus, factors that govern the timing and release pathway of microbial biomass N (see microbial death pathways in Camenzind et al. 2023) could be important in driving N dynamics among soil retention, plant uptake, and loss. Overall, the negative relationship between N incorporation in microbial biomass and N loss observed in our study (Fig. 3a) and others (Herai et al. 2006) highlights the importance of enhancing microbial N assimilation to reduce off-farm N loss.

The existing SOM content is another factor that affects the degree of N retention vs N loss (Barrett and Burke 2002; Lovett et al. 2002; Castellano et al. 2012). Soils with greater labile C substrates and high C:N ratio are likely to foster microbial immobilization of the newly added N (see meta-analysis by Cao et al. 2021). The LON soil in our study had almost twofold greater fPOM and a slightly higher C:N ratio compared to the HON soil ($P < 0.10$), and retention of the added N tended to be greater in the LON soil than in the HON soil for the microbial biomass ($P < 0.09$) and oPOM ($P < 0.07$)(Table 2). The lowest N retention occurred in the HON soil with the ammonium treatment, suggesting a high risk of fertilizer loss in soils when N immobilization is unlikely due to the relatively low C: N ratio of SOM. Long-term differences in crop rotation and nutrient inputs could lead to differences in SOC quantity and quality; however, empirical studies of soil management legacy on new N retention have shown mixed results (Jenkinson 1965; Poffenbarger et al. 2018; McDaniel et al. 2023). Nevertheless, there is cumulative evidence that microbial assimilation of fertilizer N can

be significantly enhanced with the co-amendment of organic residues (Ladd and Amato 1986; Pan et al. 2017; Zhou et al. 2023), corroborating the importance of co-managing C and N inputs for improving fertilizer use efficiency and long-term soil fertility (Drinkwater and Snapp 2007).

Soil legacy and N source effects on wheat N acquisition and biomass production

Our study highlights the importance of using legume cover crops to build SON reserves and to support plant N acquisition. Many studies have shown that management regimes with diversified rotations that include legume cover crops support greater SOM formation and increase SON storage (Drinkwater et al. 1998; McDaniel et al. 2014; Schmer et al. 2020). In our study, the HON soil, where leguminous green manures were the primary N source, had larger oPOM- and MAOM- N reserves (Table 1) and in turn supported a greater plant N acquisition compared to the LON soil managed under continuous maize and inorganic N fertilizer (Fig. 4b). In both soils, plants acquired over 90% of N from existing soil N reserves, with only 5 to 8% N from the newly added N at a rate of 22 kg N ha^{-1}. We expect this number would increase to 25–40% with a higher fertilization rate of 110 kg N ha^{-1}, assuming a linear response of fertilizer-derived N in crop to increase in fertilizer rate (see Fig. 2 in Yan et al. 2020). The importance of SON reserves for crop N supply has long been recognized. In smallholder subsistence systems that receive little if any added N, soil N content is positively correlated with grain yield and protein density (Wood et al. 2018; Fischer et al. 2020), highlighting the importance of soil N reserves for crop production in regions where accessibility of affordable synthetic fertilizers is limited. However, the discovery that SON plays a major role in crop supply even in high-input industrial systems came as a surprise. Meta-analyses of ^{15}N tracer experiments in small grain cropping systems consistently report that fertilizer N accounts for the smaller proportion of crop N uptake (32.0% ± 1.4% SE, Gardner and Drinkwater 2009) and (37.1% ± 1.1% SE, Yan et al 2020), with over half of crop N obtained from soil N reserves.

In addition to building soil fertility, the use of leguminous N may also provide other benefits in plant growth, possibly due to the alteration of soil microbiome communities. Compared to the inorganic fertilizer treatment, wheat plants receiving green manure N had higher grain yield (Fig. 5b) and tended to have greater spike number (Table S2), despite total N and P uptake being similar between the two N sources. Greater tiller number in wheat following leguminous green manure has been observed in multi-year field studies even in plots receiving high rates of mineral fertilizers (Burgess et al. 2014; Zhang et al. 2015), and the increased tiller density was associated with a greater yield in some cases (Zhang et al. 2015). In our previous greenhouse study, tiller numbers in sorghum were twofold greater in soil inoculated with microbial communities derived from soils with long-term green manure inputs (the same field where HON soils were collected in the current study) compared to soil inoculated with microbial communities from soil under continuous mineral fertilization (Gan et al. 2021). Such microbial-mediated changes in plant development can be an important mechanism underlying crop responses to different management legacies (Li et al. 2020), and we have an ongoing study investigating the metagenomic composition of the rhizosphere communities from current study.

The importance of soil oPOM and MAOM pools in supplying plant available N

Our study reveals that oPOM-N is an important soil reservoir for plant N acquisition through three lines of evidence: i) initial oPOM-N in soil is the best predictor of plant N uptake among all soil organic and inorganic N pools; ii) oPOM-N pool showed the largest decline (20–30%) after one cropping cycle; and iii) the decline in soil oPOM-N pool is positively correlated with plant N uptake. While POM is often treated as a single SOM pool (Cotrufo et al. 2019; Daly et al. 2021), oPOM and fPOM are functionally distinct pools that represent different stages of the N cycling process and differ in their accessibility to decomposers and plant roots (Drinkwater and Snapp 2022). Consisting of lightly-decomposed detritus, fPOM usually has a greater C: N ratio (23 vs 15 for C:N ratio in fPOM vs. oPOM in our study; Schipanski and Drinkwater 2011) and provides energy-rich C source for soil microbes and detritivores. In contrast, oPOM is formed after fPOM has undergone substantial biological and physiochemical transformation that results in physical protection of the resulting OM

fragments. The relative low C: N ratio in oPOM and the surrounding aggregation indicates that oPOM can serve as a nutrient-rich hotspot with relatively low labile organic C, where a cooperative dynamic between plant roots and rhizosphere microbes can develop in which roots excrete energy-rich carbohydrates to "prime" rhizosphere microbes in exchange for microbial mineralization of the oPOM-N (Finzi et al. 2015; Liu et al. 2020).

The turnover of the MAOM-N pool, as evidenced by its decline (6%) following plant harvest, could also be important in providing plant available N due to its large stock size. The positive intercept in the best-fit line between plant N uptake and the net decline in oPOM-N (Fig. 6b) also indicates other SOM pools (such as MAOM) in providing plant available N. However, the relative contribution of MAOM vs. oPOM to plant N acquisition cannot be determined in our study, partly due to the large variability associated with our MAOM estimates. The coupling of C and N cycling from root-rhizobiome cooperation associated with oPOM aggregates may also occur on the mineral surface for root acquisition of MAOM-N (Jilling et al. 2018). Acquisition of N from these sources could be affected by the spatial distribution of these SOM pools and root foraging strategies. Compared to the relatively homogeneous distribution of mineral particles, oPOM distribution is highly heterogeneous within the soil matrix (Peth et al. 2014; Rawlins et al. 2016), and nutrient density (N amount per spatial volume) can be higher within oPOM aggregates than with MAOM. As such, plant roots that have a high plasticity in root architecture development (Cahill and McNickle 2011) may preferentially proliferate within oPOM patches for N acquisition.

Implications for nutrient and SOM management

Conventional fertilizer guidelines such as 4Rs nutrient management focus on optimizing N fertilizer use efficiency and yields for a single season and do not attempt to manage SON reserves or the long-term trajectory of SON (Fixen 2020; Drinkwater and Snapp 2022). In these systems, C-limitation restricts microbial assimilation of the fertilizer N, which reduces the conversion of fertilizer N into SOM and increases the risk of environmental N loss. Taken together, this strategy poses significant challenges for linking soil health restoration to nutrient management (Grandy et al. 2022).

Our study demonstrates the value of using leguminous green manure to promote microbial processes that direct N flows into SON reserves to support crop productivity and other soil ecosystem functions. The importance of oPOM pool as a source of plant-available N suggests that identifying and breeding leguminous species or cultivars that have a high capacity to build oPOM-N is a strategy worthy of more attention (Drinkwater et al. 2021). Over the long term, increased reliance on legume-derived N creates feedback that reduces symbiotic N_2 fixation as oPOM pools increase (Blesh 2019), helping to stabilize soil N supply while limiting surpluses that lead to environmental N losses. We conclude that enhancing N incorporation into soils to build a large SON reserve should be an integrated component of nutrient management plans to support sustainable agriculture.

Acknowledgements This research was supported by funding from USDA Agriculture and Food Research Initiative Competitive Grant (grant no. 2015-67019-23588). We would like to thank Dr. Marshall McDaniel for his thorough review of a previous version of this manuscript.

Author contributions L.E.D. provided the initial study conception and design. Material preparation, data collection and analysis were performed primarily by H.G. with assistance from L.E.D. The first draft of the manuscript was written by H.G. with substantial edits from L.E.D. Both authors read and approved the final manuscript.

Funding This research was supported by funding from United States Department of Agriculture- Agriculture and Food Research Initiative Competitive Grant (grant no. 2015–67019-23588).

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing interests The authors have no relevant financial or non-financial interests to disclose.

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