Ecology, 87(8), 2006, pp. 2047–2057

NITROGEN DYNAMICS IN AN AUSTRALIAN SEMIARID GRASSLAND SOIL

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Abstract. We conducted a four-week laboratory incubation of soil from a *Themeda triandra* Forsskal grassland to clarify mechanisms of nitrogen (N) cycling processes in relation to carbon (C) and N availability in a hot, semiarid environment. Variation in soil C and N availability was achieved by collecting soil from either under tussocks or the bare soil between tussocks, and by amending soil with *Themeda* litter. We measured N cycling by monitoring: dissolved organic nitrogen (DON), ammonium (NH_4^+), and nitrate (NO_3^-) contents, gross rates of N mineralization and microbial re-mineralization, NH_4^+ and NO_3^- immobilization, and autotrophic and heterotrophic nitrification. We monitored C availability by measuring cumulative soil respiration and dissolved organic C (DOC). Litter-amended soil had cumulative respiration that was eightfold greater than non-amended soil (2000 compared with 250 μg C/g soil) and almost twice the DOC content (54 compared with 28 μg C/g soil). However, litter-amended soils had only half as much DON accumulation as non-amended soils (9 compared with 17 μg N/g soil) and lower gross N rates (1–4 compared with 13–26 μg N·[g soil]$^{-1}$·d^{-1}) and NO_3^- accumulation (0.5 compared with 22 μg N/g soil). Unamended soil from under tussocks had almost twice the soil respiration as soil from between tussocks (300 compared with 175 μg C/g soil), and greater DOC content (33 compared with 24 μg C/g soil). However, unamended soil from under tussocks had lower gross N rates (3–20 compared with 17–31 μg N·[g soil]$^{-1}$·d^{-1}) and NO_3^- accumulation (18 compared with 25 μg N/g soil) relative to soil from between tussocks. We conclude that N cycling in this grassland is mediated by both C and N limitations that arise from the patchiness of tussocks and seasonal variability in *Themeda* litterfall. Heterotrophic nitrification rate explained >50% of total nitrification, but this percentage was not affected by proximity to tussocks or litter amendment. A conceptual model that considers DON as central to N cycling processes provided a useful initial framework to explain results of our study. However, to fully explain N cycling in this semiarid grassland soil, the production of NO_3^- from organic N sources must be included in this model.

Key words: *Australia; autotrophic/heterotrophic nitrification; dissolved organic matter; nitrogen cycle; nitrogen mineralization; semiarid environment.*

Introduction

Arid and semiarid ecosystems occupy ~25% of the Earth's land area and 70% of the Australian continent. These regions of Australia are typified by highly weathered soils with extremely low organic matter contents (often <1%; Bennett and Adams 1999). Rainfall and subsequent plant productivity is also extremely variable both within and among years, with annual periods (and sometimes years) of prolonged drought (Australian Government, Bureau of Meteorology, data *available online*).[6] Conceptual frameworks of ecosystem functioning in these arid and semiarid environments also predict that spatial variability of water produces "resource islands" of greater plant productivity than surrounding areas (Noy-Meir 1973, Herman et al. 1995, Schlesinger et al. 1996). As a consequence, organic carbon (C) and nitrogen (N) concentrations and microbial activity are greater under plants relative to the bare soil between plants in arid and semiarid regions of Australia (Noble et al. 1996, Bennett and Adams 1999) and elsewhere (Kieft et al. 1987, Herman et al. 1995). Consequently, differences in C and N availability between and under plants will ultimately determine the extent and distribution of microbially mediated processes such as nitrogen (N) cycling (Bennett and Adams 1999). However, a detailed examination of the concepts and mechanisms by which C and N availability influence N cycling in arid and semiarid ecosystems is currently lacking.

Manuscript received 4 November 2005; revised 31 January 2006; accepted 3 February 2006. Corresponding Editor: P. M. Groffman.

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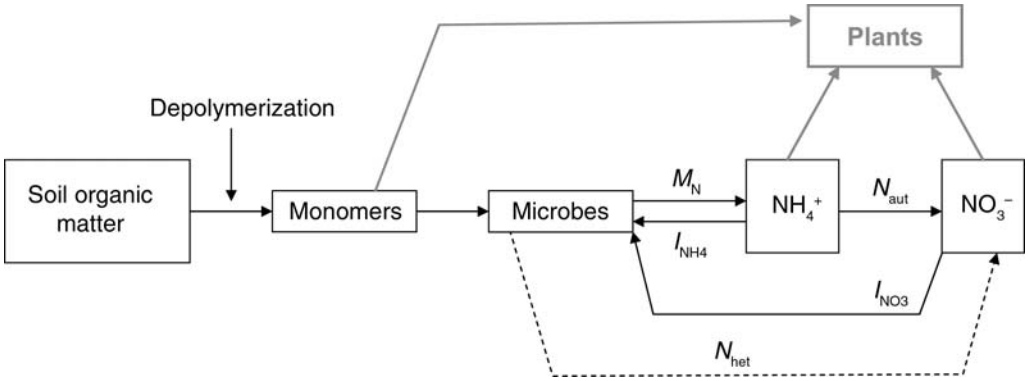

FIG. 1. Conceptual nitrogen cycling model. NH_4^+ is the ammonium pool, NO_3^- is the nitrate pool, M_N is organic N mineralization, N_{aut} is autotrophic nitrification, N_{het} is heterotrophic nitrification, I_{NH4} is ammonium immobilization, and I_{NO3} is nitrate immobilization. This figure is adapted from Schimel and Bennett (2004); the gray portions were not included in this study, and the dashed lines represent our proposed addition to the model.

Over the last decade a conceptual model has evolved that considers depolymerization of N-containing polymers rather than ammonium (NH_4^+) production as the controlling mechanism of terrestrial N cycling (summarized by Schimel and Bennett [2004] and Bardgett [2005]; Fig. 1). This model predicts that in ecosystems that are N limited, microbes compete primarily for N at the organic monomer stage, and additional inorganic N is absorbed from soil solution if available. These organic monomers are commonly water soluble and often referred to as dissolved organic N (DON). As ecosystem N availability increases relative to C availability, greater quantities of DON are released, increasing the proportion of N-rich microsites (mineralizing) to N-poor microsites (immobilizing), which, in turn, decreases microbial N limitation and net N mineralization begins. Further increasing ecosystem N availability or decreasing C availability will reduce the competition between heterotrophic soil microbes for N and provides progressively more opportunity for ammonium (NH_4^+) oxidation to NO_3^-. The variation in C and N availability that exists in arid and semiarid ecosystems provides an opportunity to test this conceptual model within, rather than between ecosystems.

The conceptual N model described in the previous paragraph predicts that nitrification, and the accumulation of NO_3^-, only occur when N is not limiting (Schimel and Bennett 2004). However, in many arid and semiarid ecosystems, typified by very low N contents, NO_3^- has been reported to accumulate in both laboratory (Kieft et al. 1987, 1998, Bennett and Adams 1999) and field (Hook and Burke 1995, Erskine et al. 1996) measurements. Nitrification is most commonly performed by autotrophic bacteria that gain energy from the oxidation of NH_4^+ and use CO_2 as a source of C. It is normally assumed that if C is not limiting, microbial heterotrophs, which use organic C as their energy source, are more competitive for NH_4^+ than autotrophic nitrifiers (Vitousek 1982, Tietema and Wessel 1992). However, low soil organic C content ($<$10 mg C/g soil) also characterizes Australian semiarid ecosystems (Bennett and Adams 1999), which may restrict heterotrophic growth and provide an opportunity for autotrophic nitrification to occur and NO_3^- to accumulate. Alternatively, nitrification is also preformed by heterotrophic bacteria or fungi (De Boer and Kowalchuk 2001) that oxidize organic N compounds and NH_4^+, and use organic C as their energy source (Paul and Clark 1996). As heterotrophic nitrifiers can compete for N at the DON stage, these bacteria and fungi may produce NO_3^- at lower N availabilities than autotrophic bacteria. If heterotrophic nitrification does play an important role in the production of NO_3^- in these ecosystems, the conceptual N model just described will require modification. Furthermore, variation in C and N availabilities within arid and semiarid ecosystems may provide an environment for both autotrophic and heterotrophic nitrification and an opportunity to test the affect of changing C and N availability on these processes.

The objective of the present study was to clarify mechanisms of N cycling in relation to C and N availability in a semiarid grassland. A range of C and N availabilities were established by collecting soil from between and under grass tussocks and through the addition of grass litter. We propose that: (1) under maximal plant productivity, greater C availability is likely to increase microbial N demand, restricting N cycling to DON; (2) progressively reducing C availability will ease the N limitation and increase the importance of NH_4^+ production and nitrification; and (3) variation in C and N availability will provide an environment for both autotrophic and heterotrophic nitrification.

METHODS

Field design and soil and tussock sampling

A detailed description of the Pilbara region and the experimental plots and soil properties are reported elsewhere (Bennett and Adams 1999, Bentley et al.

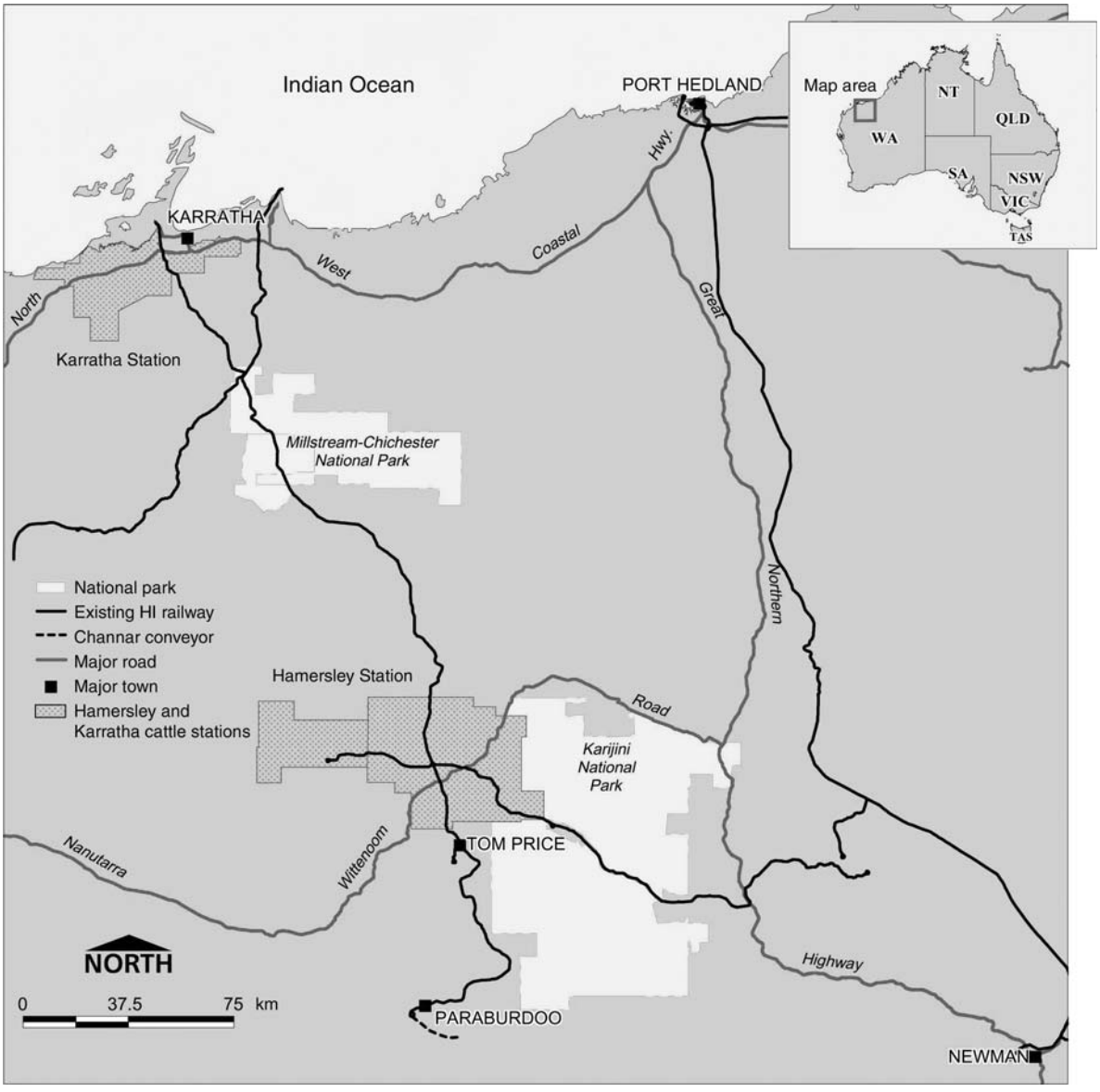

FIG. 2. Location of Hamersley Station in the Pilbara region of northern Western Australia.

1999). Briefly, experimental plots were constructed within perennial tussock grasslands (dominated by *Themeda triandra* Forsskal) located on Hamersley Station (22°17′ S, 117°40′ E) in the Pilbara region of northern Western Australia (Fig. 2). Here, growth of perennial grasses is highly responsive to rainfall, on average 350 mm/yr, but highly erratic both within and among years, and with the majority of rain falling from summer cyclones. Monthly maximum temperatures range from 24°C (July) to 40°C (December and January), and mean monthly minimum temperatures range from 11°C (July) to 26°C (January). Mean soil temperatures at 50 mm depth often exceed 30°C for six months each year. Soils are vertisols, characterized by deep cracking red clays underlain by calcrete (>600 mm depth) (Ug 5.36; Northcote 1979). These soils have a complex mineralogy containing 40% quartz, 15% kaolin, 15% smectite, 12% illite, and 10% hematite (Bennett and Adams 1999). We also assessed soil chemical composition (13% Al_2O_3, 53% SiO_2, 1% TiO_2, 16% Fe_2O_3, 2% CaO, 1% K_2O, 2% MgO) using X-ray fluorescence spectrometry (PW 1400, Philips, Eindhoven, The Netherlands). On 25 July 2004, we collected representative samples (0–50 mm depth) of soil (~5 kg) from between (BT) and under (UT) *Themeda* tussocks in four 5 × 5 m plots. Soils were sieved (<4 mm) and transported back to the laboratory within 5 days of collection. Aboveground *Themeda* litter was also collected and transported back to the laboratory, oven-dried (60°C) and finely ground (<1 mm). We assessed the composition of

the *Themeda* litter (0.3% N, 35% C, 4% lignin, 32% cellulose, and 25% hemicellulose) using combustion (CHN; LECO, St. Joseph, Michigan, USA) and fiber analysis (Goering and Van Soest 1970).

Laboratory design

Each of the eight soil samples was weighed (500 g air-dried equivalent) into two separate, 1-L, vented incubation vessels (100 mm deep). We applied ground *Themeda* litter at a rate of 15 mg/g soil to one of the two incubation vessels for each soil sample, and mixed thoroughly. This addition doubled the organic-C content of the surface soil and simulated one year of C input under maximal *Themeda* productivity (Ingram 2001). Therefore, treatments consisted of four replicates of under tussock soil (UT), under tussock soil plus *Themeda* litter (UT + T), between tussock soil (BT), and between tussock soil plus *Themeda* litter (BT + T). Distilled water was then added as a fine spray, and mixed thoroughly to establish a moisture content of between 65% to 70% field capacity. This moisture content was maintained by weighing each incubation vessel every day and adding water as a fine spray to replace mass loss (allowing for soil removed). Within two hours of weighing samples, the incubation vessels were placed in an incubator set at 40°C (±2°C) to represent the soil temperature during a summer cyclone. Soil was incubated for a period of up to 4 weeks.

Soil respiration, pH, and C and N pools

At the beginning of the incubation (day 1), soil (100 g oven-dry equivalent) was removed from each incubation vessel and incubated separately in 500-mL jars with gas septa in lids for headspace CO_2 measurement. Headspace gas was analyzed every 24 h for 10 d, and then every few days for the remaining 18 days. Soil respiration was determined by injection of 1 mL of incubation container headspace gas into an infrared gas analyzer (Series 225, Analytical Development Company, Hoddesdon, UK). Lids were then removed after each measurement from each incubation jar, and headspace gas was allowed to equilibrate (~5 min) with atmospheric CO_2. Any moisture loss during the equilibration period was replaced with distilled water using a fine spray. Lids were then replaced and the incubation continued. Results were calibrated against a 5% CO_2 standard.

A subsample of soil was collected from each incubation vessel at 1 day and 28 days, and soil pH was determined on oven-dried (50°C) soil (10 g) in 50 mL of distilled water, shaken for 1 h, and left to stand overnight.

A subsample of soil was collected from each incubation vessel at 1 day and 28 days, and total C and N were determined on oven-dried (50°C) soil using a combustion analyzer (CHN; LECO Corp., USA). A subsample of soil (40 g oven-dry equivalent) was collected from each incubation vessel 2, 4, 7, 14, 21, and 28 days after litter addition and extracted with 0.5 mol/L K_2SO_4 (1:4 solution : soil ratio). Soil NH_4^+ and NO_3^- (NO_3^- + nitrite) concentrations in the K_2SO_4 extracts were determined colorimetrically by automated segmented flow analysis (San plus stsyem, Skalar Analytical, Breda, The Netherlands). Dissolved organic N (DON) concentrations in the K_2SO_4 extracts were determined colorimetrically after alkaline persulfate oxidation (Cabrera and Beare 1993). Dissolved organic C (DOC) concentrations of the K_2SO_4 extracts were determined using a total organic C analyzer (5000A, Shimadzu, Kyoto, Japan). DOC was not determined on day 14.

Mineralization, immobilization, and autotrophic and heterotrophic nitrification rates

Rates of gross N transformations were estimated 2, 7, 14, and 28 days after litter amendment using a ^{15}N dilution technique (Murphy et al. 2003). Three subsamples of soil (each 40 g oven-dry equivalent) were collected from each incubation vessel and weighed into 300-mL vented plastic jars. To the first subsample of soil, 1 mL of NH_4Cl (60% ^{15}N enrichment) + KNO_3 (0.366% ^{15}N natural abundance) solution was added as a fine spray and thoroughly mixed by shaking the soil within the plastic jar for a few seconds, giving 2 μg N/g soil. To the second subsample of soil, 1 mL of NH_4Cl (0.366% ^{15}N natural abundance) + KNO_3 (60% ^{15}N enrichment) solution was added and thoroughly mixed by shaking the soil within the plastic jar for a few seconds, giving 2 μg N/g soil. To the third subsample of soil, 1 mL of NH_4SO_4 (60% ^{15}N enrichment) + KNO_3 (60% ^{15}N enrichment) solution was added and thoroughly mixed by shaking the soil within the plastic jar for a few seconds, giving 2 μg N/g soil. The ^{15}N-labeled soil samples were then placed back into the incubator. Subsamples of soil (10 g oven-dry equivalent) were extracted 2, 6, 12, 24, and 36 h after N application from each incubation vessel and immediately extracted with 0.5 M K_2SO_4 (1:4 solution : soil ratio). NH_4^+ and NO_3^- (NO_3^- + nitrite) concentrations in the K_2SO_4 extracts were determined colorimetrically by automated segmented flow analysis (San plus system, Skalar Analytical, Breda, The Netherlands). The NH_4^+ and NO_3^- in the K_2SO_4 extracts was recovered using a modified diffusion method (Brooks et al. 1989), and the ^{15}N:^{14}N ratio determined using isotope ratio mass spectrometery (ANCA-NT system 20/20, Europa Scientific, Cheshire, UK). Gross N rates were calculated using a ^{15}N tracing model described by Müller et al. (2004). The N transformation rates can either follow zero- or first-order kinetics and separate differential equations were defined for ^{14}N and ^{15}N pools. This numerical model was selected as it is the only one currently available that accounts for heterotrophic nitrification. Briefly, this model consists of six N pools and nine transformations in a conceptual framework presented in Fig. 3. The N pools are: N_{org} is organic N, NH_4^+ is the ammonium

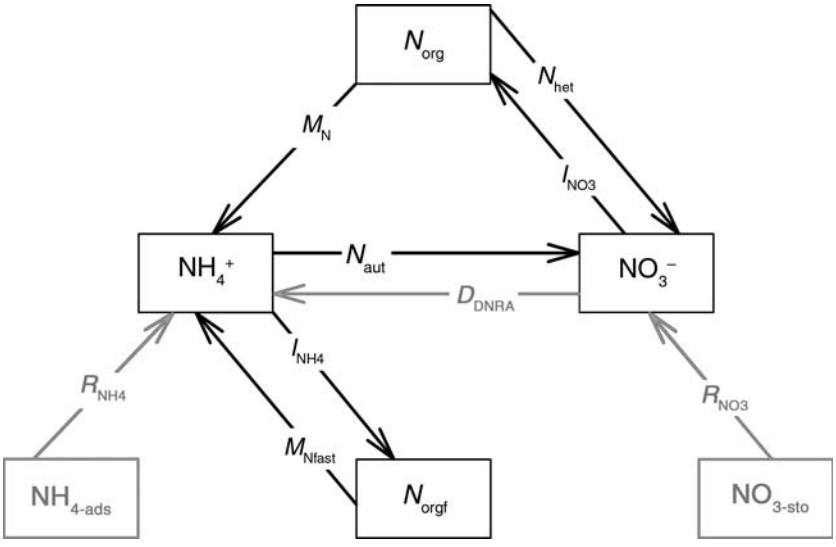

FIG. 3. Conceptual ^{15}N tracing model used to analyze nitrogen cycling in arid grassland soil. N_{org} is organic N, NH_4^+ is the ammonium pool, NO_3^- is the nitrate pool, N_{orgf} is a small, quickly cycling microbial biomass N pool, $NH_{4\text{-ads}}$ and $NO_{3\text{-sto}}$ are adsorbed NH_4^+ and stored NO_3^- pools, respectively, M_N is organic N mineralization, N_{aut} is autotrophic nitrification, N_{het} is heterotrophic nitrification, I_{NH4} is ammonium immobilization, I_{NO3} is nitrate immobilization, M_{Nfast} is microbial N mineralization, D_{DNRA} is a direct transformation from NO_3^- to NH_4^+, and R_{NH4} and R_{NO3} are the release of adsorbed NH_4^+ and stored NO_3^-, respectively. This figure is adapted from Müller et al. (2004); the gray portions were not included in this study.

pool, NO_3^- is the nitrate pool, N_{orgf} is a small microbial biomass N pool that undergoes quick N transformations, $NH_{4\text{-ads}}$ and $NO_{3\text{-sto}}$ are N pools related to the adsorption of NH_4^+ and the storage of NO_3^- (Fig. 3). $NH_{4\text{-ads}}$ and $NO_{3\text{-sto}}$ were not considered in the current study. The N transformations are: M_N is organic N mineralization, M_{Nfast} is microbial N mineralization from the N_{orgf} pool, N_{aut} is autotrophic nitrification, N_{het} is heterotrophic nitrification, I_{NH4} is ammonium immobilization, I_{NO3} includes NO_3^- immobilization by the microbial biomass and other NO_3^- consumption rates such as denitrification (note, I_{NH4} and I_{NO3} enter N_{orgf} and N_{org}, respectively, as bacteria are likely to preferentially use NH_4^+ as their N source, while other organisms, such as fungi, may prefer NO_3^- as their N source), D_{DNRA} is a direct transformation from NO_3^- to NH_4^+, and R_{NH4} and R_{NO3} are the release of adsorbed NH_4^+ and stored NO_3^- (Fig. 3). D_{DNRA}, R_{NH4}, and R_{NO3} were not considered in the current study. Model data are presented as means ± 1 SD. The optimization procedure for this model was the same as the two-step approach described by Müller et al. (2004).

Statistical analysis

The experimental design consisted of three factors: proximity to tussock (two levels, under and between, fixed), litter amendment (two levels, amended and non-amended, fixed), and sampling time (varied between different measurements, fixed), with three replicates. The optimization algorithm within the ^{15}N model calculated the residual sum of squares based on the actual mean and standard deviation of the data. This is also referred to as a "cost function" and is minimized during the optimization run. Once the model has reached the optimum, it calculates analysis of variance. All data are presented on an oven-dry basis and corrected for differences in field bulk density. Time series data were tested (Kurtosis skewness) for normality and log-transformed when necessary. Significant differences between treatments were tested using repeated sampling analysis (Webster and Oliver 1990) using GenStat Version 7.0 (GenStat 2003).

RESULTS

At the start and end of the incubation, soil C and N content and soil C/N ratio were significantly ($P < 0.05$) higher in *Themeda*-amended than non-amended soils and in soils sampled from under tussocks than between tussocks (Table 1). At the end of the incubation, soil pH,

TABLE 1. Effect of amending soil with *Themeda* litter (+T) on soil pH (in water), soil carbon and nitrogen content, and C:N ratio of soil sampled from under (UT) and between (BT) *Themeda* tussocks during a 28-day laboratory incubation.

Treatment	Day	pH	Carbon (g C/kg soil)	Nitrogen (g N/kg soil)	C:N
UT + T	1	8.21[b]	22.7[b]	1.19[a]	22.3[b]
	28	7.88[a]	18.5[a]	1.14[a]	18.9[a]
UT	1	8.20[a]	12.5[a]	1.08[a]	13.5[a]
	28	8.19[a]	12.2[a]	1.09[a]	13.1[a]
BT + T	1	8.19[b]	19.8[b]	1.10[a]	21.0[b]
	28	7.75[a]	16.2[a]	1.06[a]	17.8[a]
BT	1	8.17[a]	10.2[a]	0.98[a]	12.1[a]
	28	8.19[a]	9.8[a]	0.93[a]	12.3[a]

Notes: Treatment means followed by the same superscript letter are not significantly different ($P < 0.05$; $n = 4$ replicates per treatment). The C:N molar ratio is moles C/kg : moles N/kg.

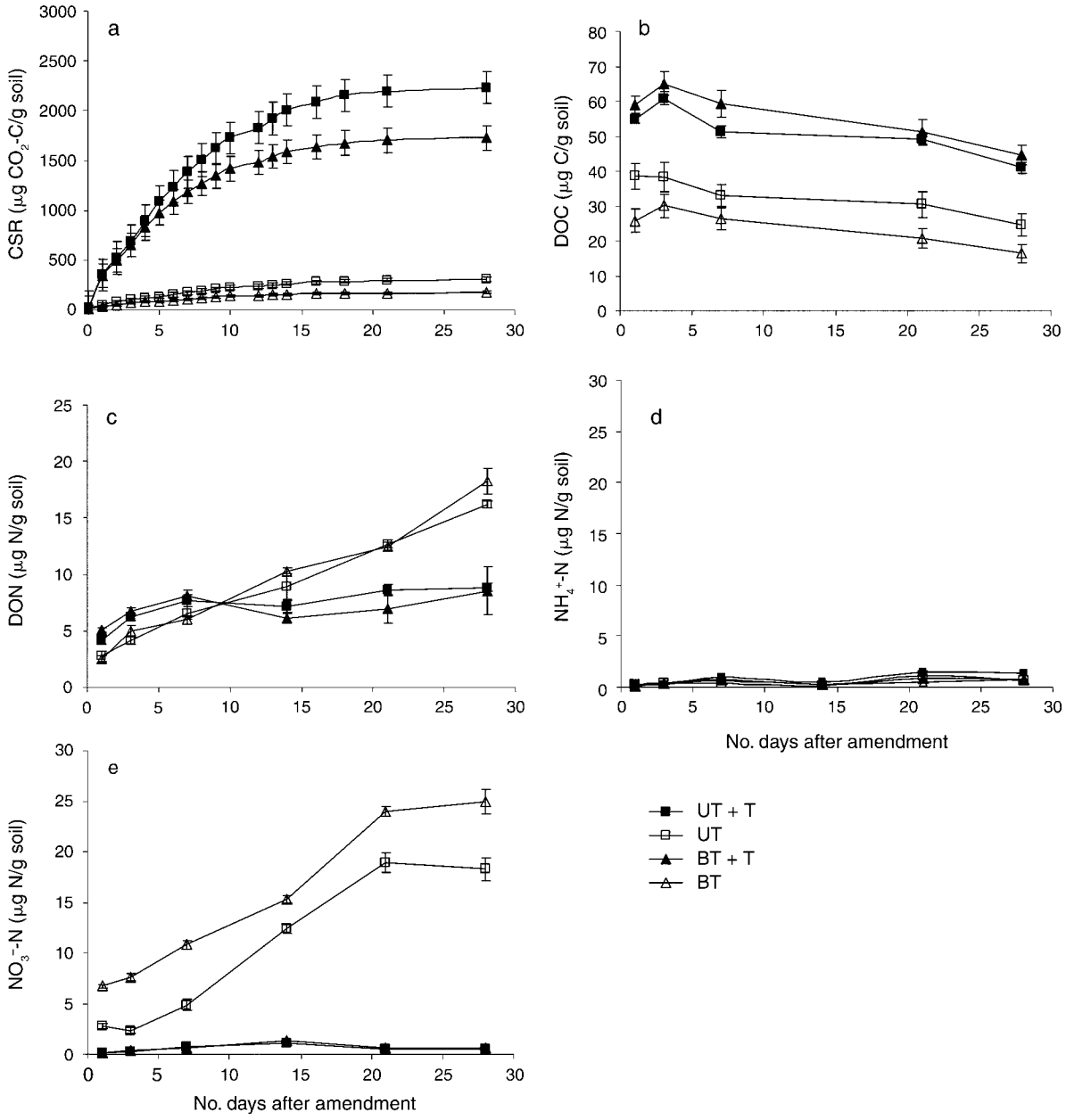

FIG. 4. The effect of amending soil with *Themeda* litter (+T) on (a) cumulative soil respiration (CSR), (b) dissolved organic carbon (DOC), (c) dissolved organic nitrogen (DON), (d) ammonium (NH_4^+), and (e) nitrate (NO_3^-) content in soil sampled from under (UT) and between (BT) *Themeda* tussocks during a 28-day laboratory incubation. Error bars are standard errors of the difference ($n = 4$).

soil C content, and C:N ratio were all significantly ($P < 0.05$) lower than at the start of the incubation in *Themeda*-amended soils; these factors did not change in non-amended soil over the incubation period (Table 1).

Cumulative soil respiration was significantly ($P < 0.05$) greater in *Themeda*-amended than non-amended soil for the entire incubation period (Fig. 4a). From day 12, and for the remainder of the incubation period, cumulative soil respiration was also significantly ($P < 0.05$) greater in soil sampled from under tussocks than between tussocks (Fig. 4a). Except an initial increase (day 2–4), DOC concentrations decreased over the incubation period by ~20 μg C/g soil, in all soils (Fig. 4b). DOC was also significantly ($P < 0.05$) greater in all *Themeda*-amended than non-amended soils and in non-amended soil sampled from under tussocks than between tussocks (Fig. 4b). Initially, DON concentrations were similar in *Themeda*-amended (~5 μg N/g soil) and non-amended (~3 μg N/g soil) soil (Fig. 4c). However, DON concentrations in non-amended soil

TABLE 2. Nitrogen transformation model parameters, rate constants, and kinetics after optimization of soil sampled from under (UT) and between (BT) *Themeda* tussocks and amended with *Themeda* litter (+T) during a 28-day laboratory incubation.

Treatment	Day	Model parameters								
		khetnit	ki_NH_4	ki_NO_3	kmin	kminfast	knit	Norginit	Norgfastinit	r^2
UT + T	2	0.1386	0.0549	0.3008	0.0703	0.0492	0.0984	300	0.0017	0.9853
	7	0.1593	0.0472	0.2525	0.0523	0.0059	0.0454	300	0.0017	0.9263
	14	0.1999	0.0324	0.3061	0.0516	1.6660	0.0964	300	0.0017	0.9342
	28	0.0193	0.0134	0.3140	0.1398	0.0000	0.1772	300	0.0017	0.9706
UT	2	0.4186	1.2955	0.1630	0.5405	0.0554	0.7850	300	0.5125	0.9999
	7	0.1103	0.5161	0.0225	0.5164	0.0000	0.0298	300	0.5125	0.9947
	14	0.0890	0.0611	0.0929	0.5050	0.1997	0.7000	300	0.5125	0.9955
	28	1.6093	0.0689	0.0928	0.5051	0.1754	0.4531	300	0.5125	0.9976
BT + T	2	0.1386	0.0100	0.3008	0.0547	3.2714	0.1294	300	0.0017	0.8941
	7	0.1386	0.0100	0.3008	0.0547	3.2714	0.1294	300	0.0017	0.8507
	14	0.1720	0.0000	0.2754	0.0682	5.0261	0.1112	300	0.0017	0.9845
	28	0.1720	0.0000	0.4052	0.0682	5.0261	0.1112	300	0.0017	0.8485
BT	2	0.6908	1.2839	0.1605	0.5141	0.0584	0.7558	300	0.5125	0.9297
	7	0.7152	0.7815	0.1573	0.5862	0.3358	0.7948	300	0.5125	0.9979
	14	0.7014	0.5004	0.1515	0.8850	0.6952	1.0759	300	0.5125	0.9905
	28	0.7014	4.3744	0.0875	0.9844	0.9254	1.2061	300	0.5125	0.9983

Notes: Abbreviations are: heterotrophic nitrification (khetnit), ammonium immobilization (ki_NH_4), nitrate immobilization (ki_NO_3), N mineralization (kmin), N mineralization from N_{orgf} (N_{orgf} is a small microbial biomass pool that undergoes quick N transformation; kminfast), autotrophic nitrification (knit) rate constants; and initial organic N (Norginit) and microbial N_{orgf} (Norgfastinit) concentrations. The values for khetnit and kmin are zero-order kinetics, while those for ki_NH_4, ki_NO_3, kminfast, and knit are first-order kinetics.

increased over the entire incubation period (~17 μg N/g soil) and were significantly ($P < 0.05$) greater than *Themeda*-amended soil after 21 days of incubation. DON did not increase in litter-amended soil after day 7 (~8 μg N/g soil; Fig. 4c). There was no effect of proximity to tussocks on DON concentration (Fig. 4c). Soil NH_4^+ contents were not affected by the treatments and remained at <1 μg N/g soil for the entire incubation period (Fig. 4d). Soil NO_3^- contents were significantly ($P < 0.05$) higher in non-amended than *Themeda*-amended soil, where they also remained <2 μg N/g soil for the entire incubation period and were not affected by proximity to tussock (Fig. 4e). Soil NO_3^- contents in non-amended soil were also significantly ($P < 0.05$) higher in soil from between tussocks compared with under tussocks for the entire incubation period (Fig. 4e).

The model parameters after optimization for the ^{15}N model are presented in Table 2. Model runs had an R^2 between 0.84 and 0.99 (Table 2). In general, N mineralization, microbial N mineralization, NH_4^+ immobilization, autotrophic nitrification, heterotrophic nitrification, and NO_3^- immobilization rates were significantly ($P < 0.05$) greater in non-amended than *Themeda*-amended soil (Table 3). On most sampling

TABLE 3. Effect of amending soil with *Themeda* litter (+T) on various N mineralization, immobilization, and nitrification rates, and the percentage of heterotrophic nitrification of total nitrification ($N_{het}/N_{het}+N_{aut}$) in soil sampled from under (UT) and between (BT) *Themeda* tussocks during a 28-day laboratory incubation.

Treatment	Day	Nitrogen flux rates (μg N·[g soil]$^{-1}$·d^{-1})						$N_{het}/N_{het}+N_{aut}$ (%)
		M_N	M_{Nfast}	I_{NH4}	N_{aut}	N_{het}	I_{NO3}	
UT + T	2	1.7 (0.2)	0.3 (0.1)	0.6 (0.3)	1.1 (0.0)	3.3 (0.2)	4.2 (0.2)	74
	7	1.3 (0.4)	0.1 (0.2)	0.8 (0.7)	0.8 (0.6)	3.8 (1.4)	4.6 (1.4)	83
	14	1.2 (0.7)	0.7 (0.8)	0.7 (2.3)	2.1 (0.6)	4.8 (2.0)	7.4 (2.3)	69
	28	3.4 (1.0)	0.0 (0.0)	0.3 (4.8)	4.6 (4.8)	0.5 (1.9)	6.9 (3.4)	9
UT	2	13.0 (2.0)	8.0 (1.6)	13.6 (1.9)	8.3 (0.6)	10.1 (0.8)	17.6 (1.7)	55
	7	12.4 (3.2)	0.0 (0.0)	11.8 (3.3)	0.7 (0.4)	2.7 (0.6)	2.2 (0.3)	79
	14	12.1 (3.7)	1.4 (4.4)	1.1 (6.0)	12.9 (3.8)	2.1 (4.2)	14.0 (2.2)	14
	28	12.1 (5.9)	2.1 (10)	2.1 (6.5)	13.6 (6.5)	38.6 (27)	47.6 (21)	74
BT + T	2	1.3 (0.1)	0.1 (0.0)	0.1 (0.0)	1.2 (0.1)	3.3 (0.2)	4.5 (0.2)	73
	7	1.3 (0.1)	0.1 (0.1)	0.1 (0.1)	1.7 (0.1)	3.3 (0.3)	5.2 (0.4)	67
	14	1.6 (0.8)	0.0 (0.0)	0.0 (0.0)	2.4 (0.8)	4.1 (0.9)	8.2 (0.8)	64
	28	1.6 (0.7)	0.3 (0.1)	0.3 (0.8)	3.0 (0.7)	4.1 (1.9)	8.2 (1.9)	58
BT	2	12.3 (0.7)	8.1 (2.5)	13.4 (1.5)	7.9 (1.6)	16.6 (1.2)	20.5 (0.6)	68
	7	14.1 (0.1)	12.5 (3.9)	13.5 (1.5)	13.7 (0.4)	17.2 (9.7)	28.3 (9.6)	56
	14	21.2 (12)	10.2 (3.0)	10.4 (2.7)	22.4 (1.2)	16.9 (1.4)	37.3 (7.0)	43
	28	23.6 (11)	75.8 (38)	84.3 (26)	23.2 (1.2)	16.8 (9.8)	35.1 (9.1)	42

Notes: Abbreviations are: gross nitrogen mineralization (M_N), gross microbial N mineralization (M_{Nfast}), gross ammonium immobilization (I_{NH4}), gross autotrophic nitrification (N_{aut}), gross heterotrophic nitrification (N_{het}), and gross nitrate immobilization (I_{NO3}). Numbers in parentheses are standard deviations (SD) ($n = 4$ replicates per treatment).

days, microbial N mineralization (day 7, 14, and 28), NH_4^+ immobilization (day 14 and 28), autotrophic nitrification (day 7, 14, and 28), heterotrophic nitrification (day 2, 7, and 14), and NO_3^- immobilization (day 2, 7, and 14) rates were significantly ($P < 0.05$) greater in soil sampled from between tussocks than under tussocks in non-amended soil (Table 3). Proximity to tussock had no affect on any gross N flux rates in *Themeda*-amended soil (Table 3). In general, gross N fluxes increased with incubation time (Table 3). Across all sampling days and treatments, total nitrification ($N_{aut} + N_{het}$) ranged from 2 to 52 μg N·(g soil)$^{-1}$·d^{-1} with heterotrophic nitrification rate accounting, on average, for >50% of total nitrification (ranged 9% to 83%; Table 3). The proportion of total nitrification explained by heterotrophic activity was not affected by proximity to tussock or *Themeda* amendment (Table 3).

Discussion

Semiarid ecosystems are often characterized by small-scale gradients in C and N availability caused by patchiness of vegetation cover and seasonal variation in plant productivity. We proposed that under conditions of maximal plant productivity, greater C availability is likely to increase microbial N demand and restrict N cycling to DON. Our results tend to agree, as amending soil with *Themeda* litter increased C availability and restricted DON content compared with non-amended soil. High microbial N demand and the rapid recycling of DON in *Themeda*-amended treatments likely restricted N mineralization and nitrification in these soils. Increases in soil C have previously been shown to contribute directly to increased microbial immobilization of N in other arid and semiarid environments, resulting in less available N for nitrification (Smith et al. 1994, Gallardo and Schlesinger 1995, Schaeffer and Evans 2005). Similarly, we found that in *Themeda*-amended soil, consumptive processes (I_{NO3}) dominated over productive (M_N and N_{het}) processes. Our results also indicate that heterotrophic nitrification was lowest in *Themeda*-amended soil, suggesting that heterotrophic nitrifiers might also recycle DON rather than release NO_3^- under restricted N supply. It is possible that under conditions of high N demand, heterotrophic immobilizers may also restrict the availability of nitrifiable DON. These results tend to support the assertion that the release of DON is central to terrestrial N cycling (Jones et al. 2004, Schimel and Bennett 2004).

Schimel and Bennett (2004) proposed that, moving along a conceptual N-availability gradient, the release of DON and the importance of N mineralization and nitrification increased. We found that, although C availability was lower in *Themeda*-amended soil from between tussocks compared with under tussocks, there was no difference in N cycling. This is likely to reflect that both *Themeda*-amended soils were equally N limited and that differences in field C and N content had little effect on N cycling under these conditions. Alternatively, under the lower C-availability conditions of non-amended soil, DON accumulated and N productive (M_N, M_{Nfast}, and N_{het}) processes dominated consumptive (I_{NH4} and I_{NO3}) processes, providing an opportunity for greater autotrophic nitrification to occur. It is generally assumed that where microbial growth is C limited, microbes will use C from dissolved organic matter to support their energy needs and release mineral N (Schimel and Bennett 2004, Bardgett 2005). This suggests that microbial C supply was limited to some extent in non-amended soil. It is also likely that this C limitation was more severe in soil from between tussocks, as C supply was lower and NO_3^- accumulation and gross N fluxes greater, than in soil from under tussocks. The release of NH_4^+ from the fast remineralization of newly formed microbial biomass (M_{Nfast}; Müller et al. 2004) was also greatest in soil from between tussocks, especially during the latter stages of the incubation. Prolonged incubation of soil that receives no other C inputs can induce a C limitation in heterotrophic microorganisms (Hart et al. 1994). Therefore, the C limitation in non-amended soil is likely to have increased over the duration of the incubation, especially in soil from between tussocks. This is likely to have either increased the turnover and release of surplus N within the microbial biomass and/or steadily increased microbial stress, resulting in biomass reduction and the release of N. Previous studies have also proposed that microbial N cycling in arid environments is C limited (Schaeffer et al. 2003, Schaeffer and Evans 2005). Our results suggest that N cycling in this semiarid grassland is likely to be mediated by both C and N limitations depending on the patchiness of vegetation cover and seasonal variation in plant productivity.

Soils in many semiarid ecosystems often accumulate NO_3^-, even though these ecosystems are characterized as N limited (Hook and Burke 1995, Erskine et al. 1996, Bennett and Adams 1999). We proposed that the variation in C and N availability that characterizes many semiarid ecosystems provides an environment for both autotrophic and heterotrophic nitrification to occur. Our results tend to confirm this hypothesis, as heterotrophic production of NO_3^- from organic N generally explained >50% of total NO_3^- production within this semiarid ecosystem. However, there was no consistent evidence that the relative importance of autotrophic and heterotrophic nitrification was affected by a variation in C and N availability. This suggests that factors mediating heterotrophic nitrification in our study were also likely to mediate NH_4^+ availability and, hence, autotrophic nitrification. As our results suggest that microbial C status and N demand are likely to mediate DON availability, these factors are likely also the major determinates of potential heterotrophic and autotrophic nitrification activity.

Previous research has indicated that heterotrophic nitrification was not important in another semiarid

environment (López et al. 2003). It is likely that heterotrophic nitrification is increasingly important in ecosystems where NH_4^+ availability is restricted (Killham 1986). López et al. (2003) found NH_4^+, rather than NO_3^-, was the dominant form of mineral N in their study, suggesting that both autotrophic and heterotrophic nitrification was limited. As nitrification is generally assumed to be sensitive to soil moisture and temperature, it is also likely that nitrification was restricted by the dry (2–4% soil moisture), cool (1°–15°C) conditions that characterized the Argentinean semiarid steppe environment on which the study of López et al. (2003) was conducted. A recent study of a Western Australian semiarid agricultural soil reported considerable gross nitrification to occur under moist (12% soil moisture), hot (40°C) conditions (Hoyle et al. 2006), but did not separate autotrophic and heterotrophic nitrification. However, autotrophic activity is often considered to be restricted at temperatures above 40°C (Malhi and McGill 1982). Therefore, our results might suggest that, in hot semiarid environments ($\geq$40°C), nitrification may predominately result from heterotrophic activity and may explain the differences among our study and that of López et al. (2003). López et al. (2003) also compared net, rather than gross, nitrification rates and used nitrapyrin to inhibit autotrophic NH_4^+ oxidizers. Net nitrification rates depend, however, on the supply of nitrifiable substrates (NH_4^+ for autotrophs and organic N compounds for heterotrophs) and the rate of NO_3^- immobilization/consumption. The measurement of gross N flux rates provides an opportunity to discern between these factors and more accurately estimate heterotrophic and autotrophic nitrification.

In the current and previous ^{15}N models (Barraclough and Puri 1995, Müller et al. 2004; Fig. 3), it is assumed that all heterotrophic nitrification originates from organic N and that NH_4^+ oxidation to NO_3^- occurs via autotrophic nitrification (Fig. 3). While NH_4^+ is not considered an important substrate for heterotrophic nitrification in forest soils (Schimel et al. 1984, Pedersen et al. 1999), heterotrophic nitrifiers have been shown to outcompete autotrophic nitrifiers for NH_4^+ at low dissolved oxygen concentrations and C:N substrate ratios of $\geq$10 (Van Niel et al. 1993). As nitrification from organic N was a significant process by which NO_3^- was formed in our study, organic-N pools such as DON are likely to mediate the majority of heterotrophic nitrification. However, autotrophic nitrification consumed, on average, 87% of NH_4^+ released in *Themeda*-amended soil, even though it is generally assumed that microbial heterotrophs are more competitive for NH_4^+ than autotrophic nitrifiers when C is non-limiting (Vitousek 1982, Tietema and Wessel 1992). This might suggest that heterotrophic activity may also play an important role in NH_4^+ oxidation in this semiarid grassland, at least under conditions of high C and low N availability.

A wide phylogenetic range of microorganisms have the potential for heterotrophic nitrification (Eylar and Schmidt 1959, Hirsh et al. 1961, Rho 1986, Stroo et al. 1986, Brierley and Wood 2001), and two general biochemical pathways have been proposed. The first pathway is known from heterotrophic nitrifying bacteria that possess ammonia and hydroxylamine oxidizing enzymes that have strong similarities with those of autotrophic nitrifiers (Moir et al. 1996). Interestingly, Verstraete and Alexander (1973) showed that heterotrophic nitrification by *Arthrobacter* sp. forms part of the response of the organism to iron limitation. If iron concentrations are limiting, chelating hydroxamates are synthesized; if iron is in excess, nitrite and NO_3^- are generated instead. Though we cannot confirm the presence of *Arthrobacter* sp. in our soil, the mineralogy of these soils (Bennett and Adams 1999) provides iron well in excess of biological requirements. A number of bacterial heterotrophic nitrifiers are also capable of aerobic denitrification (Castignetti and Hollocher 1984, Kuenen and Robertson 1987, Robertson and Kuenen 1990), a mechanism not estimated in our study. By doing so, organisms can maintain a high growth rate, which can be advantageous when energy substrates are periodically in excess (Stouthamer et al. 1997) via spatial variability of rainfall and organic matter. The second pathway is known from fungal nitrification as proposed by Wood (1988), where N compounds react with hydroxyl radicals that are produced when hydrogen peroxide and superoxide are both present. These conditions for hydroxyl radical formation are likely to occur during cell lysis and lignin or acetate degradation (De Boer and Kowalchuk 2001). As many forest soils are likely to have a dominant fungal biomass and considerable lignin-containing/acetate-forming plant litter, the fungal pathway is often considered of more importance than the bacterial pathway (Killham 1986, 1990). The *Themeda* litter in our study contained some lignin (4%). Although we are unable to associate the measured heterotrophic NO_3^- production in our study to any specific organism, these studies suggest it is possible that both bacterial and fungal pathways do occur.

The division of organic N into two pools (I_{NH4} and I_{NO3}) within the ^{15}N model used in our study is justified by the presence of physiologically different groups of organisms (Müller et al. 2004). While bacteria are thought to predominantly use NH_4^+ for their N requirements (Jasson et al. 1955), other organisms such as fungi can grow on NO_3^- (Marzluf 1997). Although we measured considerable NO_3^- accumulation in non-amended soil, substantial amounts of NO_3^- were also immobilized. This suggests that the fungal biomass may play an important role in the conservation of N in this N-limited environment, as NO_3^- production is likely to provide the major pathway for N losses in this ecosystem. However, the relative importance of the

fungal and bacteria community to N cycling in semiarid ecosystems is largely unknown.

While we measured considerable NO_3^- accumulation in soil from under tussocks, it is likely that under field conditions, accumulation may be limited, as plants are likely to outcompete nitrifiers for available NH_4^+ and also take up NO_3^-. However, NO_3^- that accumulates in the bare soil between tussocks is less likely to be utilized by plants, providing greater opportunity for N losses via denitrification and NO_3^- leaching. An estimated 60% to 80% of N_2O production in semiarid grasslands results from nitrification (Parton et al. 1988, Mummey et al. 1994). Therefore, denitrification may constitute a major mechanism for N loss in this ecosystem, especially in soil between tussocks.

In conclusion, we found that in *Themeda*-amended soil, high C availability restricted DON and mineral N release as N-consumptive processes dominated N-productive processes. In non-amended soil, C limitation provided conditions for DON accumulation, N productive processes to dominate N consumptive processes, and NO_3^- accumulation. A greater C limitation in non-amended soil from between tussocks also resulted in higher rates of gross N fluxes and NO_3^- accumulation compared with soil from under tussocks. These results suggest that DON is central to N cycling in this semiarid grassland, which is mediated by both C and N limitations, depending on the patchiness of vegetation cover and seasonal variation in *Themeda* litterfall. In our study, heterotrophic activity was generally >50% of total nitrification, but that this percentage was not affected by the proximity of soil to tussocks or litter addition. Finally, the conceptual model presented by Schimel and Bennett (2004) was useful as an initial framework by which the variation in C and N availability might explain N cycling processes in our laboratory experiment (Fig. 1). However, to more accurately reflect the N dynamics in this semiarid grassland soil, this conceptual model requires more detailed N flows related to microbial and organic N turnover. In particular, we propose that the production of NO_3^- from organic N by heterotrophic living microorganisms should be included (Fig. 1) for ecosystems where nitrification is important.

Acknowledgments

This research was conducted with the support of an Australian Research Council post-doctorial fellowship. The authors wish to thank Lidia Bednarek for her support with ^{15}N analysis and Michael Smirk for his support with soil elemental analysis.

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