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Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems

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Abstract

Agricultural systems that receive high or low organic matter (OM) inputs would be expected to differ in soil nitrogen (N) transformation rates and fates of ammonium (NH_4^+) and nitrate (NO_3^-). To compare NH_4^+ availability, competition between nitrifiers and heterotrophic microorganisms for NH_4^+, and microbial NO_3^- assimilation in an organic vs. a conventional irrigated cropping system in the California Central Valley, chemical and biological soil assays, ^{15}N isotope pool dilution and ^{15}N tracer techniques were used. Potentially mineralizable N (PMN) and hot minus cold KCl-extracted NH_4^+ as indicators of soil N supplying capacity were measured five times during the tomato growing season. At mid-season, rates of gross ammonification and gross nitrification after rewetting dry soil were measured in microcosms. Microbial immobilization of NO_3^- and NH_4^+ was estimated based on the uptake of ^{15}N and gross consumption rates. Gross ammonification, PMN, and hot minus cold KCl-extracted NH_4^+ were approximately twice as high in the organically than the conventionally managed soil. Net estimated microbial NO_3^- assimilation rates were between 32 and 35% of gross nitrification rates in the conventional and between 37 and 46% in the organic system. In both soils, microbes assimilated more NO_3^- than NH_4^+. Heterotrophic microbes assimilated less NH_4^+ than NO_3^- probably because NH_4^+ concentrations were low and competition by nitrifiers was apparently strong. The high OM input organic system released NH_4^+ in a gradual manner and, compared to the low OM input conventional system, supported a more active microbial biomass with greater N demand that was met mainly by NO_3^- immobilization.

Keywords: Microbial ecology; ^{15}N isotope pool dilution; Agroecology

1. Introduction

Agricultural systems vary in important ways with respect to nitrogen (N) and carbon (C) cycling. Systems that receive high organic matter (OM) inputs have greater labile C pools, greater microbial activity and greater soil N supplying power compared to systems that receive only mineral fertilizer (Gunapala and Scow, 1998; Kramer et al., 2002). Many studies have focused on fates of N inputs during one or more growing seasons, and many chemical and biological soil assays have been developed to predict N availability to crops (Bundy and Meisinger, 1994). Less is known about the actual rates of short-term microbial N transformations in systems that differ in C availability and soil N supplying capacity. Agricultural soils that differ in OM inputs would be expected to differ in rates of soil N transformations, competition for ammonium (NH_4^+) by immobilizers and nitrifiers, and fates of nitrate (NO_3^-).

Ammonium has been found to be the preferred form of N for assimilation by microbes in many cultivated soils (e.g. Azam et al., 1993). Nevertheless, nitrification is often considered the major fate of NH_4^+ in agricultural soil (Robertson, 1997), where NH_4^+ is usually present in low concentrations (<2 μg NH_4^+-N g^{-1} soil) compared to NO_3^-. In some agricultural soils, no NO_3^- immobilization has been observed (Shi and Norton, 2000), while in others, NO_3^- immobilization was recorded after 1–4 weeks (Schimel, 1986) or several months (Kissel and Smith, 1978). Carbon inputs often increase NO_3^- immobilization (Recous et al., 1990). In most investigations comparing NO_3^- and NH_4^+ assimilation in cultivated soils, N is applied in concentrations of ≥ 50 μg N g^{-1} soil, and microbial N immobilization in soils that do not receive concentrated N inputs has

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been less studied. A better understanding of inorganic N immobilization by microbes in differentially managed cropping systems is important since timing of inorganic N release and crop N uptake are not always synchronized, thus leading to potential N loss and environmental degradation (Robertson, 1997; Matson et al., 1997).

Drying and rewetting of soil, as it occurs in irrigated systems, may also affect the rates of production and consumption of NH_4^+ and NO_3^-. Increased net N mineralization upon rewetting air-dry soils has been demonstrated (Sparling and Ross, 1988; Appel, 1998). Nitrification activity, on the other hand, has been shown to either respond quickly to increased moisture (Davidson et al., 1993), to increase over 1–4 d after rewetting (Venterea and Rolston, 2000a), or to be inhibited by repeated drying and wetting (Franzluebbers et al., 1994). Simultaneous measurement of gross rates of ammonification and nitrification, combined with measurements of microbial N immobilization after rewetting dry soil could show relationships between production and consumption of inorganic N during key periods of crop N uptake or potential N loss.

The ^{15}N isotope pool dilution approach is well suited to measure short-term N transformations (Hart et al., 1994), but spatial variability of inorganic N concentrations, especially in agricultural soils (Wolt, 1994), can make this method unfeasible. To reduce variability of inorganic N concentrations, sieving and mixing agricultural soil has often been used (e.g. Myrold and Tiedje, 1986). Sieving can increase mineralization rates (Ross et al., 1985; Calderón et al., 2000). Leaching as an alternative to sieving also achieves the objective of reducing NO_3^- variability, but leaching can also remove soluble organic N and C that may be playing a role in mineralization/immobilization (Mengel et al., 1999). Both types of disturbances occur in agricultural soils due to tillage and rainfall or irrigation events.

We compared two irrigated cropping systems in California's summer-dry Central Valley that receive different types and amounts of C and N inputs to investigate the differences in soil N supplying capacity, short-term NH_4^+ availability, and microbial N immobilization patterns. The objectives of this study were to: (i) monitor soil N supplying capacity during a cropping season; (ii) measure rates of gross mineralization, gross nitrification, and microbial immobilization of inorganic N after rewetting dry soil; (iii) evaluate two methods, sieving and leaching, to minimize variability of soil NO_3^- concentrations, a prerequisite for using the ^{15}N isotope pool dilution technique.

2. Materials and methods

2.1. Field site

Our study site was the University of California Davis Long Term Research on Agricultural Systems (LTRAS) project in Yolo County, CA, where since 1993 several cropping systems have been maintained in six randomized replications of 60 m × 60 m plots on the same soil types. We sampled soils of an organically and a conventionally managed system (Table 1). The inputs in the organically managed tomato–corn rotation were a winter legume cover crop, animal manure (composted poultry litter), and harvest residue. Average N inputs from cover crop and manure were 350 kg ha^{-1} yr^{-1}. An additional 40 kg N ha^{-1} yr^{-1} was returned to the soil as harvest residue. Dry matter inputs in the conventionally managed tomato − wheat rotation consisted of harvest residue and fertilizer. Each crop received 165 kg N ha^{-1} as granular inorganic fertilizer or urea, in two applications each. Approximately 20 kg N ha^{-1} yr^{-1} was returned to the soil as harvest residue. Both systems were furrow-irrigated. The soil types were Yolo silt loam, a fine-silty, mixed, non-acid, thermic Typic Xerorthent and Rincon silty clay loam, a fine montmorillonitic, thermic Typic Haploxeralf.

Table 1
Soil characteristics and average yearly inputs of organic (tomato/corn/legume cover crop) and conventional (tomato/wheat) systems at LTRAS. Bulk density was measured at midseason. Values are means ($n = 3$)

Management type	Conventional	Organic
PH (H_2O 1:1)	6.8	6.5
CEC (meq 100 g^{-1} soil)	30.8	33.9
Sand (%)	23	19
Silt (%)	55	58
Clay (%)	22	23
Bulk density 0–7.5 cm (g cm^{-3})	1.07	1.12
Bulk density 7.5–15 cm (g cm^{-3})	1.24	1.29
Organic C (g kg^{-1})	10.3	12.8
Organic N (g kg^{-1})	1.0	1.4
C/N ratio	9.9	9.3
Dry matter inputs (Mg ha^{-1} yr^{-1})	3	22
N inputs (kg N ha^{-1} yr^{-1})	165	350

2.2. Potentially mineralizable N and inorganic N pools

During the 1999 tomato growing season, we sampled three replicates of each system on the following dates: May 24, June 7, July 1, July 23, and August 19. On each date, a composite sample was collected consisting of 30 cores (2 cm diameter, 0–15 cm depth) taken in diagonal patterns across each plot. An equal number of cores was taken 15, 30, and 45 cm from the centerline of the tomato beds. To avoid the confounding effects of extremely high inorganic N concentrations, the fertilizer bands (7.5 and 23 cm from the centerline) were not sampled.

On each sampling day, three 7 g subsamples of the well-mixed composite samples were extracted with 2 M KCl at a liquid/soil ratio of 5:1. The samples were shaken for 1 h on a reciprocating shaker, centrifuged, and the supernatant was collected. The rest of the soil was air-dried for about 3 d and sieved (2 mm) to standardize assay conditions between sampling dates. To determine potentially mineralizable N

(PMN), three 5 g subsamples were incubated with 25 ml of deionized H_2O under anaerobic conditions at 37 °C (Waring and Bremner, 1964). After 7 d, 25 ml of 4 M KCl was added and the samples were then extracted as earlier. For hot KCl extraction, triplicate 1 g samples were digested for 3 h in 5 ml of 2 M KCl in gas-tight Teflon capped glass tubes at 100 °C (Gianello and Bremner, 1986). The supernatant was collected after the tubes had been cooled to room temperature. Another set of subsamples of air dried, sieved soil was extracted with 2 M KCl in the above manner to determine background NH_4^+ concentrations. All extracts were frozen until analysis for NH_4^+ and $NO_3^- + NO_2^-$ with a Lachat flow injection analyzer (Lachat 8000, Zellweger, Milwaukee, WI).

2.3. ^{15}N pool dilution

The soils for determination of inorganic N production and consumption rates were collected on 22 July, 1999. Eighty cores were taken from random locations within three plots of each management type in clusters of four cores. The cores were removed using beveled PVC pipe sections (15×5 cm^2 diameter) that could be separated into 0–7.5 and 7.5–15 cm depths. Again, an equal number of cores was taken 15, 30, and 45 cm from the center of the beds.

Half of the samples were sieved by passing the soil of all four cores comprising a cluster through a 2 mm mesh. The soil was mixed, repacked into the PVC cylinders, and brought to field capacity. The other half of the samples were left undisturbed and NO_3^- was leached from those cores with two pore volumes of 0.01 M $CaCl_2$. All cores were then air dried to a gravimetric water content (w) of 0.08 g H_2O g^{-1} soil and stored in a glasshouse. Two weeks later, the soil cores were rewet and kept at 25 °C, covered with perforated parafilm. Soil water potential (Ψ_s) was estimated based on gravimetric water content by interpolating Ψ_s values from moisture retention curves generated for the organically and conventionally managed soils with a pressure plate apparatus.

Gross N transformation rates were measured by the ^{15}N pool dilution method (Hart et al., 1994). The 24 h-incubations were started either 12 or 120 h after rewetting. The ^{15}N label was added in six 1 ml injections. The $(^{15}NH_4)_2SO_4$ (99% ^{15}N) solution was 1.02 mM, and the $K^{15}NO_3$ (99% ^{15}N) solutions were 1.75 and 3.50 mM for the leached and sieved samples, respectively. The additions increased w by 3–4%, NH_4^+ concentrations by approximately 100%, and NO_3^- concentrations by 15–25%. To extract inorganic N, about 160 and 110 g of soil were used for 0 and 24 h samples, respectively, with the same procedure as described for field measurements. At 24 h, 50 g of soil was used for determination of microbial biomass N (MBN) by the 1 d chloroform-fumigation extraction method (Brookes et al., 1985). The MBN extracts and 0.5 M K_2SO_4 blanks were digested, using a modified Kjeldahl procedure to eliminate NO_3^- interference (Wyland et al., 1994). All extracts were analyzed for NO_3^- and NH_4^+ on a Lachat flow injection analyzer. The unfumigated soil samples provide a measure of dissolved organic N (DON). A small amount of soil was used for moisture determination (0 and 24 h).

To prepare the samples and blanks for ^{15}N analysis, diffusion techniques were used (Brooks et al., 1989; Stark and Hart, 1996). As NH_4^+ concentrations in the 24 h KCl extracts in the ^{15}N pool dilution experiments were low, some of the samples with similar concentrations of NH_4^+ were pooled for the diffusion procedures. The mass and isotopic composition of N due to Devarda's alloy contamination (Sigman et al., 1997) were measured several times by combusting a single tin capsule containing ten filter disks (each from one diffused blank). Isotope ratios were determined by an Europa Integra mass spectrometer (PDZ Europa Ltd, Cheshire, UK) at the UC Davis Stable Isotope Facility.

Gross ammonification, nitrification (n), and consumption rates of NH_4^+ (c_{NH4}) and NO_3^- were calculated according to Kirkham and Bartholomew (1954). Immobilization of NH_4^+ and NO_3^- by microbes was estimated from consumption rates by assuming that gaseous N losses and other possible fates of inorganic N were negligible. Microbial immobilization of NH_4^+ (i_{NH4}) was estimated as

$$i_{NH4} = c_{NH4} - n. \quad (1)$$

MBN was calculated by subtracting NH_4^+-N in the digested non-fumigated sample from the NH_4^+-N in the digested fumigated sample. All MBN data are expressed as the flush of N after fumigation with no conversion factor to account for the incomplete recovery of MBN. Immobilization of NO_3^- or NH_4^+ ($I_{NO3,NH4}$) was also calculated from ^{15}N recovery in microbial biomass, as

$$I_{NO3,NH4} = \frac{\left(N_{fum}\frac{Ae_{fum}}{100}\right) - \left(N_{non\text{-}fum}\frac{Ae_{non\text{-}fum}}{100}\right)}{Ae_{NO3,NH4}} \quad (2)$$

where N_{fum} = μg NH_4^+-N g^{-1} soil in digested fumigated samples, $N_{non\text{-}fum}$ = μg NH_4^+-N g^{-1} soil in digested non-fumigated samples, Ae_{fum} = at.% excess ^{15}N in extracts of fumigated samples, $Ae_{non\text{-}fum}$ = at.% excess ^{15}N in extracts of non-fumigated samples, and $Ae_{NO3,NH4}$ = average at.% excess ^{15}N of soil NO_3^- or NH_4^+, calculated as (at.% excess ^{15}N at 0 h + at.% excess at 24 h)/2.

To evaluate the sieving and leaching pretreatments, the coefficient of variation (CV) of NO_3^- concentrations was determined in pairs of cores from the same location in the field. Pairs (at 0 or 24 h) consisted of cores injected with either $^{15}NO_3^-$ or $^{15}NH_4^+$. The known mass of the ^{15}N added as label was subtracted, if necessary. The mean of all CVs of sieved and leached pairs was then calculated.

The data were analyzed as a split plot design with soil management type as the main plot effect and sampling date for field measurements or laboratory treatments as subplots. To test simple effects within soil management type,

Duncan's multiple range test and Fisher's protected LSD were used. In the text, means ± standard errors (SEM) are reported.

3. Results

3.1. Field measurements: seasonal changes in soil N supplying capacity

Both indicators of soil N supplying capacity, PMN and hot minus cold KCl-extracted NH_4^+, were about twice as high in the organically managed soil compared to the conventionally managed soil ($P < 0.0001$) (Fig. 1). In both soil management types, PMN declined by about 60% during the course of the growing season. In the organically managed soil, mean hot minus cold KCl-extracted NH_4^+ decreased by about 21% between May 24 and August 19 measurements, but there was no change in the conventionally managed soil. In contrast to the soil N availability indices, inorganic N pools did not differ between farming practices. Mean NH_4^+ concentrations were low (<1–2 μg NH_4^+-N g^{-1} soil) on all five sampling occasions, including the 1 July sampling that took place 3 weeks after the $(NH_4)_2SO_4$ sidedress application in the conventionally managed soil. Mean NO_3^- concentrations in these composite samples were variable, but were not different between dates.

3.2. ^{15}N pool dilution and inorganic N immobilization

Total recovery of the applied ^{15}N label was 107.1 ± 6.2% for NO_3^-, and 80.9 ± 1.0% for NH_4^+. In the samples labeled with $^{15}NO_3^-$, virtually no ^{15}N was found in excess of natural abundance as NH_4^+. The average soil water potential (Ψ_{soil}) in the four treatments ranged from −0.1 to −0.3 MPa, with no differences between the two soil types. There was a weak positive correlation between gravimetric water content (w) and gross ammonification rates ($r^2 = 0.19$, $P < 0.05$) in the organically managed soil, but regressions of nitrification rates on w and of gross ammonification rates on w in the conventionally managed soil were not significant.

Production and fate of NH_4^+ at 12 and 120 h after rewetting were summarized for the organic and conventional system by pooling the results of leached and sieved samples (Fig. 2(a)); see below for a comparison of these two pretreatments. The means of corresponding upper (0–7.5 cm) and lower layer (7.5–15 cm) samples were considered experimental units. Mean N transformation rates in each soil type and treatment did not differ between layers (*t*-tests, $P > 0.05$). Gross nitrification data of the sieved samples at 12 h after rewetting from the organic soil were not included due to the high propensity for negative values (Fig. 3). Pooling the data for pretreatment and layers in this manner highlighted the major trends by reducing variability. Gross ammonification rates were significantly

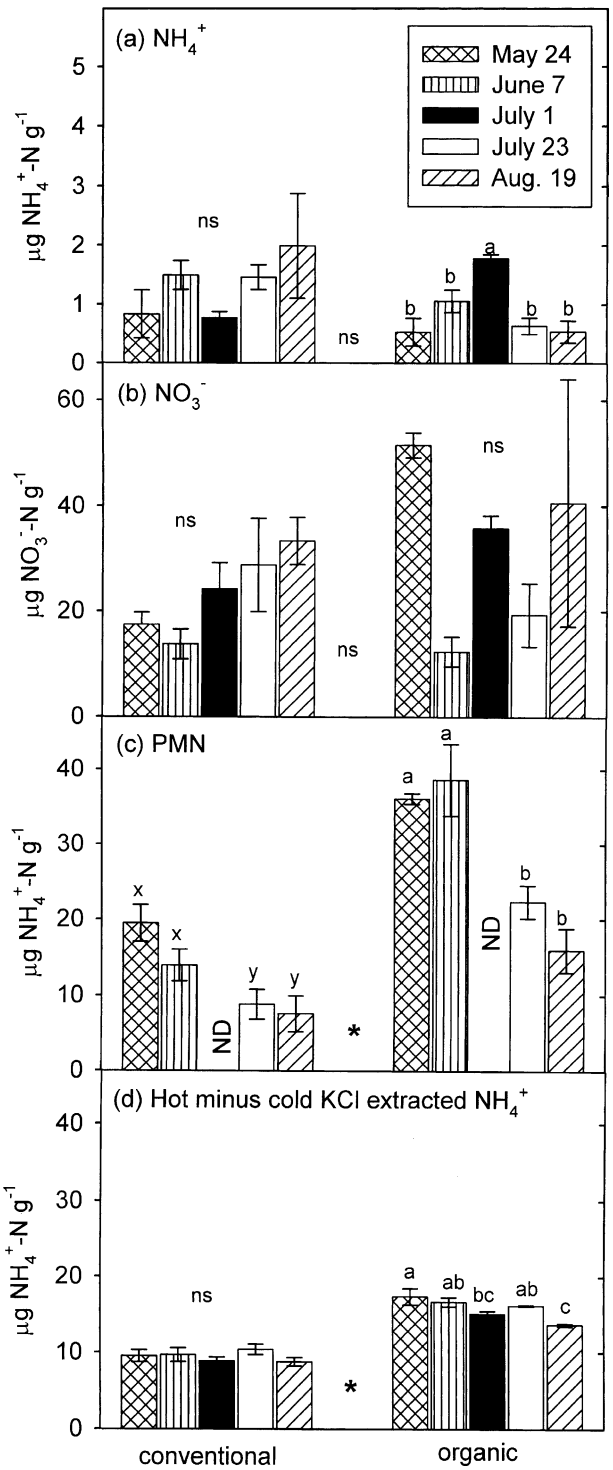

Fig. 1. Mean (±SEM) soil NH_4^+, NO_3^-, PMN, and hot minus cold KCl-extracted NH_4^+ measured at five dates during the 1999 tomato growing season in the 0–15 cm layer of organic and conventional cropping systems at LTRAS ($n = 3$). * $P < 0.0001$. For each farming system, means with the same letter are not significantly ($P > 0.05$) different.

higher in the organically than in the conventionally managed soil, but gross nitrification rates were similar in the two soils (Fig. 2(a)). Gross nitrification rates did not differ from gross ammonification rates in either soil. Time

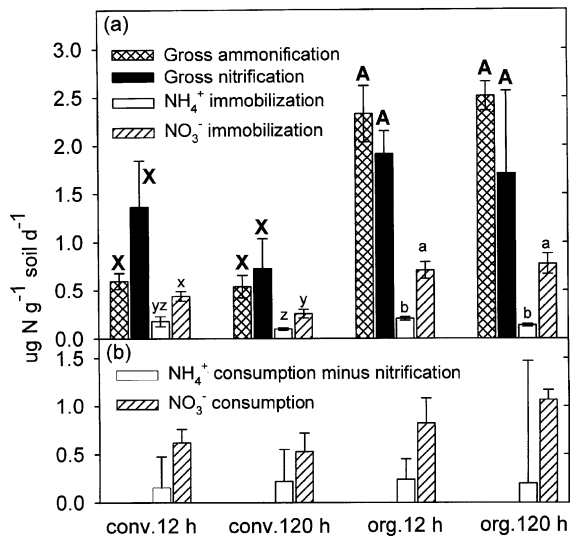

Fig. 2. (a) Mean rates of gross ammonification, gross nitrification, NH_4^+ and NO_3^- immobilization by microbes (based on ^{15}N recovery in MB) 12 and 120 h after rewetting soils from 0–15 cm depth from organic and conventional cropping systems at LTRAS. Mean comparisons are between N production rates (gross ammonification, gross nitrification) and between immobilization rates (estimated microbial NH_4^+ immobilization and NO_3^- immobilization) within each soil management type. Means (±SEM) designated with the same letter are not significantly ($P > 0.05$) different ($n = 8$). Statistical comparisons between cropping systems are given in the text. (b) Mean (+SEM) microbial N immobilization rates, estimated for NH_4^+ as gross consumption−gross nitrification rates, and for NO_3^- as gross consumption rate ($n = 8$).

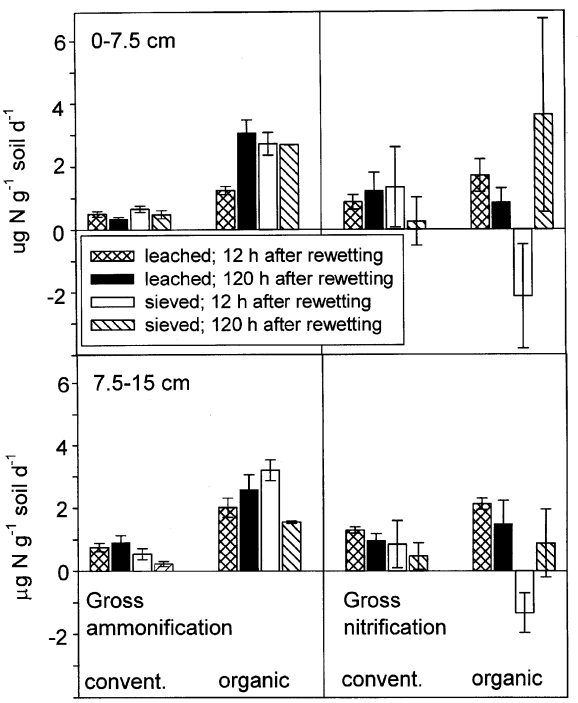

Fig. 3. Mean (±SEM) gross ammonification and gross nitrification rates (±SEM) at mid-season in soil from the 0–7.5 cm and 7.5–15 cm layer of organic and conventional plots at LTRAS, measured after differential pre-treatments (leaching or sieving) to reduce variability of NO_3^- concentrations. For each soil type ($n = 5$), no significant differences were observed between layers across treatments (*t*-tests, $P > 0.05$).

since rewetting neither affected mean gross ammonification rates nor mean gross nitrification rates.

MBN was higher in the organically than in the conventionally managed soil ($P < 0.0001$) (Table 2). In both soils at 12 and at 120 h after rewetting, microbes assimilated significantly more NO_3^- ($^{14+15}NO_3^-$-N) than NH_4^+ ($^{14+15}NH_4^+$-N) based on ^{15}N recovery in the microbial biomass (Fig. 2(a)). Mean microbial immobilization rates of NH_4^+ and NO_3^-, as calculated from gross consumption rates, showed the same trend, but there were no differences statistically because of large standard errors (Fig. 2(b)). Using ^{15}N recovery in MBN, the estimated net microbial NO_3^- assimilation rate in the conventionally managed soil was between 32 and 35% of gross nitrification rates; in the organically managed soil, the estimated net assimilation rate of NO_3^- was between 37 and 46% of gross nitrification rates. Net estimated NO_3^- assimilation per day relative to the respective mean NO_3^- pools was 2–4% in the conventional and 5–6% in the organic system. Estimated net NH_4^+ assimilation per day relative to the respective mean NH_4^+ pools was 19–24% in the conventionally, and 30% in the organically managed soil.

DON was significantly lower in the leached samples than in the sieved samples (5.7 ± 0.3 µg N g^{-1} soil vs. 6.8 ± 0.4 µg N g^{-1} soil, respectively; $P < 0.01$), but mean gross ammonification rates of sieved and leached samples in soils of both management types were not different (*t*-test; $P > 0.05$). Leaching was more effective at reducing variability of initial NO_3^- concentrations than sieving, and therefore, more consistent nitrification rates were obtained in the leached samples (Fig. 3). The mean CV of NO_3^- concentrations for leached pairs was 9 vs. a mean CV of 21 for sieved pairs. About 45% of the gross

Table 2
Inorganic N and MBN concentrations in the ^{15}N isotope pool dilution experiments. Values are means (±SEM). $n = 8$ (NH_4^+) or 10 (NO_3^-)

Experiment	µg N g^{-1} soil	
	Conventional	Organic
NH_4^+, 12 h		
t_0	1.26 ± 0.19	0.76 ± 0.05
$t_{24\ h}$	0.62 ± 0.15	0.27 ± 0.01
$MBN_{t24\ h}$	11.45 ± 1.14	23.31 ± 1.01
NH_4^+, 120 h		
t_0	0.60 ± 0.03	0.64 ± 0.04
$t_{24\ h}$	0.24 ± 0.04	0.27 ± 0.05
$MBN_{t24\ h}$	10.12 ± 0.78	19.92 ± 1.20
NO_3^-, 12 h		
t_0	10.53 ± 1.20	12.10 ± 1.40
$t_{24\ h}$	12.59 ± 2.08	12.51 ± 1.08
$MBN_{t24\ h}$	10.58 ± 1.26	23.67 ± 1.27
NO_3^-, 120 h		
t_0	11.89 ± 1.60	14.31 ± 0.10
$t_{24\ h}$	11.45 ± 1.83	15.16 ± 1.54
$MBN_{t24\ h}$	10.95 ± 1.17	22.05 ± 1.40

nitrification rates obtained using sieved soil were negative whereas 8% of those rates were negative among the leached samples.

4. Discussion

PMN, hot minus cold KCl-extracted NH_4^+ and ^{15}N isotope pool dilution experiments all demonstrated the greater soil N supplying capacity, and therefore greater NH_4^+ availability, of the organic compared to the conventional system. The most striking results were that under both management types, microbes assimilated more NO_3^- than NH_4^+ and that microbial NO_3^- immobilization rates were substantial compared to gross nitrification rates.

4.1. Soil N supplying capacity

In both soils, PMN declined as the tomato season progressed. Lower values of PMN in the second half of the growing season in similar cropping systems were also reported by Gunapala and Scow (1998). The decline in PMN could be due to a decrease in MBN or a diminishing pool of easily mineralizable non-living OM. In anaerobic incubation, killed aerobic microorganisms may be the most important part of the organic substrate being mineralized. In a range of forest soils, there was a strong correlation between the amount and isotopic composition of the NH_4^+ produced by anaerobic incubation and MBN measured via chloroform fumigation-incubation (Myrold, 1986), suggesting that those two N pools are largely identical. In agricultural soils such correlations have not always been found (Drinkwater et al., 1996), perhaps because different types of C and N inputs and removals affect substrate composition. In both our soils, concentrations of MBN and PMN were similar, when both were measured in late July, supporting the idea that NH_4^+ released by anaerobic incubation is mostly microbial N. In this study, PMN as an indicator of relative N availability showed a clear difference between organic and conventional farming practices. However, PMN provided no information on actual rates of NH_4^+ release, the fate of this newly released NH_4^+, or the extent of recycling of inorganic N via microbial biomass. The results of the ^{15}N experiments, which complement the PMN measurements, suggest a very dynamic role of MBN and highlight the importance of microbial N immobilization that is taking place simultaneously with inorganic N production.

While PMN as a biological assay measures the release of NH_4^+ from various easily mineralizable N fractions, hot KCl releases NH_4^+ from only certain organic N compounds whose chemical nature is mostly unknown (Gianello and Bremner, 1986; Curtin and Wen, 1999). In neither soil did changes in PMN closely parallel changes in hot minus cold KCl-extracted NH_4^+. By 19 August, concentrations of hot minus cold KCl-extracted NH_4^+ and PMN were no longer different in either soil. At that time, whether the NH_4^+ released by the two assays was of the same origin is not known.

In contrast to the soil N availability indices, inorganic N concentrations provided no information on actual NH_4^+ availability since NH_4^+ concentrations were always low except in the fertilizer band, where concentrations reach up to 1000 μg NH_4^+-N g^{-1} soil (Frederick and Broadbent, 1965).

4.2. Rates of soil N transformations

Nitrate immobilization by heterotrophic microbes and N process rates were greater under organic than conventional practice although gross nitrification rates were not statistically different between the two soils due to the large variability of the data. In general, gross nitrification rates plus NH_4^+ immobilization rates approximately added up to gross ammonification rates. Gross nitrification rates in the 12 h treatment of the conventionally managed soil were twice as high as gross ammonification rates probably because mean background NH_4^+ concentrations in those samples were twice as high as in the other treatments (Table 2). Thus, during that 24 h incubation, residual and freshly produced NH_4^+ were apparently nitrified, and presumably, nitrification rates would not have exceeded ammonification rates over the longer term.

An important result of this study was the high NO_3^- immobilization rates relative to the gross nitrification rates. Nitrate immobilization rates approaching gross nitrification rates have been reported for grassland and forest soils (Stark and Hart, 1997; Hatch et al., 2000), which, in contrast to agricultural soils, are considered N rather than C limited. Although in both soils microbial NO_3^- immobilization was substantial, suggesting an important function of microbes in controlling NO_3^- concentrations, there were nevertheless sizable standing pools of NO_3^- throughout the growing season possibly because soil C limited NO_3^- immobilization in both systems, and NO_3^- availability exceeded plant N demand.

In these soils, heterotrophic microbes assimilated less NH_4^+ than NO_3^- probably because NH_4^+ concentrations were low and the spatial distribution of NH_4^+ may not have been uniform due to diffusional limitations. Concentrations of NO_3^-, on the other hand, were much higher and the physical constraints to its distribution are lower (Tinker and Nye, 2000). Low concentrations of NH_4^+ only partially inhibit NO_3^- assimilation by microbes (Rice and Tiedje, 1989). Moreover, NH_4^+ may have disappeared completely from some microsites in the soil because of nitrification. The fact that microbial NH_4^+ assimilation rates were similar in the two soils, even though gross ammonification rates were much higher in the organically than the conventionally managed soil, seems to indicate strong competition for NH_4^+ between nitrifiers and N immobilizers. Thus, NO_3^- was likely

more available to microbes. If microbial immobilization of inorganic N is expressed relative to source pool sizes, as suggested by Low et al. (1997), NH_4^+ immobilization in both soils was at least five times greater than NO_3^- immobilization, lending support to the widely held notion that NH_4^+, being in the reduced state, is the preferred N form for assimilation by microbes (Paul and Clark, 1996).

It is unlikely that the addition of $K^{15}NO_3$ stimulated microbial NO_3^- immobilization since NO_3^- concentrations in the isotope pool dilution experiment were similar to those encountered in the field. Nitrate consumption rates tended to be higher than our estimated rates of microbial NO_3^- immobilization. Using a correction factor to convert the 1 d fumigation N-flush (F_N) to MBN, e.g. F_N/0.54 (Shen et al., 1984; Brookes et al., 1985), would also increase the rate of NO_3^- immobilization. Thus, our estimates of microbial NO_3^- assimilation based on ^{15}N recovery in MBN are conservative. Microbial assimilation of NH_4^+ may have been stimulated because the ambient NH_4^+ pools were approximately doubled by the application of the ^{15}N label. In addition, our estimates of microbial NH_4^+ immobilization could be inflated because some of the ^{15}N measured in the microbial biomass may have been taken up as NO_3^-.

It appears that microbial demand for N as a result of C availability, the spatial distribution of NH_4^+ and NO_3^-, and competition by NH_4^+ oxidizers must all be considered factors that determine the magnitude and proportion of NH_4^+ and NO_3^- assimilation by microbes. Because NH_4^+ and NO_3^- concentrations in the microcosms were similar as in the field, where heterotrophic microbes face additional competition for NH_4^+ by roots, our results indicate that in some agricultural soils, microbial NO_3^- immobilization may be greater than NH_4^+ immobilization.

Time elapsed since rewetting had no effect on ammonification or nitrification rates in either soil management type. Two weeks of air-drying may not have caused a flush of mineralization (Sparling and Ross, 1988) large enough to be observed in this experiment, or alternatively, the 5 d period at high water potential was possibly not long enough for mineralizable substrate depletion (Appel, 1998; Pulleman and Tietema, 1999) to occur. Nitrification activity was not impeded by prior soil drying.

4.3. Organic vs. conventional management

The high OM inputs in the organic system resulted in greater N supplying capacity well into the growing season, when N demand by plants is still high (Jackson and Bloom, 1990), compared to the low OM input conventional system. Nitrification appears to be the major fate of NH_4^+ in both systems. Measuring short-term rates of production and consumption of inorganic N highlighted the differential importance of recycling of NO_3^- in these systems. Microbial NO_3^- immobilization was unexpectedly high and, expressed as a percentage of gross nitrification rates, greater in the organic compared to the conventional soil. Highly concentrated inorganic N inputs can have detrimental environmental impacts (Matson et al., 1997; Venterea and Rolston, 2000b). Our data suggest that high OM inputs as an alternative resulted in a gradual release of inorganic N. Moreover, the greater C availability in the organic system apparently supports a more active microbial biomass with greater N demand, thus promoting immobilization and recycling of NO_3^-. The challenge in managing high OM input systems may well be in balancing C and N inputs to prevent a build-up of large standing NO_3^- pools, yet avoiding high rates of microbial N immobilization during peak periods of crop N demand.

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