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Impacts of anthropogenic N additions on nitrogen mineralization from plant litter in exotic annual grasslands

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Abstract

Urban regions of southern California receive up to 45 kg N ha^{-1} y^{-1} from nitrogen (N) deposition. A field decomposition study was done using ^{15}N-labelled litter of the widespread exotic annual grass *Bromus diandrus* to determine whether elevated soil N is strictly from N deposition or whether N mineralization rates from litter are also increased under N deposition. Tissue N and lignin concentrations, which are inversely related in field sites with high and low N deposition, determine the rate at which N moves from plant litter to soil and becomes available to plants. The effect of soil N on N movement from litter to soil was tested by placing litter on high and low N soil in a factorial experiment with two levels of litter N and two levels of soil N. The litter quality changes associated with N deposition resulted in faster rates of N cycling from litter to soil. Concentrations of litter-derived N in total N, NH_4^+, NO_3^-, microbial N and organic N were all higher from high N/low lignin litter than from low N/high lignin litter. Litter contributed more N to soil NH_4^+ and microbial N in high N than low N soil. At the end of the study, N mineralized from high N litter on high N soil accounted for 46% of soil NH_4^+ and 11% of soil NO_3^-, compared to 35% of soil NH_4^+ and 6% of soil NO_3^- from low N litter on low N soil. The study showed that in high N deposition areas, elevated inorganic soil N concentrations at the end of the summer N deposition season are a result of N mineralized from plant litter as well as from N deposition.

Keywords: Nitrogen; Mineralization; Stable isotope; Decomposition; Litter

1. Introduction

Nitrogen (N) is often an important growth-limiting nutrient in natural ecosystems. However, as the natural rate of N fixation has been doubled by human activities (Vitousek et al., 1997), there is much evidence that atmospheric reactive N deposition affects plant chemistry and physiology (Lee and Caporn, 1998), alters ecosystem processes such as mineralization (Fisk and Schmidt, 1996; Kiefer and Fenn, 1997; Falkengren-Grerup et al., 1998; Chen et al., 2002) and leads to changes in plant communities (Heil and Bruggink, 1987; Bobbink et al., 1988). In southern California, fossil fuel combustion is a major source of atmospheric reactive N (Russell et al., 1985), which contributes up to 35–45 kg N ha^{-1} y^{-1} to forested mountains and lower elevations dominated by shrubs and grasses (Bytnerowicz et al., 1987; Bytnerowicz and Fenn, 1996; Fenn et al., 2003). In high N deposition urban areas, native coastal sage scrub (CSS), a semi-deciduous Mediterranean-type shrubland (Mooney, 1988), is being replaced by exotic annual grasses such as *Bromus diandrus, B. madritensis,* and *Avena fatua* (Minnich and Dezzani, 1998) and N deposition may contribute to CSS decline by facilitating the invasion of exotic annual grasses (Allen et al., 1998; Fenn et al., 2003).

Nitrogen deposition in southern California results in increased soil N at the end of the summer N deposition season (Padgett and Allen, 1999; Sigüenza et al., 2006)

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which is then available to plants during the growing season. In areas that receive high N deposition loads, there is higher N and lower % lignin in mature tissue of the exotic annual grass species, *B. diandrus* (A. G. Sirulnik, unpub. PhD thesis, 2004). If C is not limiting to decomposers, then high litter N and low initial lignin content can result in faster decomposition rates (Schlesinger and Hasey, 1981; Melillo et al., 1982; Berg et al., 1987; Fog, 1988; Huang et al., 2003; Xuluc-Tolosa et al., 2003) and N movement from litter to soil (Vitousek, 1982; Flanagan and Van Cleve, 1983; Nadelhoffer et al., 1983; Frankenberger and Abdelmagid, 1985). If litter quality changes associated with N deposition result in greater movement of N from litter to soil, then elevated soil N concentrations in high N deposition sites at the beginning of the growing season may be attributable not just to summer N deposition, but to N mineralization from litter as well. A recent review shows that the effects of elevated N may either increase or decrease decomposition (Knorr et al., 2005), so it is not clear how N deposition affects N mineralization and movement of N from litter to soil in Mediterranean-type exotic annual grasslands.

The present study investigates how N deposition affects N release from *B. diandrus* litter via altered litter quality and increased soil N by using a ^{15}N tracer technique. To distinguish the effects of litter quality from inorganic soil N on N mineralization from litter, a litter transplant method was employed using 2 levels of inorganic soil N and 2 levels of litter N in a 2×2 factorial experimental design. *B. diandrus* grass was grown under high and low levels of ^{15}N-labeled N fertilizer. Litter decomposed in high and low soil N plots and ^{15}N was measured in soil compartments as *B. diandrus* litter decomposed. The soil compartments that were tested for litter-derived ^{15}N were inorganic N, microbial N, total N, and organic N. Further, because N fertilization can have a positive (Gallardo and Schlesinger, 1994; Hart and Stark, 1997) or negative (Söderström et al., 1983; Smolander et al., 1994; Fisk and Fahey, 2001) effect on microbial biomass, microbial N and C were compared among soil and litter N treatments to determine how N fertilization affects microbial N in Mediterranean-type exotic annual grasslands.

The hypotheses that were tested were (1) as high and low N *B. diandrus* litter decomposed, high N litter would contribute more N to soil N pools than would low N litter and (2) *B. diandrus* litter decomposing on the surface of high N soil would contribute more N to soil N pools than would litter decomposing on the surface of low N soil.

2. Materials and methods

2.1. Generation of ^{15}N-labeled grass litter

To avoid site and climate-related effects on litter quality, *B. diandrus* was grown under controlled watering and fertilization conditions. *B. diandrus* seeds were collected from field grass at a low N deposition site: Lake Skinner in the Western Riverside County Multi-Species Reserve near Temecula, CA (33.6035°N, 117.0172°W). Seeds were air dried and stored at 20 °C until sown. Seeds were sown in the soil 'UC mix #3' (75% fine quartz sand, 25% ground peat moss) and fertilized with a mineral nutrient solution designed to mimic field conditions (see Padgett and Allen (1999) for details) for 4 months in a lath house. N was applied in a combination of $K^{15}NO_3$ (99 at%) and $^{14}NH_4Cl$, in a 1–10 ratio of ^{15}N–^{14}N, that was adjusted to achieve a total N application of either 4 or 40 μg N g^{-1} soil, referred to as low or high N, respectively. These levels of N application were selected to resemble inorganic N concentrations available to germinating seeds in high and low N deposition sites at the beginning of the winter growing season (Padgett et al., 1999; Wood et al., 2005; Sigüenza et al., 2006): The highest measured inorganic N concentration at a high N deposition coastal sage scrub site in the region is 95 μg N g^{-1} soil (Padgett et al., 1999). Aboveground grass biomass was harvested after grass senescence commenced and was dried at 60 °C to constant mass. Seeds were removed and grass from each N treatment was chopped into 8 cm lengths and thoroughly mixed by hand to evenly distribute leafy and structural grass segments. A subsample was analyzed for total C and N using a Carlo Erba CNS combustion analyzer. Initial C/N ratios of high and low N litter were 14.2 (±0.05 SEM) and 23.47 (±0.17), respectively ($P<0.001$). Initial %N values of high and low N litter were 2.75 (±0.01) and 1.61 (±0.02), respectively ($P<0.001$). Initial % lignin values of high and low N litter were 3.23 (±0.17) and 6.93 (±0.27), respectively ($P<0.001$).

2.2. Site description

Decomposition occurred in field microcosms that were placed in fertilized and control plots of a long-term N fertilization experiment at Lake Skinner (see above for location details). Soil is granitic alluvium and vegetation is primarily coastal sage scrub with an exotic annual grass component between shrubs. The climate is Mediterranean-type with little to no precipitation from approximately May–October, and most plant growth occurs from November to April. Average annual precipitation from 1997 to 2003 was 21.0 cm (California Irrigation Management Information System). Ten fertilized and 10 control plots, each 5×5 m, were established in a randomized complete block design. Fertilized plots have received two applications per year of 30 kg N ha^{-1} y^{-1} as NH_4NO_3 (a total of 60 kg N ha^{-1} y^{-1}) since 1994. This fertilization regime was implemented to accelerate the establishment of soil N levels that would have resulted from 45 years of chronic N deposition, as seen in high N deposition sites in the region (Padgett et al., 1999). Fertilized and control soils will be referred to as high and low N, respectively, throughout this paper. Five of the 10 blocks were used for this experiment.

2.3. Experimental design

To measure N mineralization from high and low N litter in high and low N soil, microcosms constructed of PVC pipe segments 20 cm long × 15.2 cm diameter were placed in a 2 × 2 factorial design with 2 soil N treatments and 2 litter N treatments. In 5 high N and 5 low N plots, PVC pipes were inserted 10 cm into the soil, 2.00 ± 0.05 g dried litter (either high or low N) was placed inside each pipe directly on top of the soil, and pipes were covered with a 2 mm wire mesh screen to prevent animal disturbance. The goal of using PVC pipe was to eliminate plant root interference so that microbially mediated processes could be examined with minimal confounding effects of plants on decomposition or competition for inorganic N between plants and soil microorganisms. The experiment was timed to measure the N flush from litter that coincided with early plant growth. Microcosms were set out on 13 January 2003. Five microcosms per treatment were destructively sampled after 41, 68 and 137 days. The experiment ended when vegetation outside the microcosms began to senesce. Cumulative precipitation from day one to each consecutive sampling date was 7.1, 19.6, and 23.5 cm (California Irrigation Management Information System). Microcosms were taken intact to the lab on ice. The top 6 cm of soil was removed and sieved through a 2-mm wire mesh. Sieved soil was analyzed for ^{15}N-labeled litter-derived N in inorganic N, microbial N, total N, and organic N. Litter mass loss rates were not recorded because litter was not confined in litter bags; additional litter bag experiments showed that changes in *B. diandrus* litter quality related to increased N availability resulted in increased mass loss rates (Sirulnik, 2004 loc.cit.).

2.4. Inorganic soil N determination

The extraction procedure for NH_4^+ and NO_3^- was modified from methods described by Maynard and Kalra (1993). Subsamples of soils were removed for gravimetric water content measurements. Ten grams of soil were shaken with 40 ml of 2 M KCl for 60 min at room temperature. Extracts were filtered through 0.4 μm Millipore® Isopore™ Polycarbonate Membrane Filters and stored in polyethylene scintillation vials at −20 °C until analyzed. A subsample of extract was analyzed for N–NH_4^+ and N–NO_3^- on a Technicon Autoanlyzer II continuous flow analyzer (Technicon Industrial Systems, Industrial Method # 487-77A, 1977; Technicon Industrial Systems, Industrial Method # 98-70W, 1973). The remaining extract was analyzed for ^{15}N at% in NH_4^+ and NO_3^- by diffusing the ions onto acid filter disks to capture N for isotopic analysis (Stark and Hart, 1996). Acid filter disks were analyzed for ^{15}N by continuous flow isotope ratio mass spectrometer (IRMS) at the UC Davis Stable Isotope Facility. Litter-derived inorganic soil N calculations are described below.

2.5. Microbial N and C determination

Microbial N and C were released by fumigating soils for 24 h with $CHCl_3$. Prefumigated and postfumigated soils were extracted with 0.05 M K_2SO_4 in a soil to solution ratio of 1:4, shaken for 60 min and filtered through Whatman 42 paper filters (Brookes et al., 1985). Extracts were divided into two subsamples. To measure microbial C, subsamples of K_2SO_4 extracts were analyzed for total organic carbon (TOC) on a Shimadzu TOC Vcsh/csn combustion analyzer. Soil microbial C was calculated as the difference in TOC between prefumigated and postfumigated soils with a K_{EC} of 0.45 (Wu et al., 1990). Remaining extracts were treated with persulfate oxidation to convert organic and inorganic N to NO_3^- (persulfate N) (Cabrera and Beare, 1993). Persulfate treated extracts were divided into two subsamples. One subsample was analyzed for N–NO_3^- on a Technicon Autoanlyzer II continuous flow analyzer. The other subsample was analyzed for ^{15}N–NO_3^- at% by acid trap diffusion as above. Soil microbial N was calculated as the difference in persulfate N between prefumigated and postfumigated soils with a conversion factor (K_{EN}) of 0.45 (Jenkinson, 1988). Litter-derived soil microbial N calculations are described below.

2.6. Total N and lignin determination

To measure total soil ^{15}N and N, soil subsamples were air dried to constant mass and analyzed by continuous flow isotope ratio mass spectrometer (IRMS) as above. Total N includes indigenous organic and inorganic N as well as organic N and inorganic N derived from ^{15}N-labeled litter. At each sampling date, litter C and N were measured using a Carlo Erba CNS combustion analyzer. Litter-derived total soil N calculations are described below. Before decomposition commenced, lignin was measured in litter by sulfuric acid and heat (Bath et al., 1978).

2.7. Calculations

The amount of N in each compartment that was derived from litter was calculated according to Hauck and Bremner (1976) as reported in Powlson and Barraclough (1993):

$$F = T(A_s - A_B)/A_F,$$

where F is the mass of N derived from labeled material (litter); T the total mass of N in sample (N–NH_4^+, total N, N–NO_3^-, or prefumigated and postfumigated soils for microbial N calculation); A_s the atom% excess ^{15}N in sample; A_B the atom% excess ^{15}N in control sample (from soils in sample plots outside of microcosms, before microcosms were inserted); A_F the atom% excess ^{15}N in labeled material (litter).

Litter N recovered in microbial N was calculated as litter-derived N in prefumigated samples subtracted from litter-derived N in postfumigated samples (Davidson et al., 1991). Total organic N in soils was calculated as the

difference between total soil N and inorganic N (Glasener et al., 1998).

2.8. Statistical analysis

Data were analyzed for soil N and litter N treatment effects by ANOVA with post hoc multiple comparisons made with Tukey $HSD_{0.05}$ test. Two-way comparisons between high and low N soils and between high and low N litters were made with students *t*-test. Statistical analyses were performed with JMP 5.0 software (SAS Institute Inc).

3. Results

3.1. Litter chemistry

Differences between high and low N litter in litter % N and C/N remained throughout the experiment (Fig. 1), but the difference narrowed as decomposition progressed. Litter quality affected the rate of N loss from litter as high N litter lost a greater percentage of the initial concentration of labeled N on days 41, 68, and 137 ($P<0.001$) (Fig. 2).

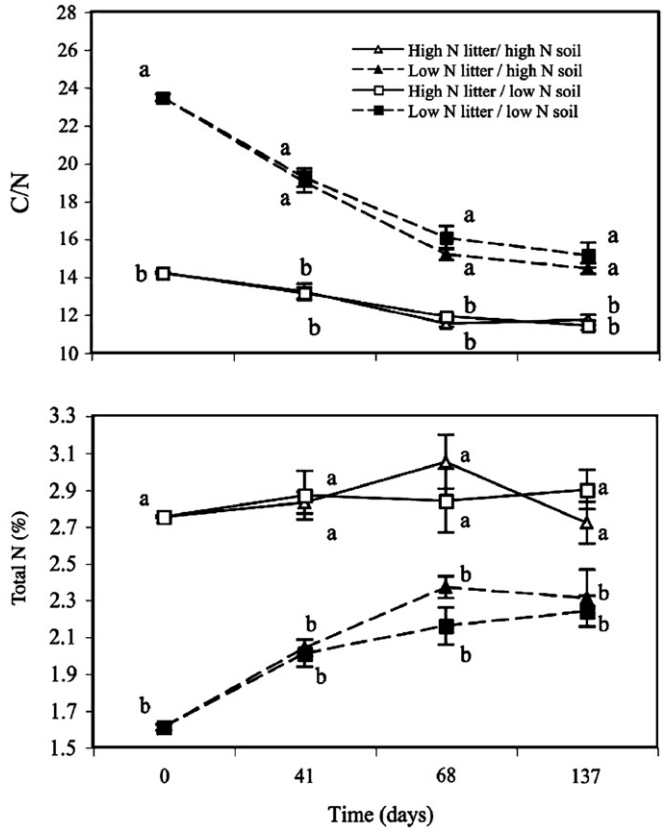

Fig. 1. Total %N and C to N ratio of *Bromus diandrus* litter throughout decomposition. Values are means ($n=5$) with ±standard error of the mean (SEM). At each time period, different letters indicate a significant difference at $P<0.05$ according to Tukey test.

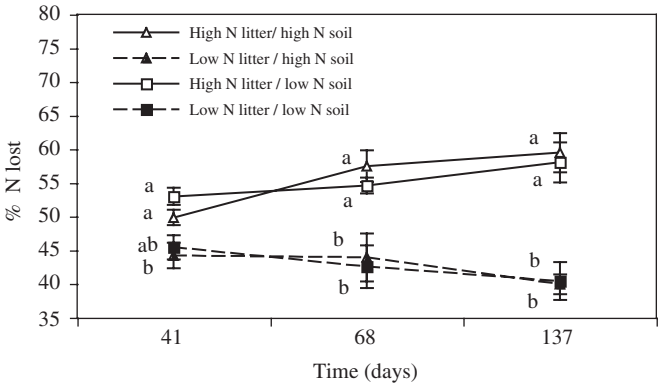

Fig. 2. % original labeled N lost at each collection date. Values are means ($n=5$) with ±standard error of the mean (SEM). At each time period, different letters indicate a significant difference at $P<0.05$ according to Tukey test.

3.2. Soil N

Differences among all four-treatment combinations are reported in Figs. 3–5. Throughout the ^{15}N litter incubation, high N litter contributed more N to most N pools than did low N litter. For some N pools, more N originated from litter if the microcosms were in high soil N plots.

3.2.1. Soil N–NO_3^- and N–NH_4

At the beginning of incubation, total soil N in high N soil and low N soil was 765.1 (SEM ±41.0) and 527.9 (±95.2) μg N g soil^{-1}, respectively ($P=0.052$). NO_3^- concentrations in high and low N soil were 40.7 (±7.0) and 18.3 (±2.7) μg N g soil^{-1}, respectively, ($P=0.020$). NH_4^+ concentrations in high and low N soil were 55.0 (±13.4) and 6.2 (±1.1) μg N g soil^{-1}, respectively ($P=0.007$).

Litter and soil N affected concentrations of soil NO_3^- and NH_4^+ (Fig. 3) and the amount of N that litter contributed to soil NO_3^- and NH_4^+ (Fig. 4). Highest extractable NH_4^+ concentrations were found in high N soil with high N litter throughout incubation, and low N litter resulted in decreased extractable N concentrations in soils with high initial extractable N concentrations (Fig. 3). High N litter contributed more N to soil NO_3^- than did low N litter on day 41 ($P=0.003$), although there was no significant difference among treatment combinations (Fig. 4). High N litter contributed more N to soil NH_4^+ than did low N litter on day 137 ($P<0.001$). Litter on high N soil contributed more N to soil NH_4^+ than did litter on low N soil on days 41 ($P=0.028$) and 137 ($P<0.001$), although there was no significant difference among treatment combinations on day 41 (Fig. 4). There was a soil N × litter N interaction effect on the amount of soil NH_4^+ that was derived from litter on days 68 ($P=0.037$) and 137 ($P=0.001$): high N litter on high N soil contributed more N to NH_4^+ than any other treatment combination (Fig. 4). A greater percentage of soil N–NO_3^- (litter-derived + indigenous) was comprised of high N litter-derived N–NO_3^- than low N litter-derived N–NO_3^- on days

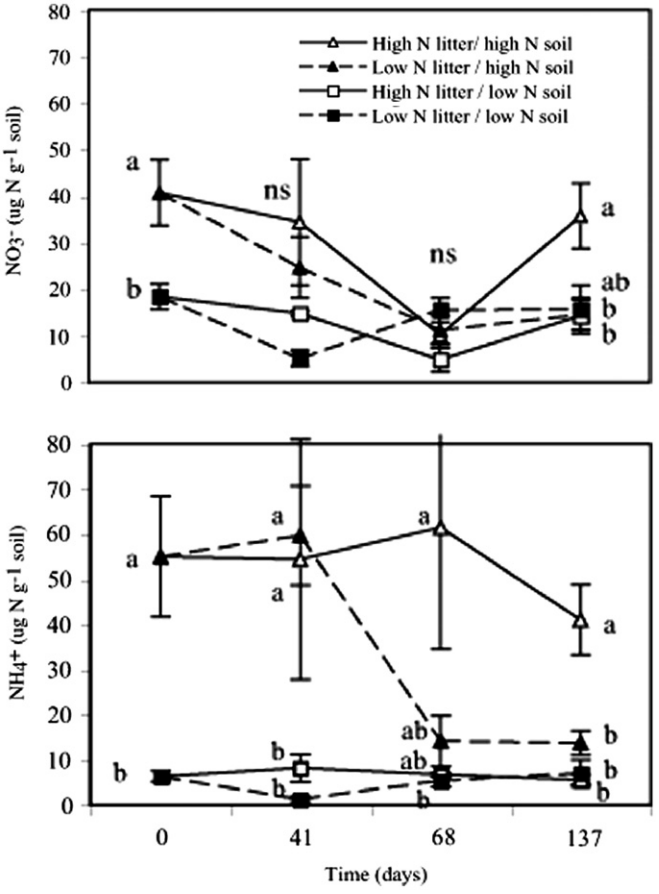

Fig. 3. Concentrations of inorganic soil N throughout decomposition. Values are means ($n = 5$) with $\pm$standard error of the mean (SEM). At each time period, different letters indicate a significant difference at $P < 0.05$ according to Tukey test. ns = no significant difference among treatment combinations.

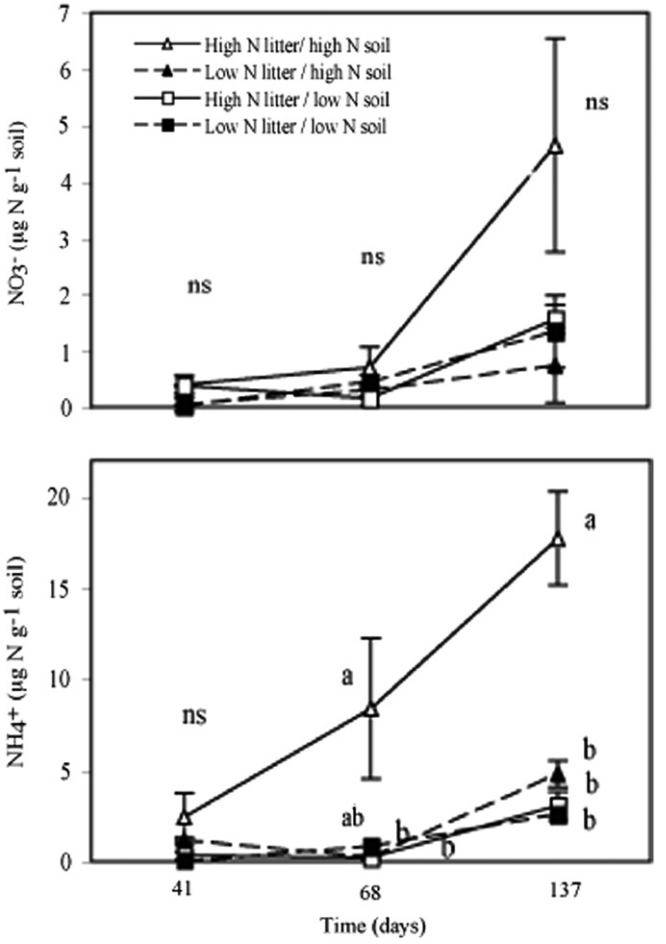

Fig. 4. Concentrations of litter-derived N (^{15}N-labeled) in inorganic soil N. Values are means ($n = 5$) with $\pm$standard error of the mean (SEM). At each time period, different letters indicate a significant difference at $P < 0.05$ according to Tukey test. ns = no significant difference among treatment combinations.

41 ($P = 0.002$) and 137 ($P = 0.020$). By day 137, 11.4% ($\pm$2.3) of soil N–NO_3^- was comprised of N–NO_3^- from high N litter on high N soil while 6.3% ($\pm$1.8) of soil N–NO_3^- was from low N litter on low N soil (data not shown, no significant difference among treatment combinations). On day 41, a greater percentage of soil N–NH_4^+ was comprised of litter-derived N–NH_4^+ on high N soil than on low N soil ($P = 0.012$). A greater percentage of soil N–NH_4^+ was comprised of high N litter-derived N–NH_4^+ than low N litter-derived N–NH_4^+ on days 41 ($P = 0.002$) and 137 ($P = 0.001$). By day 137, 46.2% ($\pm$5.1) of soil N–NH_4^+ was comprised of N–NH_4^+ from high N litter on high N soil while 34.8% ($\pm$4.2) of soil N–NH_4^+ was from low N litter on low N soil (data not shown, no significant difference among treatment combinations).

3.2.2. Litter-derived soil organic N, total soil N, and soil microbial N

On days 41, 68, and 137, high N litter contributed more N to litter-derived soil organic N than did low N litter ($P < 0.001$), respectively) (Fig. 5). On each of those days, high N litter also contributed more N to litter-derived total soil N (organic N + inorganic N) than did low N litter ($P < 0.001$) (Fig. 5). On day 137, there was a litter N × soil N interaction effect on litter-derived total N ($P = 0.020$). Soil microbial ^{15}N results from day 41 showed that high N litter and litter on high N soil contributed more N to litter-derived soil microbial N than did low N litter or litter on low N soil ($P = 0.01$). There was no significant interaction effect, but litter in microcosms with high N litter and high N soil contributed more N to microbial N than all other combinations (Fig. 5). Also on day 41, a greater percentage of labeled organic N was comprised of labeled microbial N in high N soil than in low N soil ($P = 0.018$). Microbial ^{15}N data for days 68 and 137 included negative values for litter-derived soil microbial N at both sampling periods (data not shown). Negative values would suggest that there was more litter-derived N in prefumigated than postfumigated soils, which indicates that microbial ^{15}N was so low that it was within the experimental error. As the low

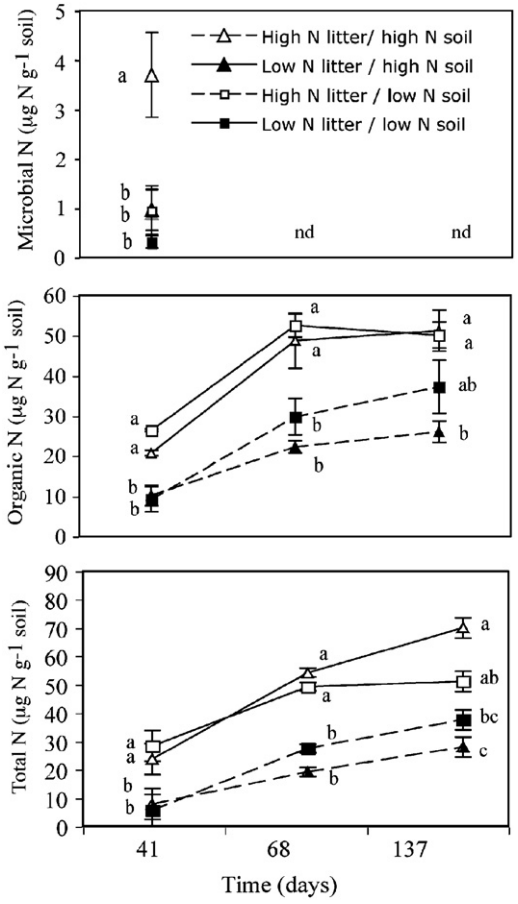

Fig. 5. Concentrations of litter-derived N (^{15}N-labeled) in soil microbial N, organic N, and total N. Values are means ($n = 5$) with ±standard error of the mean (SEM). At each time period, different letters indicate a significant difference at $P < 0.05$ according to Tukey test. nd = no data.

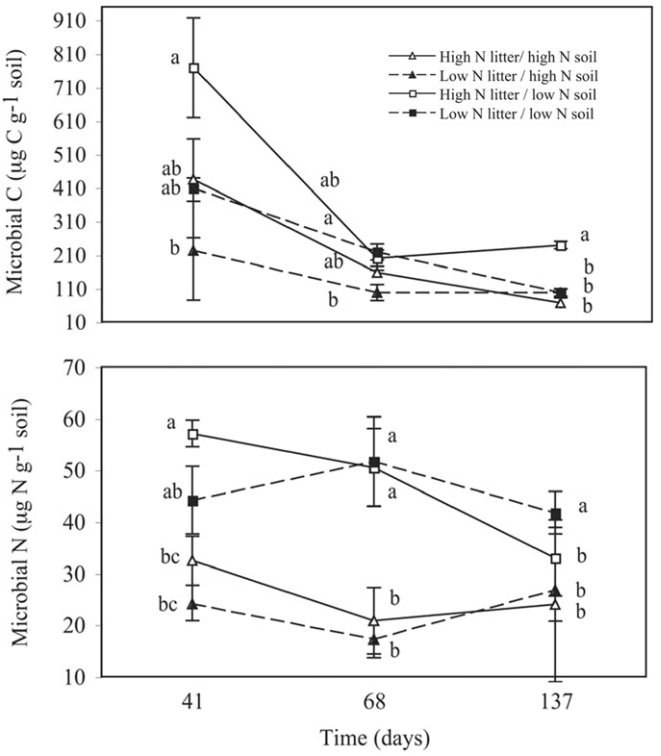

Fig. 6. Soil microbial N and C under high and low soil and litter N concentrations. Values are means ($n = 5$) with ±standard error of the mean (SEM). At each time period, different letters indicate a significant difference at $P < 0.05$ according to Tukey test.

total microbial N (Fig. 6) shows a 4–8 fold reduction over time, there may have been a similar reduction in microbial ^{15}N.

3.3. Microbial N and C

Effects of N on soil microbial N (^{15}N labeled + indigenous N) and soil microbial C varied among sampling dates and between N treatments (litter N or soil N) (Fig. 6). On day 41, soil with high N litter had more microbial N and microbial C than did soil with low N litter ($P = 0.034$ and 0.015, respectively) and high N soil had less microbial N and microbial C than did low N soil ($P < 0.001$ and 0.024, respectively). Likewise, on day 68, high N soil had less microbial N and microbial C than did low N soil ($P < 0.001$ and 0.011, respectively). On day 137, soil with high N litter had more microbial C than did soil with low N litter ($P = 0.007$) and high N soil had less microbial C than did low N soil ($P < 0.001$). There was also a soil N × litter N interaction effect on soil microbial C ($P < 0.001$). On day 68, high N soil had greater soil microbial C/N than did low N soil ($P = 0.002$).

4. Discussion

4.1. N loss from litter to soil N pools

This study showed that litter quality affects N loss from litter and that litter quality and inorganic soil N concentrations affect N movement from litter to various soil compartments. High N availability has resulted in high N and low lignin concentrations in *B. diandrus* under artificial fertilization (this study) as well as under high rates of N deposition (Sirulnik, 2004 loc.cit.). N additions to other grass species have shown the same trend (Lee and Lee, 2000). Studies have shown that the amount of N that is released from decomposing plant material is correlated with N content and that the rate of N mineralization from litter can be related to lignin composition (Frankenberger and Abdelmagid, 1985; Dinesh et al., 2001). The current study suggests that N release from *B. diandrus* could be related to both N and lignin content. Because N deposition alters litter N and litter lignin content simultaneously, it is not known what influences the rate of N movement from *B. diandrus* litter more, litter N or lignin. However, it can be said that changes in litter quality associated with N deposition result in faster N movement from litter to soil.

4.1.1. Inorganic soil N

During the first few weeks of decomposition, high N litter contributed more N to soil NO_3^- than did low N litter

but there was no litter N effect on litter-derived soil N–NH_4^+, suggesting that litter-derived NH_4^+ had been nitrified or immobilized by the first sampling date. If litter-derived soil NH_4^+ were immobilized proportionately to litter-derived microbial N, then the effect of litter quality on concentrations of litter-derived soil NH_4^+ would have been disguised. At the end of the experiment, high N litter contributed more N to soil NH_4^+ than did low N litter but there was no litter N effect on litter-derived N–NO_3^-. Likewise, higher concentrations of litter-derived soil N–NH_4^+ from litter on high N soil did not translate to a soil N effect on nitrification of the litter-derived NH_4^+. These results are in agreement with Clein and Schimel (1995) who found no correlation between soil NH_4^+ concentrations and nitrification and Hart et al., (1994) who found that gross rates of mineralization and nitrification were not correlated.

The litter and soil N effects on litter-derived N–NH_4^+ show that elevated soil NH_4^+ concentrations in high N deposition sites at the beginning of the growing season (Sirulnik, 2004 loc.cit.) are due, in part, to mineralization from litter. At the end of the experiment, there was a litter N effect on the fraction of soil N–NH_4^+ that was comprised of litter-derived N–NH_4^+: 46.2% of soil N–NH_4^+ was comprised of N–NH_4^+ from high N litter on high N soil while 34.8% was from low N litter on low N soil. The higher concentrations of NH_4^+ mineralized from high N litter throughout the winter growing season is likely to have important ecological implications. Plants that become photosynthetically active earliest, such as annual grasses (Chiariello, 1989), may gain a competitive advantage over plants with delayed activity, such as native annuals and perennial shrubs, as a large flush of NH_4^+ becomes available when the winter rainy season commences. Results of this study suggest that elevated soil NO_3^- in high N deposition areas of southern California is primarily from NO_3^- deposition and perhaps from nitrification of soil-derived NH_4^+ rather than from litter. By the end of the experiment, although there was a litter N effect on the fraction of soil N–NO_3^- that was comprised of litter-derived N–NO_3^-, only 11.4% of soil N–NO_3^- was comprised of N–NO_3^- from high N litter on high N soil and 6.3% was from low N litter on low N soil.

4.1.2. Soil microbial and organic N

During the early stages of decomposition, higher concentrations of litter-derived soil microbial N in soil with high N litter and in high N soil could be evidence that the soil microbial community preferentially utilized litter N over soil N. This could have occurred because the C-limited soil microbial community was stimulated by the litter substrate (Olfs et al., 2004) and because the less humified litter-derived organic N compounds were more metabolically available to the soil microbial community than was native soil N (Kelly and Stevenson, 1987; Hart et al., 1994). Higher concentrations of litter-derived soil organic N from high N litter and from litter on high N soil was probably due to increased microbial N, as suggested by a greater percentage of labeled organic N that was comprised of labeled microbial N in high N soil than in low N soil.

After day 41, there was a 4–8 fold reduction in microbial N. This resulted in negative microbial ^{15}N values and microbial ^{15}N values that were so low that they were within the experimental error. Because microbial N pool turnover is rapid (Anderson and Domsch, 1980) and the microbial N pool was small, after a few weeks in the field the ^{15}N in the microbial pool may have been too diluted to accurately detect with the fumigation extraction technique. During a 31 week incubation of ^{15}N-labeled ryegrass amended soils, the proportion of added ^{15}N found in microbial N declined exponentially (Thomsen et al., 2001).

Even though high soil N resulted in greater concentrations of litter-derived microbial N, there were lower concentrations of total (litter-derived and indigenous) microbial N and C in high N soil than in low N soil. These results support other studies that found that microbial biomass is not necessarily a good indicator of N cycling (Hart et al., 1994; Fisk and Fahey, 2001). Because plant roots were excluded from the microcosms, soil microorganisms from the present study obtained C primarily from aboveground litter and indigenous SOM. Soil microorganisms in high N soil may have been C limited because faster litter decomposition rates associated with high N soil (Sirulnik, 2004 loc.cit.) resulted in earlier C depletion. Additionally, soil organic C in high N soils may have been less accessible to microorganisms than in low N soils due to repression of Basidiomycetes and/or repression of ligninolytic and cellulytic enzymes (Fog, 1988; Freeman et al., 2001; Carreiro et al., 2002; Deforest et al., 2004) and chemical transformations involving N that result in more recalcitrant compounds (Nömmik and Vahtras, 1982). Results from other studies on the effects of inorganic N on microbial biomass are variable. Some studies have shown a positive N fertilization effect on microbial biomass (Gallardo and Schlesinger, 1994; Hart and Stark, 1997), whereas others have shown that N fertilization negatively affects microbial biomass (Söderström et al., 1983; Smolander et al., 1994; Fisk and Fahey, 2001).

There is some evidence from this study that high soil N has a greater fungi:bacteria ratio than low N soil. Bardgett et al. (1999) found that short-term additions of N can result in a fungal dominated community. However, this result occurred on only one day and the soil N effect on microbial C/N was just one instantaneous measurement within what is probably a dynamic and variable seasonal pattern. From this one occurrence, a conclusion cannot be made about the general effects of soil N on soil microbial C/N.

5. Conclusions

The rate at which N moves from litter to soil, and thus becomes available to plants, is correlated with high litter N

and low lignin (Vitousek, 1982; Flanagan and Van Cleve, 1983; Nadelhoffer et al., 1983). The current study showed that soil N also increased N movement from litter to soil and that there was a cumulative effect when there were higher concentrations of N in both litter and soil. In addition to affecting N movement from litter to soil, inorganic N can stimulate soil N mineralization (Fisk and Schmidt, 1996; Kiefer and Fenn, 1997; Falkengren-Grerup et al., 1998; Chen et al., 2002) and a recent study has shown that in the same plots as this study, soil N mineralization was also higher in high N than low N soil (Sirulnik, 2004 loc.cit.). At the beginning of the growing season, exotic annual grass seedlings that are exposed to elevated rates of N deposition in southern California are presented with a rapid and large flush of inorganic N from (1) N deposition, (2) soil N mineralization, and (3) litter N mineralization. N deposition could enhance the competitive advantage for N that early germinating (Chiariello, 1989) nitrophilous species such as *B. madritensis* (Yoshida and Allen, 2001; Yoshida and Allen, 2004) have over later native species. Thus, the conversion of CSS to exotic annual grasslands may be related to high rates of N deposition coupled with increased N mineralization rates.

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