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Soil Biology & Biochemistry 39 (2007) 1737–1743

Soil Biology & Biochemistry

www.elsevier.com/locate/soilbio

Potential importance of bacteria and fungi in nitrate assimilation in soil

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Received 4 October 2006; received in revised form 16 January 2007; accepted 23 January 2007
Available online 5 March 2007

Abstract

Soil microorganisms can use a wide range of N compounds but are thought to prefer NH_4^+. Nevertheless, ^{15}N isotope dilution studies have shown that microbial immobilization of NO_3^- can be an important process in many soils, particularly relatively undisturbed soils. Our objective was to develop a method for measuring NO_3^- immobilization potential so that the relative contributions of bacteria and fungi could be determined. We modified and optimized a soil slurry method that included amendments of KNO_3, glucose, and methionine sulfoximine (an inhibitor of N assimilation) in the presence of two protein synthesis inhibitors: chloramphenicol, which inhibits bacteria, or cycloheximide, which inhibits fungi. By adding ^{15}N-labeled KNO_3, we were able to measure gross rates of NO_3^- production (i.e., gross nitrification) and consumption (i.e., gross NO_3^- immobilization). We found that bacteria, not fungi, had the greatest potential for assimilating, or immobilizing, NO_3^- in these soils. This is consistent with their growth habit and distribution in the heterogeneous soil matrix.

Keywords: NO_3^- assimilation; NO_3^- immobilization; Bacteria; Fungi; ^{15}N isotope dilution; Allylthiourea; Methionine sulfoximine; Chloramphenicol; Cycloheximide

1. Introduction

Nitrogen is essential for the growth and activity of all soil microorganisms. Both bacteria and fungi have the genetic potential to use organic and inorganic sources of N (Merrick and Edwards, 1995; Marzluf, 1997), but it is generally accepted that heterotrophic microorganisms in soils preferentially use NH_4^+ over NO_3^- (Recous et al., 1990; Jansson et al., 1955). This preference is consistent with a recent meta-analysis that found that the importance of NO_3^- assimilation decreased as N mineralization increased (Booth et al., 2005), which fits with empirical studies with soils that have shown that NO_3^- assimilation is regulated by NH_4^+, being inhibited at high NH_4^+ concentrations (Rice and Tiedje, 1989; McCarty and Bremner, 1992). Nevertheless, ^{15}N isotope dilution studies with soils from many ecosystems have often found significant rates of NO_3^- immobilization in soil (Booth et al., 2005). Booth et al. (2005) also report that, in general, NO_3^- immobilization is higher in forest and grassland soils than in cultivated agricultural soils, but exceptions exist (Burger and Jackson, 2003).

The importance of NO_3^- immobilization in a given soil is likely dependent upon a variety of factors, such as the rate of nitrification, which supplies NO_3^-, and the availability of C, which fuels the growth and activity of heterotrophic microorganisms (Schimel and Bennett, 2004; Booth et al., 2005). For example, NO_3^- immobilization has been shown to be stimulated by the addition of readily available C sources (Azam et al., 1988; Trinsoutrot et al., 2000; Nishio et al., 2001). The positive correlation of NO_3^- immobilization with nitrification is a bit more puzzling because high rates of nitrification are also associated with high availability of NH_4^+ (Booth et al., 2005), but NH_4^+ should inhibit NO_3^- assimilation (Rice and Tiedje, 1989; McCarty and Bremner, 1992). One explanation for this conundrum is that within the heterogeneous soil matrix, there are microsites of high available C and low NH_4^+ where NO_3^- can be assimilated (Schimel and Bennett, 2004; Booth et al., 2005). Evidence for this microsite hypothesis includes

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0038-0717/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.soilbio.2007.01.033

observations that NO_3^- immobilization is greater in undisturbed compared to physically disrupted soils (Booth et al., 2005) and small-scale studies of microbial assimilation of NO_3^- close to decomposing residues high in C (Cliff et al., 2002a; Moritsuka et al., 2004). One study using soil bacteria in liquid culture suggested that, even in the absence of microsite variability and diffusional constraints, at least some bacteria will assimilate NO_3^- even when NH_4^+ is available (Bengtson and Bengtsson, 2005), suggesting that the composition of the microbial community may also be important in determining the relative importance of NO_3^- immobilization in soil.

The relative proportion of bacteria and fungi is one measure of microbial community composition. Although the fungal:bacterial ratio is perhaps a broad metric, it is thought to be important because the morphological, physiological, and lifestyle differences between bacteria and fungi predispose them to potentially occupy different ecological niches and to play different roles in C and nutrient cycling in soils (Bardgett and McAlister, 1999; Imberger and Chiu, 2001; Bailey et al., 2002; Klein and Paschke, 2004). Many approaches have been taken to quantify the fungal:bacterial ratio, such as microscopy (Klein et al., 1998; Bottomley et al., 2006), phospholipid biomarkers (Bardgett and McAlister, 1999; Bailey et al., 2002; Högberg et al., 2007), and most recently quantitative PCR (Fierer et al., 2005), but the only method to assess the relative proportion of fungal and bacterial activities is the use of selective inhibitors (Anderson and Domsch, 1975; Imberger and Chiu, 2001; Bailey et al., 2002). Although selective inhibitors have been used primarily in the substrate-induced respiration assay, they can be applied to measure other soil processes, such as N mineralization and nitrification (Landi et al., 1993) and denitrification (Laughlin and Stevens, 2002).

Our objectives were (1) to adapt the potential NO_3^- assimilation assay of McCarty and Bremner (1992) for use in forest and meadow soils and (2) to modify the procedure to determine the relative potential for bacteria and fungi to assimilate NO_3^- by using selective inhibitors. The second objective includes the use of selective inhibitors in combination with ^{15}N isotope dilution to separate gross from net rates of potential N cycling processes.

2. Materials and methods

2.1. Soils

Surface (0–10 cm) mineral soils were collected in early summer from adjacent meadow and forest areas at Carpenter Mountain within the H.J. Andrews Experimental Forest (44.2°N, 122.2°W) in the Western Cascade Mountains of Oregon, USA. Detailed characteristics of these sites and soils have been reported previously (Mintie et al., 2003; Rich et al., 2003). The meadow soil is classified as a Lithic Cryandept, the forest soil as a Pachic Haplumbrept. The meadow soil had less organic C (95 mg C kg^{-1} soil) than the forest soil (132 mg C kg^{-1} soil) but baseline concentrations of NO_3^- were two-fold greater in the meadow soil (2.9 vs. 1.5mg N kg^{-1} soil).

2.2. NO_3^- assimilation assay development

We evaluated and adapted the slurry method of McCarty and Bremner (1992). Slurries were prepared with 15–18 g soil (dry weight) from Carpenter meadow and 100 mL of 1.0 mM potassium phosphate buffer solution (pH 7.2) in 250-mL Erlenmeyer flasks capped with foam stoppers, and shaken at 300 rpm at room temperature (about 20 °C) for up to 48 h. Slurry samples were taken at various time intervals, extracted with an equal volume of 4 M KCl, and filtrates collected for inorganic N analysis.

We tested the need to supplement slurries with NO_3^- (0.50 mM KNO_3) and readily available C (0.84 mM glucose). Because NO_3^- assimilation rates based on the net change in NO_3^- concentration do not take into account the production of NO_3^-, we tested the effect of adding the nitrification inhibitor 1-allyl-2-thiourea (ATU; Sigma-Aldrich, St. Louis, Missouri) at 0.10 mM (Hooper and Terry, 1973). The method of McCarty and Bremner (1992) included L-methionine sulfoximine (MSX; Sigma-Aldrich, St. Louis, Missouri), an inhibitor of glutamine synthetase, so we evaluated its effect on NO_3^- assimilation using MSX at 0.33 mM. The results of these initial experiments were used to define the assay conditions we used in subsequent experiments.

2.3. Effect of bacterial and fungal inhibitors

The relative importance of bacteria and fungi in NO_3^- assimilation was determined using selective inhibitors of protein synthesis (e.g., Anderson and Domsch, 1975; Landi et al., 1993). We used chloramphenicol (CAM; Sigma-Aldrich, St. Louis, Missouri) as our bacterial inhibitor and cycloheximide (CYC; Sigma-Aldrich, St. Louis, Missouri) as our fungal inhibitor. Several concentrations of CAM (0, 100, 500 mg l^{-1}) or CYC (0, 10, 50, 100, 500 mg l^{-1}) were tested in both soils to determine their optimal concentrations for inhibiting bacterial or fungal NO_3^- assimilation.

2.4. ^{15}N isotope dilution experiment

Soil from Carpenter meadow was used in a ^{15}N isotope dilution experiment to evaluate the potential for bacteria and fungi to assimilate NO_3^-. A three-by-two factorial arrangement of treatments was used. The three levels of antibiotic treatment were control, 100 mg CAM l^{-1} to inhibit bacterial protein synthesis, and 10 mg CYC l^{-1} to inhibit fungal protein synthesis. The antibiotic treatment was crossed with two levels of MSX: a control without MSX and 0.33 mM MSX to prevent N assimilation. Each treatment was replicated four times. All samples received 0.84 mM glucose and 0.5 mM KNO_3 labeled at 10.4 atom% ^{15}N. Soil slurries with the appropriate amendments

were sampled after 8 and 32 h for the analysis of isotopic composition and concentrations of inorganic and total N. Rates of NO_3^- production and consumption were calculated using the equations of Kirkham and Bartholomew (1954), assuming a value of 0.3663 atom% ^{15}N for the source of the NO_3^- that is being produced.

2.5. Analytical methods

Filtered KCl extracts were analyzed colorimetrically for NH_4^+ and NO_3^- using an automated analyzer (Astoria-Pacific Inc., Clackamas, Oregon): the alkaline salicylate-hypochlorite method was used for NH_4^+ and Cd reduction followed by diazotization was used for NO_3^-. We found that MSX and CAM solutions contributed measurable amounts of NH_4^+ when assayed by the alkaline salicylate-hypochlorite method; consequently, we corrected for these contributions.

We used standard sequential diffusion techniques to prepare inorganic N in the KCl extracts for ^{15}N analysis (Stark and Hart, 1996). The soil remaining after filtration of the KCl extracts was rinsed to remove residual inorganic N, dried, and ground for analysis of organic N. The ^{15}N abundance of the diffused NH_4^+ and NO_3^- samples, and soil organic N was determined using an isotope ratio mass spectrometer (PDZ Europa Ltd., Crewe, Cheshire, England).

2.6. Statistical analysis

Treatment effects were tested with analysis of variance using either the ANOVA or GLM procedures of SAS release 8.2 (SAS Institute Inc., Cary, North Carolina), depending upon the design of the particular experiment. If analysis of variance showed significant treatment effects, treatments were compared using Fisher's least significant difference test ($p = 0.05$).

3. Results

3.1. Assay development

Background concentrations of NO_3^- were just a few mg N kg^{-1} soil, which were too low for measuring potential rates of NO_3^- disappearance; therefore we supplemented with 0.5 mM KNO_3 (Fig. 1A). With NO_3 supplementation, there was a lag of 6–12 h before a significant disappearance of NO_3^- was observed. The addition of glucose and/or MSX sustained the linear rate of NO_3^- disappearance from 24 to 48 h. For soils amended with NO_3^-, rates of NO_3^- disappearance were not affected by MSX, but were about 10% greater ($p < 0.05$) with added glucose (Table 1). Assimilation of inorganic N was effectively blocked by the addition of MSX, with almost immediate accumulation of NH_4^+ (Fig. 1B). The rate of NH_4^+ accumulation in treatments supplemented with NO_3^- was the same as the rate of NO_3^- disappearance, suggesting that both provided accurate rates of NO_3^- assimilation (Table 1). One caveat, however, was that addition of MSX when NO_3^- and glucose were not added also resulted in accumulation of NH_4^+ (10.3 ± 0.6 mg N kg^{-1} soil day^{-1}) even though there

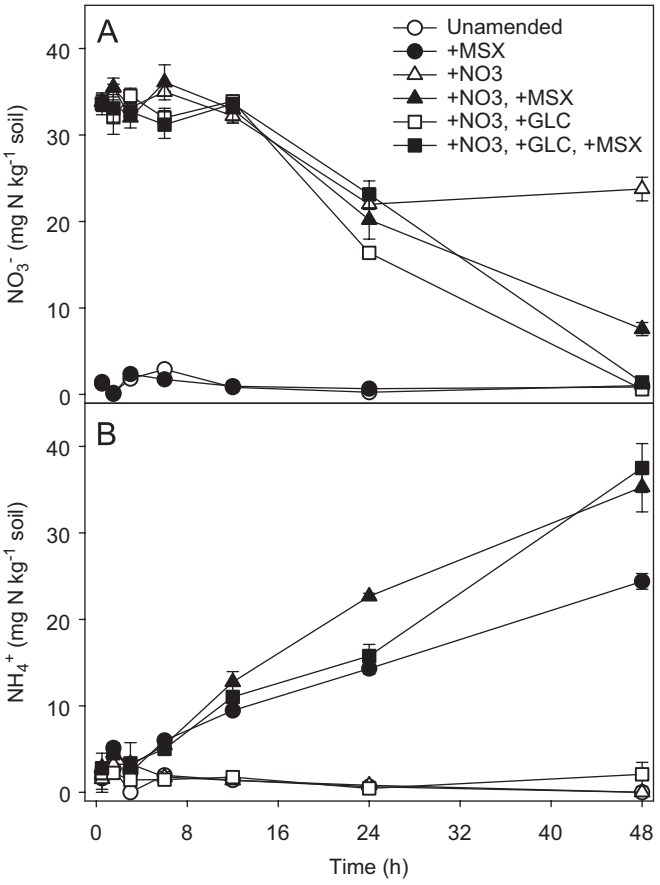

Fig. 1. Changes in concentrations of NO_3^- (A) and NH_4^+ (B) during optimization of assay conditions. Soil from Carpenter meadow was used. Circles represent background levels of soil NO_3^- and C, triangles represent addition of 0.5 mM NO_3^-, and squares represent addition of both 0.5 mM NO_3^- and 0.84 mM glucose (GLC). Open symbols are without methionine sulfoximine (MSX) and closed symbols are with 0.33 mM MSX. Data are means with standard error bars ($n = 3$).

Table 1
Potential rates of NO_3^- assimilation in Carpenter meadow soil as affected by additions of glucose (0 and 0.84 mM) and methionine sulfoximine (MSX, 0 and 0.33 mM)

Treatment	NO_3^- disappearance (mg N kg^{-1} soil day^{-1})	NH_4^+ accumulation (mg N kg^{-1} soil day^{-1})
−Glucose, −MSX	17.8 ± 0.7[a]	Na[b]
−Glucose, +MSX	16.7 ± 0.9	16.5 ± 1.7
+Glucose, −MSX	19.6 ± 0.6	Na
+Glucose, +MSX	18.5 ± 0.8	18.1 ± 1.6

Data are means ± standard errors ($n = 3$) based on linear regressions of inorganic N concentrations from 6 to 48 h.

[a]Rate calculated from 6 to 24 h because NO_3^- consumption stopped after 24 h.

[b]Not applicable because NH_4^+ did not accumulate in the absence of MSX.

Table 2
Rates of N cycle processes of Carpenter meadow soil during the isotope dilution experiment as influenced by antibiotic (CAM, chloramphenicol; CYC, cycloheximide) and methionine sulfoximine (MSX) additions

MSX (mM)	Antibiotic ($mg\,l^{-1}$)	Gross NO_3^- production ($mg\,N\,kg^{-1}\,soil\,day^{-1}$)	Gross NO_3^- consumption ($mg\,N\,kg^{-1}\,soil\,day^{-1}$)	Net change in NO_3^- ($mg\,N\,kg^{-1}\,soil\,day^{-1}$)	Net change in NH_4^+ ($mg\,N\,kg^{-1}\,soil\,day^{-1}$)	Net N mineralization ($mg\,N\,kg^{-1}\,soil\,day^{-1}$)
0	0	1.4±3.4 a	28.1±0.7 b	−26.8±0.7 b	−1.4±0.2 a	−28.2±0.9 a
	100 CAM	4.9±1.7 a	1.0±1.8 a	3.9±1.8 a	3.3±0.6 ab	8.8±0.6 bc
	10 CYC	2.9±0.7 a	27.6±0.8 b	−24.7±0.3 b	0.1±1.9 ab	−24.6±2.0 a
0.33	0	15.2±1.9 b	43.6±1.8 c	−28.4±0.3 b	47.7±4.3 c	19.3±4.3 c
	100 CAM	1.4±1.4 a	3.2±2.5 a	−1.9±2.5 a	9.0±1.4 b	7.2±3.6 b
	10 CYC	13.6±4.6 b	42.3±5.0 c	−29.2±0.7 b	45.7±5.2 c	16.5±5.5 bc

All treatments received 0.5 mM NO_3^- and 0.84 mM glucose. Data are means with standard error bars ($n = 4$). Within a column, rates followed by the same letter are not significantly different ($p = 0.05$).

was no significant decrease in NO_3^- concentration (Fig. 1A). We found that ATU had no effect on the rate of NO_3^- disappearance ($p > 0.05$, data not shown). Based on these results, subsequent assays contained 0.5 mM KNO_3, 0.84 mM glucose, and 0.33 mM MSX, but no ATU.

We found that either 100 or 500 mg CAM l^{-1} (about 625 or 3125 mg CAM kg^{-1} soil) depressed NO_3^- disappearance by about 88% relative to the control in both meadow and forest soil (data not shown). Additions of CYC to meadow or forest soils at concentrations from 10 to 500 mg CYC l^{-1}, which bracket the commonly used value of 2 g CYC kg^{-1} soil (about 250 mg CYC l^{-1}), had no significant effect on NO_3^- disappearance relative to the control, and there were no significant differences among the concentrations tested (data not shown). In fact, addition of CYC tended to increase the rate of NO_3^- disappearance. Based on these results, we used the lowest concentrations tested: 100 mg CAM l^{-1} to block bacterial protein synthesis and 10 mg CYC l^{-1} to block fungal protein synthesis.

3.2. Isotope dilution experiment

Nitrate was consumed in all treatments except those receiving CAM, the bacterial inhibitor (Table 2). Rates of net NO_3^- consumption were near zero for the CAM treatment and ranged from 25 to 29 mg N kg^{-1} soil day^{-1} for all other treatments, which were not significantly different from each other. Small, but significant, amounts of NH_4^+ accumulated in the presence of CAM, independent of the presence of MSX. For the other treatments, there was no net change in NH_4^+ concentration without MSX, but large amounts of NH_4^+ accumulated in the presence of MSX. Unlike the results obtained during method development (Table 1), MSX resulted in much more NH_4^+ accumulation than NO_3^- disappearance (Table 2).

As with inorganic N concentrations, little change in the isotopic composition of N pools was observed for treatments with CAM (Fig. 2). The ^{15}N abundance of the NO_3^- pool did not change during the incubation except in the presence of MSX in the control and CYC treatment,

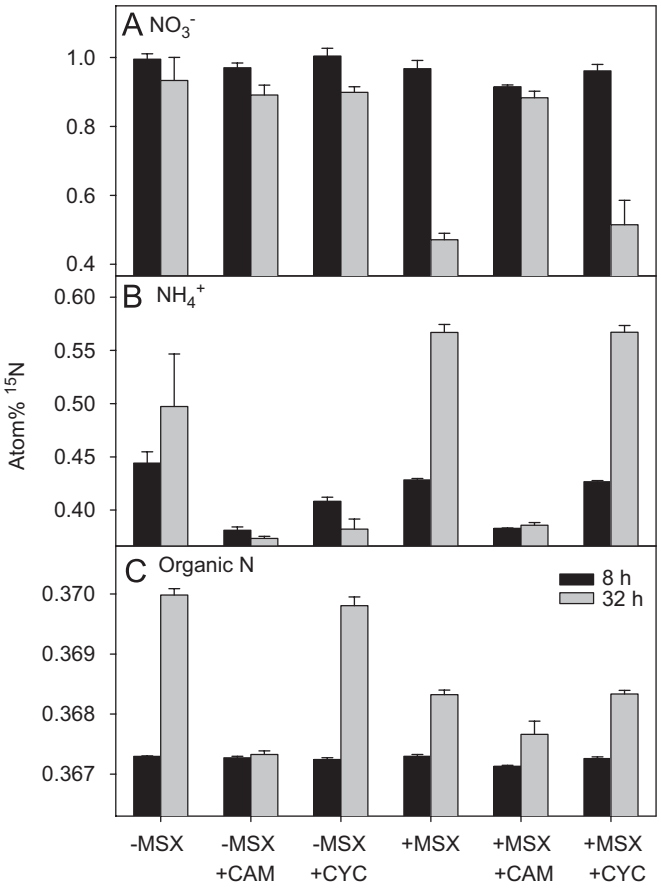

Fig. 2. Changes in atom% ^{15}N of NO_3^- (A), NH_4^+ (B), and organic N (C) during the isotope dilution experiment. Soil from Carpenter meadow was used. All treatments received 0.5 mM NO_3^- and 0.84 mM glucose. Treatments are designated by CAM (0 or 100 mg l^{-1} chloramphenicol), CYC (0 or 10 mg l^{-1} cycloheximide), and MSX (0 or 0.33 mM methionine sulfoximine). Black bars represent initial sampling at 8 h and gray bars represent final sampling at 32 h. Data are means with standard error bars ($n = 4$).

suggesting the production of NO_3^-, presumably from autotrophic nitrification (Fig. 2A). In fact, gross NO_3^- production was three- to 10-fold higher for these two treatments (Table 2), which were also the two treatments

that accumulated large concentrations of NH_4^+ (Table 2). Significant increases in ^{15}N abundance of NH_4^+ occurred only with MSX in the control and CYC treatments (Fig. 2B), suggesting that MSX blocked assimilatory NO_3^- reduction at the stage of NH_4^+ incorporation. Isotope dilution calculations confirmed that CAM blocked gross NO_3^- consumption, but the presence of CYC had no effect (Table 2). Furthermore, MSX increased gross NO_3^- consumption by 50%, presumably because the extra NO_3^- produced from nitrification in the presence of MSX was subject to assimilatory NO_3^- reduction. In the absence of MSX, gross NO_3^- consumption was the same as the net change in NO_3^- concentration; in the presence of MSX, gross NO_3^- consumption was the same as the net change in NH_4^+ concentration (Table 2). Gross NO_3^- consumption rates in the presence of MSX agreed with estimates calculated by dividing the accumulation of ^{15}N in the NH_4^+ pool by the average atom% ^{15}N of the NO_3^- pool (42.7 ± 2.9, 3.8 ± 0.6, and 37.8 ± 2.3 mg N kg^{-1} soil day^{-1} for the control, CAM, and CYC treatments, respectively), further confirming the mechanism of MSX action.

At 8 h, the ^{15}N abundance of the soil organic N pool was close to that of unlabeled soil, about 0.3667 atom% ^{15}N (Fig. 2C). During the subsequent 24 h, a significant increase in ^{15}N was found for all treatments except those receiving CAM. A mass balance based on the decrease of ^{15}N in the inorganic N pool agreed with the increase of ^{15}N in the organic N pool within 25% for the control and CYC treatments that were not amended with MSX. With MSX, a mass balance based on the decrease of ^{15}N in the inorganic N pool found that no ^{15}N should have accumulated in the organic N pool during the incubation, but we observed an increase in the atom% ^{15}N of the organic pool. This discrepancy was most likely caused by incomplete removal of ^{15}N-labeled NH_4^+ during the rinsing step, which would have had a greater influence in the MSX treatments because both the NH_4^+ concentration and ^{15}N abundance were high.

Net N mineralization was negative and basically equivalent to gross NO_3^- consumption for the control and CYC treatments without MSX (Table 2). A small amount of net N mineralization occurred in the presence of CAM. Most of this increase in inorganic N came in the form of NH_4^+, suggesting that some amount of ammonification is related to the activity of extracellular enzymes or fungi. A large increase in net N mineralization was found in the control and CYC treatments in the presence of MSX. Because MSX should block biological NH_4^+ assimilation, net N mineralization in the presence of MSX presumably equates to enzymatic and microbial ammonification processes. If we assume that MSX has no effect on ammonification, then one can estimate rates of NH_4^+ assimilation to be 16.5 ± 5.3, 5.6 ± 3.8, and 13.8 ± 5.8 mg N kg^{-1} soil day^{-1} for the control, CAM, and CYC treatments without MSX.

4. Discussion

The potential NO_3^- assimilation assay required the addition of both NO_3^- and glucose to provide consistent results. We found that 0.5 mM KNO_3 and 0.84 mM glucose (10:1 molar ratio of C:N) worked well and was similar to that used by McCarty and Bremner (1992). Under these conditions, we typically observed a lag of 6–12 h before a linear rate of NO_3^- consumption began (Fig. 1), presumably because synthesis of proteins involved in assimilating NO_3^- was required. This is consistent with previous studies that pre-incubated soils in the presence of glucose for 16–24 h before measuring NO_3^- disappearance (Rice and Tiedje, 1989; McCarty and Bremner, 1992). With our soils, the addition of 0.5 mM KNO_3 and 0.84 mM glucose permitted assays lasting up to 48 h. We would recommend using a 24-h incubation, with samples taken at 8 and 32 h after the assay begins.

Our initial studies using unlabeled NO_3^- suggested that the rate of NO_3^- consumption in the soils we used was not affected by nitrification or the addition of MSX. In fact, in the presence of MSX, we found that rates of NO_3^- consumption and NH_4^+ accumulation were the same in NO_3^--amended soils (Table 1). Our subsequent experiment with $^{15}NO_3^-$ revealed that MSX stimulated nitrification and consequently inflated rates of gross NO_3^- consumption (Table 2). We do not know the reason for this discrepancy in the effect of MSX; the only difference was the length of time that soils had been stored at 4 °C. Although we detected no increase in soil NO_3^- concentrations during storage, it is possible that the nitrifier population increased during the additional four months of cold storage (e.g., Brooks et al., 1998). If the nitrifier population was active and large enough, the NH_4^+ that accumulated in the presence of MSX could have stimulated nitrification rates. Ammonium may have accumulated either because MSX blocks NH_4^+ assimilation or because MSX itself can be metabolized to produce NH_4^+ (Landi et al., 1999; Gelsomino et al., 1999). As a consequence of these results, it is probably best to run the potential NO_3^- assay without MSX.

Specific inhibitors must always be used with caution because they may have non-target effects or other unintended consequences, and optimal concentrations may vary by soil (Anderson and Domsch, 1975; Bailey et al., 2002; Nakamoto and Wakahara, 2004). Although we could not calculate the additivity ratio as done by others because we did not have a treatment with both antibiotics (Anderson and Domsch, 1975; Bailey et al., 2002; Nakamoto and Wakahara, 2004), the sum of the gross rates of NO_3^- production and consumption measured with each antibiotic agreed with the activity of the control (Table 2), suggesting that the antibiotics were working as intended. In our soils, we found that 100 mg CAM l^{-1} (about 625 mg CAM kg^{-1} soil) inhibited bacterial enzyme synthesis and activity, which is similar to the amount used in assays of denitrification potential (e.g., Myrold, 2002) or

substrate-induced respiration (Nakamoto and Wakahara, 2004). We found no significant difference in the inhibitory effect of CYC over a 50-fold range of concentrations that bracketed the commonly used value of $2\,g\,CYC\,kg^{-1}$ soil (about $250\,mg\,CYC\,l^{-1}$; Anderson and Domsch, 1975; Landi et al., 1993; Nakamoto and Wakahara, 2004).

To our knowledge, only one other study has used selective inhibitors of bacterial or fungal protein synthesis coupled with measurements of gross rates of N processes in soil (Laughlin and Stevens, 2002) and their focus was on denitrification. The combination of selective inhibitors and ^{15}N isotope dilution provides unique insights into the roles that bacteria and fungi have on specific N cycling processes. Gross NO_3^- production, or nitrification, was completely inhibited by CAM, the bacterial protein synthesis inhibitor, but unaffected by CYC, the fungal protein synthesis inhibitor. This suggests that autotrophic nitrification, which is done solely by prokaryotes, is responsible for nitrification in this soil, which is in agreement with previous work by Mintie et al. (2003). The inhibition of nitrification by CAM further suggests that in this soil, the response of nitrification to increased concentrations of NH_4^+ requires protein synthesis, which is consistent with studies of pure cultures of ammonia- and nitrite-oxidizers (Gerards et al., 1998; Tappe et al., 1999; Geets et al., 2006).

Gross NO_3^- consumption could be due to assimilatory and dissimilatory processes, such as denitrification or reduction to NH_4^+. Mass balance calculations showed no losses of ^{15}N from the system, suggesting that denitrification was not a significant process. Although there may have been some dissimilatory reduction of NO_3^-–NH_4^+ during the first 8 h of the incubation, there was subsequently no significant increase in the atom% ^{15}N of the NH_4^+ pool (in the absence of MSX), suggesting that gross NO_3^- consumption rates we calculated are equivalent to the rate of NO_3^- immobilization. The inhibitor data showed that potential NO_3^- immobilization was unaffected by CYC but almost completely inhibited by CAM. These observations suggest that bacteria are primarily responsible for assimilating NO_3^- in this soil and require *de novo* synthesis of proteins, which is consistent with the lag observed for NO_3^- assimilation in the absence of CAM. This finding is reasonable because bacteria are relatively immobile in soil and are more likely than fungi to exist in a variety of microsites, some of which are likely C rich but N poor. Given that NO_3^- is much more diffusible in soil than NH_4^+ (Cliff et al., 2002b), bacteria trapped in C-rich microsites, such as those associated with residues or the interior of aggregates, are likely to be preferentially exposed to, and therefore assimilate, NO_3^-. Fungi, on the other hand, because of their exploratory, filamentous growth form (Klein and Paschke, 2004), can access less mobile sources of N, such as NH_4^+. It may also be possible that the neutral pH and physical disruption of the slurry assay were not conducive to fungal activity, but we did not address this question as part of the current study.

In summary, we demonstrate that a soil slurry assay using $^{15}NO_3^-$ in combination with inhibitors of bacterial or fungal protein synthesis can provide useful information on the potential importance of these to microbial groups in nitrification and NO_3^- assimilation. In a natural meadow soil we found bacteria to dominate these two processes. As with all potential assays, it would be informative to see if the same potential is expressed in the field. But this awaits further studies that couple the use of selective inhibitors and ^{15}N isotope dilution in soils in their native state.

Acknowledgements

We thank Peter Bottomley, Kermit Cromack Jr., and Mona Högberg for discussions about this research, John Cliff for analytical assistance, and the rest of the Microbial Observatory crew for their help in the field and laboratory. The comments of two anonymous reviewers helped to improve quality of this paper. This research was supported by the Microbial Observatory program of the National Science Foundation. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the authors and do no necessarily reflect the views of the National Science Foundation.

References

Anderson, J.P.E., Domsch, K.H., 1975. Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21, 314–322.

Azam, F., Mahmood, T., Malik, K.A., 1988. Immobilization-remineralization of NO_3–N and total N balance during decomposition of glucose, sucrose and cellulose in soil incubated at different moisture regimes. Plant and Soil 107, 159–163.

Bailey, V.L., Smith, J.L., Bolton Jr., H., 2002. Fungal-to-bacterial ratios in soils investigated for enhanced C sequestration. Soil Biology & Biochemistry 34, 997–1007.

Bardgett, R.D., McAlister, E., 1999. The measurement of soil fungal:-bacterial biomass ratios as an indicator of ecosystem self-regulation in temperate meadow grasslands. Biology and Fertility of Soils 29, 282–290.

Bengtson, P., Bengtsson, G., 2005. Bacterial immobilization and remineralization of N at different growth rates and N concentrations. FEMS Microbiology Ecology 54, 13–19.

Booth, M.S., Stark, J.M., Rastetter, E., 2005. Controls on nitrogen cycling in terrestrial ecosystems: a synthetic analysis of literature data. Ecological Monographs 75, 139–157.

Bottomley, P.J., Yarwood, R.R., Kageyama, S.A., Waterstripe, K.E., Williams, M.A., Cromack Jr., K., Myrold, D.D., 2006. Responses of soil bacterial and fungal communities to reciprocal transfers of soil between adjacent coniferous forest and meadow vegetation in the Cascade Mountains of Oregon. Plant and Soil 289, 35–45.

Brooks, P.D., Williams, M.W., Schmidt, S.K., 1998. Inorganic N and microbial biomass dynamics before and during spring snowmelt. Biogeochemistry 43, 1–15.

Burger, M., Jackson, L.E., 2003. Microbial immobilization of ammonium and nitrate in relation to ammonification and nitrification rates in organic and conventional cropping systems. Soil Biology & Biochemistry 35, 29–36.

Cliff, J.B., Bottomley, P.J., Gaspar, D.J., Myrold, D.D., 2002a. Exploration of inorganic C and N assimilation by soil microbes with time of flight secondary ion mass spectrometry. Applied and Environmental Microbiology 68, 4067–4073.

Cliff, J.B., Bottomley, P.J., Haggerty, R., Myrold, D.D., 2002b. Modeling the effects of diffusion limitations on nitrogen-15 isotope dilution. Soil Science Society of America Journal 66, 1868–1877.

Fierer, N., Jackson, J.A., Vilgalys, R., Jackson, R.B., 2005. Assessment of soil microbail community structure by use of taxon-specific quantitative PCR assays. Applied and Environmental Microbiology 71, 4117–4120.

Geets, J., Boon, N., Verstraete, W., 2006. Strategies of aerobic ammonia-oxidizing bacteria for coping with nutrient and oxygen fluctuation. FEMS Microbiology Ecology 58, 1–13.

Gelsomino, A., Landi, L., Cacco, G., Nannipieri, P., 1999. Determination and depletion kinetics of L-methionine-sulphoxime in soil. Soil Biology & Biochemistry 31, 561–566.

Gerards, S., Duyts, H., Laanbroek, H.J., 1998. Ammonium-induced inhibition of ammonium-starved *Nitrosomonas europaea* cells in soil and sand slurries. FEMS Microbiology Ecology 26, 269–280.

Högberg, M.N., Högberg, P., Myrold, D.D., 2007. Is microbial community composition in boreal forest soils determined by pH, C–to–N ratio, the trees, or all three? Oecologia 150, 590–601.

Hooper, A.B., Terry, K.R., 1973. Specific inhibitors of ammonia oxidation in *Nitrosomonas*. Journal of Bacteriology 115, 480–485.

Imberger, K.T., Chiu, C.-Y., 2001. Spatial changes of soil fungal and bacterial biomass from a sub-alpine coniferous forest to grassland in a humid, sub-tropical region. Biology and Fertility of Soils 33, 105–110.

Jansson, S.L., Hallam, M.J., Bartholomew, W.V., 1955. Preferential utilization of ammonium over nitrate by micro-organisms in the decomposition of oat straw. Plant and Soil 4, 382–390.

Kirkham, D., Bartholomew, W.V., 1954. Equations for following nutrient transformations in soil, utilizing tracer data. Soil Science Society of America Proceedings 18, 33–34.

Klein, D.A., Paschke, M.W., 2004. Filamentous fungi: the indeterminant lifestyle and microbail ecology. Microbial Ecology 47, 224–235.

Klein, D.A., Paschke, M.W., Redente, E.F., 1998. Assessment of fungal—bacterial development in a successional shortgrass steppe by direct integration of chloroform-fumigation extraction (FE) and microscopically derived data. Soil Biology & Biochemistry 30, 573–581.

Landi, L., Badalucco, L., Pomarě, R., Nannipieri, P., 1993. Effectiveness of antibiotics to distinguish the contributions of fungi and bacteria to net nitrogen mineralization, nitrification, and respiration. Soil Biology & Biochemistry 25, 1771–1778.

Landi, L., Barraclough, D., Badalucco, L., Gelsomino, A., Nannipieri, P., 1999. L-Methionine-sulphoxime affects N mineralization-immobilization in soil. Soil Biology & Biochemistry 31, 253–259.

Laughlin, R.J., Stevens, R.J., 2002. Evidence of fungal dominance of denitrification and codenitrification in a grassland soil. Soil Science Society of America Journal 66, 1540–1548.

Marzluf, G.A., 1997. Genetic regulation of nitrogen metabolism in the fungi. Microbiology and Molecular Biology Reviews 61, 17–32.

McCarty, G.W., Bremner, J.M., 1992. Regulation of assimilatory nitrate reductase activity in soil by microbial assimilation of ammonium. Proceedings of the National Academy of Sciences of the United States of America 89, 453–456.

Merrick, M.J., Edwards, R.A., 1995. Nitrogen control in bacteria. Microbiology and Molecular Biology Reviews 59, 604–622.

Mintie, A.T., Heichen, R.S., Cromack Jr., K., Myrold, D.D., Bottomley, P.J., 2003. Ammonia-oxidizing bacteria along meadow-to-forest transects in the Oregon Cascade mountains. Applied Environmental Microbiology 69, 3129–3136.

Moritsuka, N., Yanai, J., Mori, K., Kosaki, T., 2004. Biotic and abiotic processes of nitrogen immobilization in the soil-residue interface. Soil Biology & Biochemistry 36, 1141–1148.

Myrold, D.D., 2002. Quantification of nitrogen transformations. In: Hurst, C.J., Knudsen, G.R., McInerney, M.J., Stetzenbach, L.D., Walter, M.V. (Eds.), Manual of Environmental Microbiology, second Edition. ASM Press, Washington, DC., pp. 583–590.

Nakamoto, T., Wakahara, S., 2004. Development of substrate induced respiration (SIR) method combined with selective inhibition for estimating fungal and bacterial biomass in humic andosols. Plant Production Science 7, 70–76.

Nishio, T., Komada, M., Arao, T., Kanamori, T., 2001. Simultaneous determination of transformation rates of nitrate in soil. Japan Agricultural Research Quarterly 35, 11–17.

Recous, S., Mary, B., Faurie, G., 1990. Microbial immobilization and ammonium and nitrate in cultivated soils. Soil Biology & Biochemistry 22, 913–922.

Rice, C.W., Tiedje, J.M., 1989. Regulation of nitrate assimilation by ammonium in soils and in isolated soil microorganisms. Soil Biology & Biochemistry 21, 597–602.

Rich, J.J., Heichen, R.S., Bottomley, P.J., Cromack Jr., K., Myrold, D.D., 2003. Community structure and functioning of denitrifying bacteria from adjacent meadow and forest soils. Applied Environmental Microbiology 69, 5974–5982.

Schimel, J.P., Bennett, J., 2004. Nitrogen mineralization: challenges of a changing paradigm. Ecology 85, 591–602.

Stark, J.M., Hart, S.C., 1996. Diffusion technique for preparing salt solutions, Kjeldahl digests, and persulfate digests for nitrogen-15 analysis. Soil Science Society of America Journal 60, 1846–1855.

Tappe, W., Laverman, A., Bohland, M., Braster, M., Ritterhaus, S., Groeneweg, J., van Verseveld, H.W., 1999. Maintenance energy demand and starvation recovery dynamics of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* cultivated in a retentostat with complete biomass retention. Applied and Environmental Microbiology 65, 2471–2477.

Trinsoutrot, I., Recous, S., Mary, B., Nicolardot, B., 2000. C and N fluxes of decomposing ^{13}C and ^{15}N *Brassica napus* L.: effects of residue composition and N content. Soil Biology & Biochemistry 32, 1717–1730.