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Seasonal variation in nitrogen uptake and turnover in two high-elevation soils: mineralization responses are site-dependent

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Abstract In arctic and alpine ecosystems, soil nitrogen (N) dynamics can differ markedly between winter and summer months, and nitrogen losses can be measurable during the spring and fall transitions. To explore the effect of seasonality on biogeochemical processes in a temperate alpine environment, we used a combination of field incubations (year-round) and ^{15}N tracer additions (late fall, early spring, summer) to characterize soil N dynamics in a wet and dry meadow in the Sierra Nevada, California. The snowmelt to early summer season marked a period of high ^{15}N uptake and turnover in the two soils, coincident with the increase in microbial N pools at the start of snowmelt (wet and dry meadow); an increase in net N mineralization and net nitrification as snowmelt progressed (wet meadow only); and measureable net production of $^{15}N\text{-}NH_4^+$ in mid-summer (wet and dry meadow). Whereas fluctuations in microbial biomass were generally synchronous between the wet and dry meadow soils, only wet meadow soils appeared to mineralize N in response to declines in the microbial N pool. Net N mineralization and net nitrification rates in the dry meadow soil were negligible on all but one sampling date, in spite of periodic decreases in biomass of up to 60%. Across both sites, high ^{15}N recoveries in microbial biomass N, rapid $^{15}N\text{-}NH_4^+$ turnover, and low or negative net $^{15}N\text{-}NH_4^+$ fluxes suggested tight cycling of N, particularly in the late fall and early spring.

Keywords Alpine · Winter · Snowmelt · Seasonal transitions · Nitrification · Microbial N dynamics

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Introduction

The concept of the "growing season" has been deeply ingrained in ecology for many years, and for

much of that time there has been an implicit assumption that the "decomposition season" matched the growing season. However, increasingly, we are recognizing that these seasons are not necessarily synchronous, that important processes occur during the winter (e.g., Brooks et al. 1996; Hobbie and Chapin 1996; Schimel et al. 2004), and that a warming climate may enhance the cumulative effect of these processes (Piao et al. 2008).

In arctic and alpine soils, net N mineralization tends to occur during the winter months, whereas net N immobilization tends to dominate during the summer (Giblin et al. 1991; Brooks et al. 1998; Schmidt et al. 1999; Schimel et al. 2004). The transition from winter to summer, marked by snowmelt, is a period of high microbial activity (Brooks et al. 1996) and turnover (Fisk et al. 1998; Lipson et al. 1999; Schadt et al. 2003; Monson et al. 2006) in many high elevation soils. In the alpine, the growing season has generally been considered to be limited to the summer months following snowmelt and/or soil thaw, when plant growth and N uptake reach a maximum ($\sim$June–September; Mooney and Billings 1960; Galen and Stanton 1995; Jaeger et al. 1999). Long-term records from temperate, boreal, and alpine ecosystems show elevated stream nitrate concentrations during the non-growing season, with 30–90% of annual N export occurring during the winter and spring (e.g., Brooks et al. 1998; Campbell et al. 2000; Sickman et al. 2003; Likens 2004; Jones et al. 2005). The majority of this exported NO_3^- is derived from soil N (Campbell et al. 2002; Sickman et al. 2003), highlighting both the importance of overwinter nitrification in maintaining soil NO_3^- pools and the role of plant uptake in curbing N losses.

Asynchrony between nitrogen availability and demand during seasonal transitions has the potential to lead to N-limitation in many environments. The assumption that increased N losses during the non-growing season are directly related to reductions in plant N uptake has been inferred from descriptive studies in eastern hardwood (Mitchell et al. 1996; Houlton et al. 2003) and boreal forest (Kielland et al. 2006), and from N uptake experiments in arctic and continental alpine environments (Bilbrough et al. 2000). The role of seasonal transitions in the annual N cycle has received little attention in temperate alpine ecosystems, as most research has been conducted in environments where soils fall well below 0°C during the winter. However, large areas in western North America experience near-zero winter temperatures and deep snowpacks (PRISM Group 2006), and the processes that regulate nitrogen turnover and export in these systems are poorly understood.

In a winter soil environment characterized by near-zero temperatures and few freeze-thaw events, it is unclear whether microbial dynamics would show the same seasonality as has been reported from colder environments (Brooks et al. 1998; Lipson et al. 1999, 2002). Likewise, it is unclear whether net N mineralization could be maintained throughout the winter in such an environment, as snowpack manipulations have indicated both increased (Brooks and Williams 1999; Schimel et al. 2004) and decreased rates of net N mineralization (Groffman et al. 1999) associated with increased snow depths. Finally, we have little understanding of when, and to what extent, plant N uptake could mitigate potential N losses during the non-growing season.

To examine the effect of seasonality on biogeochemical processes in a temperate alpine environment, we tracked soil N dynamics in a wet and dry meadow in the southern Sierra Nevada, USA, that share similar climatic conditions (mild winter temperatures and deep snowpack) but differ in vegetation and hydrology. We measured short-term N uptake and turnover at three times during the year (late fall; early spring; summer), in combination with a longer time-series of field incubations, in order to determine (1) whether nitrogen uptake and turnover varied seasonally, (2) whether periods of nitrogen availability and nitrogen uptake were temporally distinct, and (3) whether the response to seasonality, if any, was consistent across sites.

Methods

Study sites

The study was conducted in two high elevation meadows located on the western slope of the southern Sierra Nevada, in Sequoia-Kings Canyon National Park. The first site was located in a seasonally wet subalpine meadow at the inlet of Emerald Lake (36° 35′ 49″N, 118° 40′ 29″W, elev. 2,800 m) and was dominated by Sierra willow (*Salix orestera* Schneid.)

and bluejoint reedgrass (*Calamagrostis canadensis* (Michx.) Beauv.). Soils and vegetation comprise 22 and 20%, respectively, of cover in the basin (Sickman et al. 2001), with 90% of the basin's N storage contained in soils, soil solution, and litter (Williams et al. 1995). The second site was approximately 5 km to the northeast, in an alpine dry meadow west of Topaz Lake (36° 37′ 30″N, 118° 38′ 11″W, elev. 3,215 m). Vegetation in the dry meadow was dominated by low-growing graminoids and shrubs, including threadleaf sedge (*Carex filifolia* Nutt. var. *erostrata* Kuk), Parry's rush (*Juncus parryi* Engelm.), and dwarf bilberry (*Vaccinium caespitosum* Michx.). Soils and vegetation comprise 41 and 32% of cover in the basin. Soils at both sites are classified as Lithic Cryumbrepts underlain by slightly weathered granodiorite, with pH ranging from 4.7 to 5.0 (Huntington and Akeson 1987). Soil organic matter (SOM), carbon (C) and nitrogen (N) contents are similar at the two sites, referred to hereafter as 'wet meadow' and 'dry meadow' (Miller et al. 2007).

The sites are characterized by deep snowpacks (mean annual precipitation 1,510 mm at the wet meadow site; 954 mm at the dry meadow site) that limit growing season length (Sickman et al. 2001). The snowpack generally develops by late November–early December, after which time soils remain above 0°C for the duration of the winter (~December–June). Snowmelt begins once the snowpack becomes isothermal, usually in April, with peak discharge usually occurring in June (Sickman et al. 2003). Snow can persist into late June–early July, and soils at the wet meadow site can remain saturated well into August.

We recorded soil temperature hourly between November 2002 and April 2004 at the two sites using HOBO data loggers (Onset Computer Corporation, Pocasset, MA) installed at 50 and 300 mm depths. Soil moisture, recorded between January 2003 and April 2004, was monitored using time-domain reflectometer (CS616-L, Campbell Scientific, Inc., Logan, UT) and frequency-domain reflectometer (ECHO probe, Decagon Devices Inc., Pullman, WA) probes installed at approximately 100 mm depth. We used soil temperature and moisture data from an existing meteorological station near the dry meadow site (Sickman et al. 2003) to complete the record for soil temperature (April–November 2002), and soil moisture (April 2002–January 2003; Fig. 1).

Net N mineralization and nitrification

Net N mineralization was measured using the intact soil core method described by DiStefano and Gholz (1986). In June 2002, we established two 50 m transects at each site. Six sets of duplicate cores were collected at approximately 8 m intervals along each transect by driving 40 mm diameter PVC cylinders to a depth of 100 mm into the ground. One set of cores was returned to the lab within 48 h for determination of gravimetric soil moisture and initial (T_0) exchangeable NH_4^+ and NO_3^-. A mixed-bed ion exchange resin (J. T. Baker, IONAC NM-60 H^+/OH^-; Phillipsburg, New Jersey, USA) enclosed in a fine mesh nylon bag was inserted into the base of the second set of cores to catch throughflow N, and the cores were then returned to their original holes to incubate for 4–6 weeks during the snow-free season (~June–October) or 6–8 weeks during the winter (late November–May). The second set of cores (T_1) was collected following each sampling interval and analyzed as outlined below.

Due to the difficulty in accessing cores through a 3–4 m snowpack, we installed a cluster of replicate cores within an approximately 5 m × 5 m area at each site in November of each year (cf. Schimel et al. 2004). We harvested replicate sets of soil cores on twelve sampling dates: June 4, 2002; July 16, 2002; September 21, 2002; October 14, 2002; October 27, 2002; December 14, 2002; April 4, 2003; June 28, 2003; August 28, 2003; October 6, 2003; November 3, 2003; and January 26, 2004. Overwinter estimates of net N mineralization were calculated from November to April at the wet meadow site, and from November to June at the dry meadow site. At each harvest, soils from mineralization cores were hand sorted to remove roots and particles greater than 2 mm, homogenized, and a 10 g subsample was extracted in 0.5 M K_2SO_4 for 2 h. Winter soil cores were broken into small pieces and homogenized as completely as possible. Ion-exchange resin bags were removed from all harvested T_1 cores, rinsed with deionized water and extracted in 2 M KCl for 2 h. All soil and resin extracts were passed through pre-rinsed Whatman #1 filters and analyzed for inorganic N on a Lachat autoanalyzer (Lachat Instruments, Milwaukee, WI); NH_4^+ was analyzed using the diffusion method (Lachat Method 31-107-06-5-A), and NO_3^- was analyzed using the Griess-Ilovsay reaction after Cd reduction (Lachat method 12-107-04-

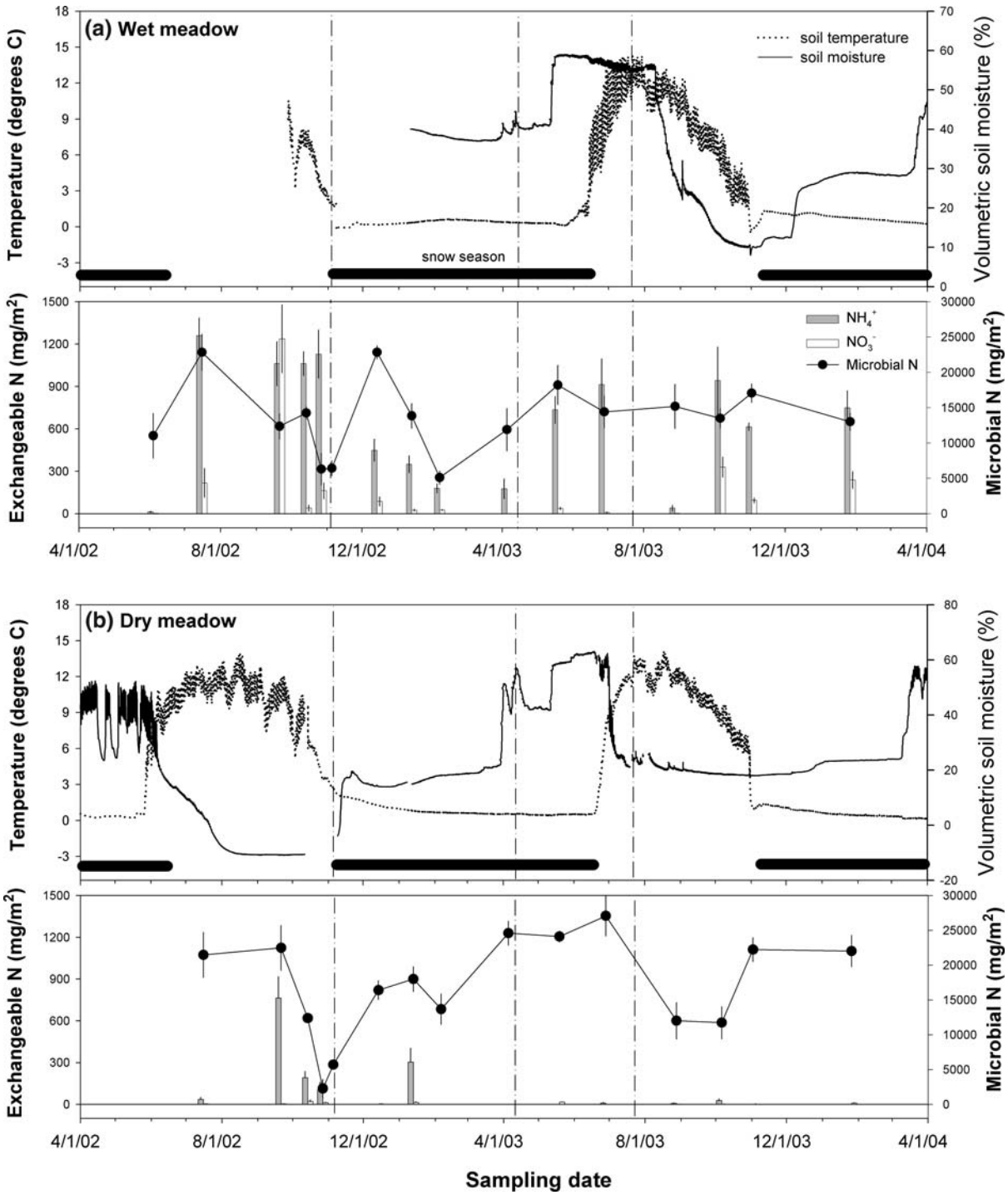

Fig. 1 Seasonal variation in soil temperature (50 mm depth) and moisture (100 mm depth), exchangeable N pools, and microbial biomass N in **a** wet and **b** dry meadow soils. Existing meteorological data were used to complete the record for soil temperature (April–November 2002) and soil moisture (April 2002–January 2003) in the dry meadow. Snow season length at each site is indicated by *horizontal bars* below the temperature–moisture curves. Exchangeable N and microbial biomass N are shown as means ±1 SE ($n = 6$–11). Dates of ^{15}N tracer additions (late fall, early spring, and summer) are indicated by *vertical dashed lines*

1-B). Net N mineralization was calculated as the difference in total N between T_0 (exchangeable N) and T_1 (exchangeable N + leachate N collected on resins), for each time period. Net nitrification was calculated as the difference in NO_3^- between T_0 and T_1. We used mean bulk densities of 0.40 g/cm^3 (wet meadow) and 0.61 g/cm^3 (dry meadow) to estimate soil N pools on per unit area basis, assuming a sample depth of 100 mm. Annual net N mineralization was estimated by summing over each sampling interval between July 2002 and June 2003.

Microbial biomass N was determined at each harvest using the chloroform fumigation-extraction method (Vance et al. 1987) over a 3-day fumigation period. Soil extracts were analyzed for total N using a persulfate digestion technique (Doyle et al. 2004), and biomass was calculated as the difference between fumigated and unfumigated digest concentrations. A correction factor was applied to chloroform-labile N ($K_{EN} = 0.54$; Brookes et al. 1985) values to estimate microbial biomass ($n = 6$).

^{15}N uptake

We measured short-term ^{15}N uptake in the late fall, immediately prior to snowfall (29 October and 5 November 2002); in the early spring, at the beginning of spring snowmelt (7 April 2003); and in mid-summer, at peak biomass (22 July 2003). Hereafter, we use 'November' to refer to the fall tracer addition. Prior to the ^{15}N additions, we installed ten 75 mm-diameter PVC rings within a 10 m × 10 m area of homogeneous meadow at each site. The rings were used to mark the location of the labeled cores, with nitrogen treatments randomly assigned. We injected a ^{15}N-NH_4^+ (99% atom enrichment as $^{15}NH_4Cl$) or ^{15}N-NO_3^- (99% atom enrichment as $K^{15}NO_3$) tracer to a depth of 100 mm within the PVC rings, slowly withdrawing the needle to deliver 1–2 μg ^{15}N/g-soil to each column of soil ($n = 5$ cores per N treatment per site). This was equivalent to an application rate of approximately 40–80 mg N/m^2, or approximately 5–45% of the ambient NH_4^+ pool and 50–100% of the NO_3^- pool, depending upon date. Wet meadow soils received 13 injections (26 ml) of a 1.0 mM ^{15}N solution, and dry meadow soils received 17 injections (34 ml) of a 0.5 mM solution. Injection points were evenly spaced in a radial pattern in each core. About 24 h after each ^{15}N addition, we harvested the ^{15}N-labeled soils by driving 150 mm sections of 75 mm-diameter, thin-walled steel pipe to a depth of 105 mm and removing the intact cores marked by the PVC rings. Volumetric soil moisture measured at least 20% on all sampling dates (Table 2).

In April, we dug snow pits to the soil surface to inject the ^{15}N tracer, quickly re-filled the pits to keep the soils insulated, and then re-excavated the pits 24 h later to harvest the cores. Snow depths at the time of the ^{15}N labeling ranged from 2.5 to 3.0 m. We did not measure short-term ^{15}N-NO_3^- uptake under snowpack due to logistical constraints. Instead, we labeled an additional set of cores ($n = 5$) in the dry meadow with either ^{15}N-NH_4^+ or ^{15}N-NO_3^- and returned to harvest them 45 days later, in early June.

All labeled cores were transported to the lab and processed within 48 h of collection. Above and belowground plant tissue was separated from soil, washed in tap water to remove soil, immersed in 0.5 mM $CaCl_2$ for 2–3 min to remove adsorbed ^{15}N, rinsed well with deionized water, and dried to constant weight at 55°C. Dried plant tissue samples were divided into live shoots, coarse (>1 mm) and fine (≤1 mm) live roots, and each tissue component was weighed, ground, and passed through a 40 mesh screen. Ground shoot and fine root tissues were analyzed for ^{15}N on an elemental analyzer (EA-MS) connected to a Europa Integra mass spectrometer (PDZ Europa, Ltd., Crewee, Cheshire, UK) at the Stable Isotope Facility, University of California, Davis.

Soil from labeled cores was hand sorted, homogenized and divided into subsamples for determination of the following: (a) gravimetric soil moisture; (b) extractable inorganic N; (c) microbial biomass N; (d) bulk soil N. The ^{15}N enrichment of extractable inorganic N and microbial biomass N pools were determined on 20 g subsamples of soil extracted in 100 ml of 0.5 M K_2SO_4, as outlined above (*Methods*: *Net N mineralization*). All soil extracts were diffused at 22°C with slow shaking for 8 days following the methods of Stark and Hart (1996). ^{15}N-NH_4^+ was collected on acidified quartz filter disks (Whatman QMA) enclosed in teflon tape and ^{15}N enrichment determined by EA-MS at UC Davis, as above.

The mass of ^{15}N in plant tissue derived from the tracer was calculated from the equation of Hauck and Bremner (1976), as cited in Knowles and Blackburn (1993):

$$F = [T(A_S - A_B)]/A_F$$

where F is the mass of ^{15}N derived from the tracer, T = total mass of N in the sample, A_S = atom % excess ^{15}N in the enriched sample, A_B = atom % excess ^{15}N in the control sample, and A_F = atom % excess ^{15}N in the tracer. The mass of ^{15}N in extractable inorganic N and microbial biomass N pools was determined from ^{15}N recoveries on diffusion disks, and from the difference in atom % ^{15}N enrichment between fumigated and unfumigated digests. Percent recovery of ^{15}N in above and belowground plant tissue, extractable inorganic N pools, and microbial biomass N was estimated as the mass of ^{15}N in each pool derived from the tracer (F), divided by the total mass of ^{15}N added to soil cores. Total ^{15}N recoveries were calculated as the sum of microbial, DIN, DON, and plant fine root recoveries in soil cores. We report ^{15}N uptake only for belowground plant tissue (fine roots, rhizomes) on the November and April sampling dates, as aboveground parts had senesced and/or partly decomposed. ^{15}N uptake is reported for above + belowground tissues on the July sampling date.

^{15}N turnover

In conjunction with the ^{15}N partitioning work, we measured gross N mineralization and nitrification rates using an in situ core pool dilution technique (Stark 2000). Approximately 2–3 weeks prior to each ^{15}N tracer addition, 40 mm-diameter PVC cores ($n = 5$) were installed in triplicate to a depth of 100 mm in the sampling areas used for field incubations, above. On the day of the ^{15}N tracer addition, two cores in each triplicate received 5–10 ml of a 1.0–2.0 mM solution of either ^{15}N-NH_4^+ (99% atom enrichment) or ^{15}N-NO_3^- (99% atom enrichment) at the same application rate used in the tracer addition ($\sim$1–2 μg ^{15}N/g-soil). The unlabeled core and the first labeled core were extracted immediately (<5 min after labeling) in 2 M KCl in the field, for determination of initial ambient and initial ^{15}N-labeled pools. The second labeled core within each triplicate was harvested and extracted in the field 24 h later. Sample extracts were filtered and then diffused to collect ^{15}N-NH_4^+ and ^{15}N-NO_3^-, as above. Where sample N concentrations were below the detection limit required for analysis by EA-MS, we spiked with 50 μg ^{14}N and back-calculated initial ^{15}N. Gross N production and consumption rates for NH_4^+ and NO_3^- were calculated using the isotope dilution equations of Kirkham and Bartholomew (1954), as outlined in Stark (2000):

$$\text{Gross production rate (GPR)} = \frac{P_0 - P_t}{t} \times \frac{\log(I_0/I_t)}{\log(P_0/P_t)}$$

$$\text{Gross consumption rate} = \text{GPR} - \frac{P_t - P_0}{t}$$

where P_0 is nutrient concentration at the beginning of the incubation; P_t is nutrient concentration at the end of the incubation; I_0 is the relative amount of isotope, in excess of background, present in the nutrient pool at the beginning of the incubation (atom % excess); I_t is the relative amount of isotope, in excess of background, in the nutrient pool at the end of the incubation; and t is the length of the incubation period. Initial ^{15}N-NH_4^+ and ^{15}N-NO_3^- pool sizes were estimated from KCl extracts of unlabeled cores at the time of the ^{15}N addition. Low exchangeable NO_3^- concentrations ($\leq$detection limit) in soils and poor ^{15}N-NO_3^- recoveries from diffusions precluded the estimation of ^{15}N-NO_3^- turnover on most sampling dates, with the exception of the wet meadow soils in the early spring (April).

Trace gas flux

Winter CO_2, N_2O, and CH_4 fluxes at the wet meadow site were estimated from snowpack concentration gradients (e.g., Fahnestock et al. 1998) on two sampling dates in the spring of 2003, and three dates in the winter/spring of 2004. At each sampling date, a stainless steel gas sampling probe connected with Teflon tubing to a portable pump was used to sample gas concentrations at 8–10 intervals along a 50 m transect. Trace gas concentrations at approximately 2 m above the snowpack, at the snow-soil interface, and at 3–5 depths in the snowpack were used to estimate the concentration gradient at each point along the transect. All samples were collected in 20 ml glass syringes, transferred to evacuated serum vials capped with rubber stoppers, and analyzed by gas chromatography at the University of California, Santa Barbara, within 1–2 weeks of collection. A portable infrared gas analyzer (EGM-2, PP Systems, Amesbury, MA) was used in the field as a check on

GC-derived CO_2 concentrations on the April 2, 2003 sampling date.

Diffusional loss from the soil to the atmosphere was calculated using a derivation of Fick's law:

$$J_g = D_g(d[g]/dz)f$$

where J_g is the gas flux, D_g is the diffusion coefficient, g is the measured trace gas concentration, z is the snow depth, and f is the porosity of the snowpack (calculated as $[1 - \rho]/\rho_{ice}$). Flux estimates were corrected for temperature, pressure, and for nonlinear diffusional flow (i.e., tortuosity) resulting from differences in snow density (ρ) throughout the snowpack (Sommerfeld et al. 1993). A diffusion coefficient (D_g) of 0.139 was used for CO_2 and N_2O, and a D_g of 0.22 was used for CH_4, as found by Sommerfeld et al. (1993). Estimated accuracy of this method for flux calculations through snow is ±11% (Sommerfeld et al. 1996).

Summer trace gas flux was measured on three sampling dates in 2003 using chambers constructed from PVC (150 mm inside diameter) installed to a depth of 100 mm, with a headspace volume of between approximately 0.75–1 l. Chambers ($n = 10$) were located at approximately 10 m intervals along the same transects used for the in situ N mineralization measurements. We used screw-top lids fitted with a rubber gasket and septum to cap the chambers approximately 10–15 min before sampling, and then sampled the headspace at approximately 1 h intervals for 3 h. All samples were collected in glass syringes, transferred to evacuated serum vials, and analyzed as above.

Statistical analyses

We analyzed for within-site seasonal differences in total $^{15}N\text{-}NH_4^+$ recovery, $^{15}N\text{-}NH_4^+$ uptake by plants and microbes, and net $^{15}N\text{-}NH_4^+$ flux within sites using the GLM procedure in SAS with date as a fixed effect (SAS Institute, Cary NC). Data expressed as percentages were arcsine-square root transformed prior to analysis. A Tukey's Studentized Range (HSD) test was used to examine a posteriori differences among treatment means. The relationship between net N mineralization and net nitrification was analyzed across sites and sampling dates using simple linear regression.

Results

Soil microclimate

Soil temperature and moisture varied seasonally, but were moderated by winter snow cover and high water content in the late spring. Freeze-thaw events were uncommon. At the wet meadow site, minimum soil temperatures at 50 mm reached −0.05 and −0.55°C, respectively, in early November of the first and second years. The freeze-thaw period lasted approximately 5–10 days and occurred around the time of the first winter storms, prior to snowpack development. As snow depths increased, soils warmed to a winter maximum of approximately 0.2°C (dry meadow) and 0.6–0.8°C (wet meadow) by mid-February, but cooled to approximately 0.1°C by mid-May. This cooling was associated with the rapid increase in soil moisture during the latter stages of snowmelt (Fig. 1a). In spite of winter soil temperatures that were on average 0.1–0.2°C colder than in the wet meadow, dry meadow soils never fell below 0°C, even during the transition seasons (Fig. 1b). Snowmelt began at roughly the same time at the two sites, but the dry meadow soils dried out almost 2 months earlier than those in the wet meadow.

Microbial N dynamics and net N mineralization

Fluctuations in microbial N were relatively synchronous between sites (Fig. 1a, b). Over the course of the study, microbial N pools varied by as much as 4-fold at the wet meadow site (Fig. 1a), and by nearly 10-fold at the dry meadow site (Fig. 1b). Reductions in microbial N were generally accompanied by increases in soil exchangeable N in the wet meadow, but not in dry meadow soils. Both sites showed a decline (49–90%) in microbial N in the fall of the first year (September–November) and during the early part of the winter (24–63%) as soils warmed (January, February), with microbial pools recovering by the start of snowmelt (April; Fig. 1a, b). Whereas microbial N continued to increase into the summer in the dry meadow, it decreased by approximately 40% in the wet meadow during the latter stages of snowmelt (May–June). A third, marked decline (55%) in microbial N occurred in the dry meadow late in the summer of the second year (July–September; Fig. 1b), but was not apparent in the wet meadow soils. In the wet meadow, a late fall/early

winter freeze-thaw event (early November) occurred in both years, yet appeared to have little effect on microbial biomass; i.e., biomass had declined prior to the freeze-thaw event in the first year, and had increased slightly in the second year (Fig. 1a).

Net N mineralization and net nitrification in wet meadow soils was several orders of magnitude greater than in dry meadow soils, where net rates were negligible during all but one sampling interval. In the wet meadow soils, overwinter net N mineralization was low (October–April; $\sim$2 mg m^{-2} day^{-1}), but increased 6-fold during the snowmelt season (April–June; $\sim$13 mg m^{-2} day^{-1}; Table 1), when it accounted for 34% of annual production. Together, net N mineralization during the winter and spring months accounted for 44% of annual N production. Likewise, winter and spring contributions to net nitrification ($\sim$8 mg m^{-2} day^{-1}; Table 1) accounted for approximately 66% of annual NO_3^- production. During the mid- to late summer of the first year (July–September), daily net N mineralization and net nitrification rates in the wet meadow soils were 2–10 times greater than during any other season. Net N mineralization during the same period in the dry meadow soils was great enough to offset net N immobilization during the rest of the year (Table 1).

At both sites, high rates of net N mineralization during the first summer (July–September) were followed by a period of N immobilization in the early fall (September–October). On average, NO_3^- comprised roughly 15 and 40% of total exchangeable N in the wet and dry meadow sites, respectively. Annual net N mineralization ranged from approximately 60 mg N/m^2 in the dry meadow to 1,500 mg N/m^2 in the wet meadow (Table 1). Annual estimates of net nitrification ranged from <10 mg N/m^2 in the dry meadow to 2,200 mg N/m^2 in the wet meadow. Net nitrification rates were positively related to net N mineralization ($y = 0.30x + 2.27$; $r^2 = 0.40$; $P < 0.01$; Fig. 2) and were driven by variation at the wet meadow site. When the fall immobilization event at the wet meadow site was included, the strength of the relationship increased ($y = 0.43x + 2.04$; $r^2 = 0.70$; $P < 0.001$; data not shown).

^{15}N recovery and uptake rates

Total ^{15}N recoveries varied seasonally ($P < 0.001$) and by site ($P < 0.001$; Fig. 3). Recoveries of ^{15}N-NH_4^+ (80–84%) and ^{15}N-NO_3^- (44–46%) in microbial + plant + soil N pools were comparable between the two sites in the late fall, but diverged on

Table 1 Cumulative net N mineralization and nitrification measured in intact soil cores, 2002–2004

Incubation period	No. of days	Wet meadow		Dry meadow	
		Net N mineralization (mg N/m^2)	Net nitrification (mg N/m^2)	Net N mineralization (mg N/m^2)	Net nitrification (mg N/m^2)
July–September 2002	68	1,755 ± 302	1,024 ± 218	729 ± 154	0.2 ± 3
September–October 2002	34	−1,662 ± 354	−853 ± 255	−655 ± 201	12 ± 3
October 2002–April/June 2003[a]	161–200	318 ± 445	1,338 ± 384	−14 ± 1	−14 ± 0
April–June 2003	85	1,087 ± 327	690 ± 389	ND	ND
June–August 2003	60	−952 ± 24	−11 ± 0.9	−11 ± 13	3 ± 2
August–October 2003	39	ND	ND	ND	0.1 ± 2
October–November 2003	28	−347 ± 169	−249 ± 55	ND	ND
November 2003–January 2004	86	240 ± 110	197 ± 47	11 ± 9	11 ± 9
Estimated annual total (mg N m^{-2} $year^{-1}$)		1,499	2,198	60	−1.8

Annual totals are calculated from estimates of net N mineralization and net nitrification measured over 4–5 incubation periods between July 16, 2002 and June 28, 2003

ND no data

[a] Overwinter mineralization rate was estimated between October 27, 2002 and April 4, 2003 for the subalpine wet meadow, and between October 27, 2002 and June 28, 2003 for the alpine dry meadow. Means ± 1 SE (n = 6–11)

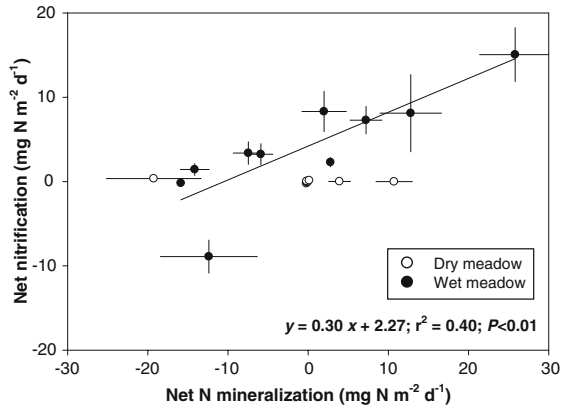

Fig. 2 Relationship between net N mineralization and net nitrification rates is driven by variation in the wet meadow soils. Means ±1 SE ($n = 6$)

subsequent sampling dates when the dry meadow immobilized considerably more ^{15}N. At both sites, the majority of added $^{15}N\text{-}NH_4^+$ was recovered in microbial biomass (32–103%; Fig. 3), although ^{15}N recoveries in plant tissue (10–20%) and soil inorganic N (10–17%) were also substantial in the wet meadow.

Microbial ^{15}N uptake rates were greatest in the fall (both sites), whereas ^{15}N uptake by plants was at a maximum in the spring and summer (wet meadow; $P < 0.05$; Table 3). In some cases, apparent recovery of ^{15}N in the microbial biomass exceeded 100%, potentially due to N contamination and/or analytical error. At the wet meadow site, ambient N concentrations in plant roots and rhizomes increased by more than 50% between snowmelt (April) and mid-summer (July), and remained high into the late fall (Table 2). Plant ^{15}N uptake in the dry meadow did not vary seasonally, nor did root N concentrations vary through time. Plant belowground biomass averaged approximately 5.2 mg/m^2 in the wet meadow and 6.0 mg/m^2 soil in the dry meadow (data not shown).

We found little redistribution of added ^{15}N through time in the dry meadow, where we tracked the fate of $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$ over a 45-day period during snowmelt (April–June; Fig. 4). Recovery of $^{15}N\text{-}NH_4^+$ in microbial biomass decreased by >50% between the 1- and 45-day harvests without a concomitant increase in plant or soil N pools. Recoveries of $^{15}N\text{-}NH_4^+$ and

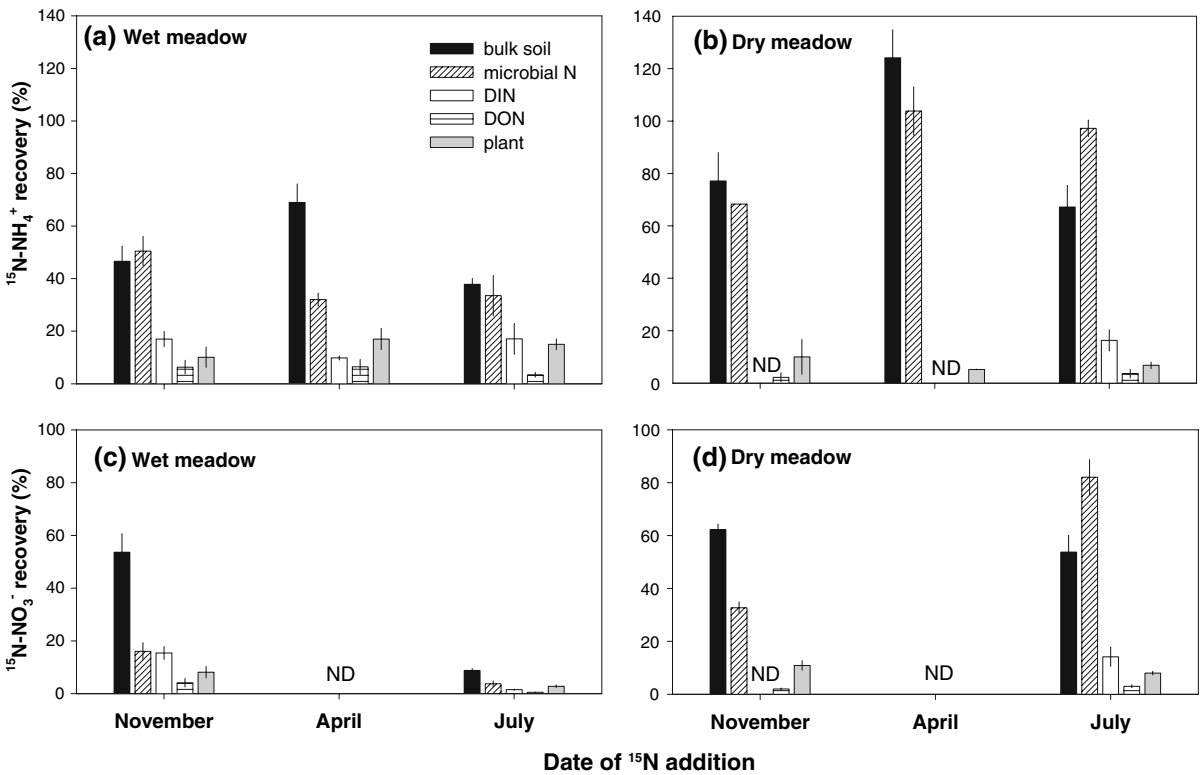

Fig. 3 Percent $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$ recovery after 24 h in plant, microbial, and soil N pools on three sampling dates in wet (**a**, **c**) and dry (**b**, **d**) meadow soils. Soils were labeled under the snowpack in April. Means ± 1 SE ($n = 5$). *ND* no data

Table 2 Nitrogen measured in ambient exchangeable N pools, DON, microbial biomass, and fine roots on late fall (November), early spring (April) and summer (July) ^{15}N tracer addition dates in 2002–2003

Site	Date	Exchangeable N		Microbial biomass			
		NH_4^+ (mg N/m^2)	NO_3^- (mg N/m^2)	N (mg N/m^2)	C (g C/m^2)	Fine root N (%)	Soil moisture (%)
Wet meadow	November	668 ± 110	32 ± 13	6,880 ± 1,500	60.6 ± 18.8	1.3 ± 0.08	28.9
	April	1,268 ± 83	0 ± 0	16,280 ± 760	ND	0.9 ± 0.05	38.9
	July	1,710 ± 258	29 ± 3	25,570 ± 3,900	358.2 ± 43.6	1.4 ± 0.06	55.1
Dry meadow	November	59 ± 22	11 ± 2	6,120 ± 700	86.1 ± 19.9	0.7 ± 0.03	ND
	April	21 ± 8	110 ± 27	24,650 ± 2,400	ND	0.6 ± 0.06	40.8
	July	127 ± 58	38 ± 3	25,770 ± 1,100	467.4 ± 30.6	0.7 ± 0.04	23.7

Microbial biomass C (g C/m^2) is included for reference. Volumetric soil moisture is the mean for that date, recorded by TDR. All other means ± 1 SE (n = 5–7)

ND no data

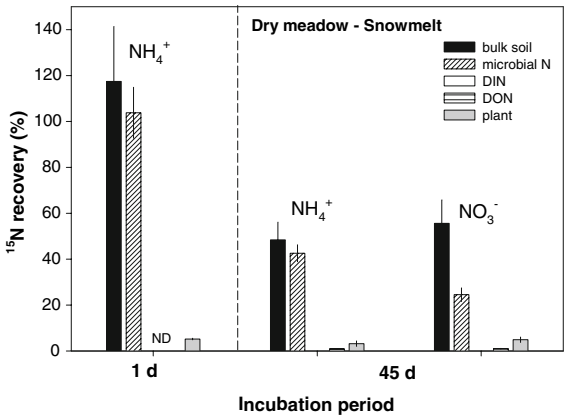

Fig. 4 Percent ^{15}N-NH_4^+ and ^{15}N-NO_3^- recovery in plant, microbial, and soil pools labeled under snowpack and harvested 1 day (^{15}N-NH_4^+) and 45 day (^{15}N-NH_4^+ and ^{15}N-NO_3^-) later at the dry meadow site. Short-term (1-day) ^{15}N-NO_3^- uptake was not measured under snowpack. Means ± 1 SE (n = 5). *ND* no data

^{15}N-NO_3^- in plant and bulk soil N pools were almost equivalent 45 days after the tracer addition (Fig. 4), suggesting that initial (1-day) recoveries could have been comparable, as well.

^{15}N turnover

Net ^{15}N-NH_4^+ flux showed strong seasonal variation in the wet ($P < 0.01$) and dry ($P < 0.001$) meadow soils, with net production of ^{15}N-NH_4^+ measured only in the summer (July; Table 3). Net ^{15}N-NH_4^+ fluxes measured by pool dilution were an order of magnitude greater than net NH_4^+ production rates measured with intact soil cores during the June–August interval (e.g., wet meadow: −16 mg N m^{-2} day^{-1}; dry meadow: −0.1 mg N m^{-2} day^{-1}; Table 1). However, net nitrification measured for April–June in the wet meadow (8 mg N m^{-2} day^{-1}) approximated the net ^{15}N-NO_3^- flux for that site in April (9 mg N m^{-2} day^{-1}; Table 3).

Gross N consumption can be stimulated by ^{15}N additions (Davidson et al. 1990), particularly when soils are severely N-limited, and it is possible that enhanced ^{15}N-NH_4^+ consumption contributed to the net negative N fluxes measured on most sampling dates. The highest rates of gross ^{15}N-NH_4^+ production were recorded in the late fall when microbial biomass was at a minimum (Table 2; Fig. 2). At that time, mean residence time of ^{15}N-NH_4^+, calculated as the extractable N pool divided by the gross N production rate (cf. Booth et al. 2005), was estimated to range from <0.1 (dry meadow) to 1.3 day (wet meadow). Estimated residence times on the other sampling dates were longer, ranging from 3 to 4 days in July to approximately 6 days (wet meadow) in April, under snowpack.

Trace gas flux

Trace gas flux from the wet meadow site was substantial during the spring snowmelt and early summer months (early April to late July). Although CO_2 fluxes approximately doubled as the site became snow-free, winter (January–March) and spring (April–May) fluxes accounted for approximately 4 and 22% of annual CO_2 release at the site. Differences in snow

Table 3 Seasonal variation in in situ gross N production and consumption rates and short-term $^{15}N\text{-}NH_4^+$ and $^{15}N\text{-}NO_3^-$ uptake rates by microbes and plant roots in wet and dry meadow soils

Site	N form	Date	^{15}N turnover (mg N m^{-2} day^{-1})			^{15}N uptake (mg N m^{-2} day^{-1})	
			Production	Consumption	Net N flux	Microbial	Plant
Wet meadow	$^{15}N\text{-}NH_4^+$	November	522 ± 69	885 ± 124	−363 ± 58[b]	39 ± 5	5 ± 2[b]
		April	208 ± 93	579 ± 262	−253 ± 156[b]	25 ± 2	14 ± 3[a]
		July	397 ± 156	195 ± 87	287 ± 85[a]	27 ± 6	12 ± 2[a]
	$^{15}N\text{-}NO_3^-$	November	ND	ND	ND	11 ± 4	6 ± 1[a]
		April	183 ± 20	174 ± 26	9 ± 28	ND	ND
		July	ND	ND	ND	3 ± 1	2 ± 0[b]
Dry meadow	$^{15}N\text{-}NH_4^+$	November	954 ± 114	1,364 ± 166	−410 ± 52[b]	71 ± 11	5 ± 1
		April	ND	ND	ND	57 ± 4	3 ± 0
		July	37 ± 32	0 ± 0	37 ± 32[a]	54 ± 2	4 ± 1

Lower case letters denote differences in net ^{15}N flux and plant and microbial ^{15}N uptake rates among sampling dates ($P < 0.05$). Means ± 1 SE ($n = 5$)

ND no data

depth and soil temperature may have contributed to the approximately three-fold difference in CO_2 flux measured in April of both years. Soils appeared to be a net sink for N_2O in early April 2003 (Table 4), but had become a net source by mid-May. Efflux of CO_2 ranged from ~351 to 625 mg C m^{-2} day^{-1} during that period, with CO_2, N_2O, and CH_4 fluxes peaking in late June, when soils were warming but still saturated (Fig. 1; Table 4). Multiplying the mean daily N_2O flux by each measurement interval yielded an annual N_2O flux of approximately 8.9 mg N m^{-2} $year^{-1}$ for the wet meadow. The winter contribution to annual N_2O flux was substantial, accounting for approximately 50% of annual N_2O release. Snow-covered soils were always a net sink for CH_4; we did not measure net CH_4 efflux until soils were snow-free, in late June.

Discussion

Nitrogen uptake and turnover varied seasonally, but the response to seasonality was not consistent across sites. Although microbial N pools followed similar trajectories in the wet and dry meadow, a fundamental difference between the two sites was the degree to which soil N pools tracked fluctuations in microbial N. In the wet meadow, net N mineralization rates

Table 4 Trace gas flux at the subalpine wet meadow site, 2003–2004. Winter fluxes were measured through the snowpack

Date	Snow depth (m)	Soil temperature (°C)	CO_2 (mg C m^{-2} day^{-1})	N_2O (μg N m^{-2} day^{-1})	CH_4 (μg C m^{-2} day^{-1})
2003					
April	2.75	0.07	350.9 ± 64.6	−36.5 ± 2.9	ND
May	2.34	0.02	353.9 ± 71.3	15.0 ± 3.0	−64.6 ± 20.5
June	–	10.38	783.9 ± 181.8	60.0 ± 2.5	85.5 ± NA
July	–	14.39	625.4 ± 145.0	10.4 ± 3.7	47.9 ± 4.0
August	–	12.16	368.9 ± 57.7	24.8 ± 19.5	28.3 ± 1.8
2004					
January	1.95	0.59	30.4 ± 6.3	38.5 ± 5.4	−124.5 ± 24.7
March	2.40	0.28	36.3 ± 6.4	27.0 ± 2.8	−108.9 ± 25.8
April	1.80	0.01	107.7 ± 17.6	ND	ND

Summer fluxes were measured using chambers installed in the soil. Soil temperatures (100 mm depth) represent the mean for that sampling date. Means ± 1 SE ($n = 4$–11)

were greatest in the fall and spring, following periods of microbial decline. In the dry meadow, net N mineralization rates were generally insensitive to changes in the microbial N pool, and soils immobilized N through the winter and into the spring. Freeze-thaw events were uncommon; we recorded sub-zero temperatures only in the wet meadow, and only during the fall/winter transition.

Net ^{15}N flux was positive at both sites in mid-summer, at the peak of the growing season, and at the wet meadow site in the early spring, at the start of snowmelt. This spring-summer period of N production coincided with a period of high plant and microbial ^{15}N uptake in the wet meadow, indicating that both plants and microbes could exploit seasonally available N. Net N mineralization rates measured during the summer incubations (June–August) were negative at both sites, consistent with the high ^{15}N recoveries in microbial biomass. Both results, i.e., high ^{15}N recoveries and net N immobilization, suggested that while gross NH_4^+ production was substantial, the soil microbial community had the capacity to assimilate most of the N produced.

Nitrogen availability in the wet meadow was greater than in the dry meadow at all times of the year, and this difference was reflected in all of our measurements: i.e., inorganic N pools, net N mineralization rates, and growing season gross mineralization rates were greater, and ^{15}N retention was lower, in the wet meadow. Estimated annual net N mineralization (1.5 g m^{-2} $year^{-1}$) and nitrification (2.2 g m^{-2} $year^{-1}$) rates at the wet meadow site were comparable to rates measured in alpine (1.2–5 g m^{-2} $year^{-1}$; Fisk and Schmidt 1995; Rehder and Schäfer 1978) and moist subarctic tundra (4.7 g m^{-2} $year^{-1}$; Hart and Gunther 1989). Rates in the dry meadow were more than an order of magnitude lower, comparable to those in arctic tussock tundra ($\leq$0.5 g m^{-2} $year^{-1}$; Giblin et al. 1991).

Overwinter N mineralization

Deep snowpacks at both sites kept soils at temperatures near 0°C for most of the winter. In the wet meadow, net N mineralization and net nitrification during the winter months (November–April) accounted for approximately 10 and 45%, respectively, of annual net N production. In contrast, dry meadow soils immobilized N over the course of the winter. Overwinter immobilization by microbes has been reported infrequently in other ecosystems (e.g., Schmidt et al. 1999), but, in general, N assimilated by microbes is released back to soils prior to or during the spring thaw, resulting in net N mineralization (Brooks et al. 1996; Lipson et al. 1999; Schmidt et al. 1999; Schimel et al. 2004; Edwards et al. 2006). Our observations of net N immobilization in the dry meadow soils are consistent with previous laboratory incubations (Miller et al. 2007) and suggest that they could be N-limited at temperatures $\geq$0°C. For example, mineralized C:N was relatively high in dry meadow soils incubated at 0 and 5°C in the lab, suggesting a high microbial demand for N (Miller et al. 2007). However, at −2°C the mineralized C:N ratios were lower, suggesting that microbes might shift to N-rich materials as soils freeze.

Freeze-thaw events were uncommon in the field, and we did not record soil temperatures <0°C in the dry meadow. That said, it is certainly possible that freeze-thaw events could occur during one or both of the transition seasons. Soil freezing has been shown to increase microbial respiration and net N mineralization in some soils (e.g., Schimel and Chapin 1996; Neilsen et al. 2001), presumably due to the release of cell contents (simple sugars and free amino acids) upon cell death (Ivarson and Sowden 1970), and/or the shift by microbes to more labile C compounds (Schimel and Mikan 2005). However, the rate and severity of freezing may determine the response of the microbial community, and both alpine (Lipson et al. 2000) and eastern hardwood forest soils (Neilsen et al. 2001) have been found to be relatively tolerant of milder freeze-thaw regimes. In the wet meadow, a freeze-thaw event occurred each year in the late fall/early winter (November), but it is unclear whether this event enhanced N turnover, as microbial biomass appeared to be unaffected.

Both wet and dry meadow soils experienced a mid-winter decline in the microbial N pool that suggested C-limitation. Similar declines in biomass have been reported from arctic (Edwards et al. 2006) and alpine soils (Lipson et al. 2000), although in both cases the decline occurred later, during the thaw period. Here, it is possible that sustained mid-winter temperatures of up to 0.6–0.8°C may have supported increases in microbial biomass and/or activity that could have resulted in a depletion of available C. We did not track changes in soil C availability, but steep declines in soluble C recorded post-snowmelt (Lipson et al. 2000;

Edwards et al. 2006) and the apparent sensitivity of winter microbial communities to temperatures >0°C (Lipson et al. 2002) have been cited as possible explanations for the spring decline in microbial biomass elsewhere. In this study, the decline in microbial N was followed by measurable net N mineralization in the wet meadow soils. In the dry meadow soils, net N immobilization continued in spite of microbial turnover, again suggesting that soils were extremely N-limited.

Spring transition—snowmelt

The spring transition (snowmelt) represents a critical period in the annual nitrogen cycle of arctic and alpine environments. In the wet meadow, net nitrification rates measured during the winter and spring were approximately 50% as great as rates measured during the growing season, but nevertheless accounted for approximately two-thirds of annual net NO_3^- production. Microbial biomass declined by approximately 20% during the latter stages of snowmelt (May–June), during which time net N mineralization rates increased more than 6-fold. Net N mineralization and net nitrification during the snowmelt period (April–June) thus accounted for approximately 34 and 23%, respectively, of annual net N production.

In the dry meadow, microbial N increased continuously during the late winter-early spring (February–June), but then declined by more than 50% post-snowmelt. The loss of microbial biomass N following snowmelt did not result in an increase in exchangeable N, suggesting that nitrogen was being rapidly re-immobilized. However, it was only during this post-snowmelt period (July) that we also measured net production of ^{15}N. In soils labeled at the start of snowmelt (April), total recoveries of ^{15}N-NH_4^+ and ^{15}N-NO_3^- ranged from 50 to 60% by the end of the snowmelt period (June). These rates are comparable to or exceed ^{15}N recoveries reported from arctic tundra (Tye et al. 2005) and coastal forest (Perakis et al. 2005) over similar time scales, and are noteworthy given that they were measured during the thaw period, when large volumes of meltwater would have been flushing the dry meadow soils.

As in the wet meadow, the spring thaw period has been shown to be important to annual N budgets in boreal forest (Kielland et al. 2006) and continental alpine (Brooks et al. 1998; Lipson et al. 1999) communities, as well. In the Colorado alpine, soil N concentrations increase slowly over the winter and reach a maximum just after the start of snowmelt (Brooks et al. 1996, 1998), and microbial biomass declines as sites become snow-free (Brooks et al. 1998; Lipson et al. 1999, 2002). Here, we found that N availability in the wet meadow increased roughly 3-fold between the onset of snowmelt (early April) and peak discharge (late May), concurrent with a 50% increase in microbial biomass. Exchangeable N concentrations continued to increase into the latter stages of snowmelt (late June), apparently fueled by the decline in microbial N that occurred after peak discharge. The general pattern that we found in the wet meadow was similar to that reported by Brooks et al. (1998), although the increase in microbial biomass N at the start of snowmelt was less pronounced, and the decline in microbial N occurred earlier, before sites were snow-free. Furthermore, we found no evidence of freeze-thaw events at either site during snowmelt, in contrast to the spring thaw dynamics reported from other sites (Brooks et al. 1998; Groffman et al. 1999; Lipson et al. 1999; Edwards et al. 2006).

The release of soluble N from microbial biomass during the spring transition is thought to provide an important source of N to plants (Lipson et al. 1999). An increase in plant ^{15}N uptake has been found to coincide with the decrease in the microbial ^{15}N pool during the spring thaw (Grogan and Jonasson 2003), and alpine plants have been shown to acquire up to 12% of their seasonal N requirement from nitrogen released during snowmelt (Bilbrough et al. 2000). In our study, plants recovered between 10 and 20% of added ^{15}N across all sampling dates, in agreement with other studies in grassland systems (Hart et al. 1993) and wet arctic tundra (Schimel and Chapin 1996). In the wet meadow, plant uptake of ^{15}N-NH_4^+ was at a maximum at the beginning of snowmelt (14 mg N m^{-2} day^{-1}), suggesting that plants could readily exploit N released during the latter stages of melt. Microbial biomass was still increasing at the start of snowmelt, and microbial uptake was roughly equivalent to levels recorded during the growing season (25–27 mg N m^{-2} day^{-1}). Assuming an annual N demand of approximately 2,300 mg N/m^2 (A. Miller, unpublished data), nitrogen released during snowmelt could account for up to 45% of annual plant N requirements at the wet meadow site.

Fall transition

Although snowmelt appears to be an important control over annual soil N budgets, the conditions preceding snowpack development may also be critical. The fall transition resulted in increased N availability at both the wet and dry meadow sites, but only in the first year of the study. At the wet meadow site, the summer-fall transition (July–September) was marked by a roughly 50% decline in microbial N and a period of measurable net N mineralization followed by net N immobilization (September–November). At the dry meadow site, the summer-fall decline in microbial N was steeper (90%), occurred later in the season (September–November), and coincided with a period of net N immobilization rather than N mineralization. At both sites, plants and microbes had the capacity to exploit this fall pulse of N.

Fall conditions have been shown to control the winter soil microclimate (Schimel et al. 2004); i.e., late snowfall leads to rewetting and/or freeze-thaw events that can result in the release of N-rich material from soil organic matter (Edwards and Cresser 1992) or damaged roots and soil microorganisms (DeLuca et al. 1992; Ruess et al. 2003). At the Sierra sites, we measured the greatest exchangeable N concentrations and highest net N mineralization rates in late summer of the first year (July–September), and the highest rates of gross ^{15}N-NH_4^+ production and consumption in the late fall (October–November). In the interim, we also measured high net N immobilization rates (September–October) in wet and dry soils, perhaps owing to soil rewetting following early fall storms (NOAA-NCDC; https://ols.nndc.noaa.gov; accessed for Visalia, CA, 6/3/08), and in both years a freeze-thaw event (early November) in the wet meadow soils. These events (rewetting, freeze-thaw) may have released labile carbon from litter and/or microbial biomass, thus promoting N immobilization, at least in the short-term.

Consistent with the fall immobilization event, microbial ^{15}N uptake rates were at a maximum and mean residence times for ^{15}N-NH_4^+ were low in the late fall (November), suggesting that the surviving microbial pool was actively cycling available N. Plants also had the capacity to assimilate considerable N at this time, in spite of the fact that all aboveground parts had senesced. Similar ^{15}N recoveries have been reported for plants in a subarctic heath community at the onset of winter (Grogan and Jonasson 2003), suggesting that plants could acquire N released from microbial biomass during the fall transition, as well.

In the dry meadow, high ^{15}N retention rates suggested that microbes were N limited. Rapid turnover of microbial biomass in alpine and montane forest soils (Lipson et al. 1999; Schadt et al. 2003; Monson et al. 2006) has been shown to result in sustained microbial N demand and high N retention (Schmidt et al. 2007). Assuming that nitrification depends on the amount of NH_4^+ available to nitrifiers (Myrold 1998), the low nitrification rates in the dry meadow suggest that most NH_4^+ produced by mineralization was rapidly assimilated. Relatively large microbial N pools, rapid ^{15}N uptake by microbes, high gross ^{15}N-NH_4^+ production and consumption rates, and low net ^{15}N-NH_4^+ flux also indicated rapid turnover of a small pool of available N. Low, temporally stable N concentrations in plant belowground tissue suggested severe N-limitation. It is noteworthy, then, that plants in the dry meadow showed measurable ^{15}N-NH_4^+ uptake in the late fall, when exchangeable N pools were at a maximum, and that NO_3^- concentrations in stream water draining the dry meadow basin have historically peaked during the fall, rather than during spring snowmelt (Sickman et al. 2003).

Nitrogen export

Earlier and deeper snowpacks have been shown to contribute to greater overwinter N mineralization in alpine catchments (Brooks and Williams 1999), and to greater N yields during snowmelt (Brooks et al. 1999; Sickman et al. 2001). In the Emerald Lake watershed, which includes the wet meadow site, NO_3^- release during snowmelt can account for up to 50–90% of annual N export, most of which is derived from soils (Sickman et al. 2003). Our results from the wet meadow suggest that although N accumulates in the soil during the winter months, sustained nitrification during snowmelt could also be an important contributor to NO_3^- export. Furthermore, it appears that N release during snowmelt is due to increased NO_3^- production rather than to asynchrony between N availability and N demand, per se. The decline in microbial N and high rates of net N mineralization and net nitrification over the course of snowmelt

(April–June), and the net production of $^{15}N\text{-}NO_3^-$ at the onset of snowmelt (April), are consistent with the hypothesis that NO_3^- release during spring runoff is due to microbial turnover (Sickman et al. 2001).

We measured a mean soil NO_3^- concentration of approximately 0.4 kg/ha within days of maximum NO_3^- export from the wet meadow in 2003 (N yield = 0.13 kg N ha^{-1} day^{-1}; J. Sickman, unpublished data), suggesting that as much as one-quarter of the NO_3^- produced during snowmelt could have been immediately lost from the system. If microbial and plant uptake of NO_3^- in the spring was comparable to levels measured in the fall (0.05–0.10 kg N ha^{-1} day^{-1}), it is likely that at least an equal fraction of NO_3^- was retained. This possibility is supported by Schimel et al. (2004), who reported N immobilization during snowmelt in arctic tundra soils, at a time when fresh inputs of labile C should have been lacking.

Over the past two decades, spring and autumn temperatures have increased in the northern latitudes (Piao et al. 2008). In the Sierra Nevada, increased warming is predicted to result in increased winter precipitation, a greater proportion of precipitation falling as rain at mid-elevations (Knowles et al. 2006), and increasingly early snowmelt dates (Stewart et al. 2005; Maurer et al. 2007). In this study, net N mineralization and nitrification rates showed strong seasonal variation, suggesting the importance of temperature and moisture to soil N dynamics at both sites. Previous lab incubations had indicated that the wet meadow soils would mineralize N only at temperatures $\geq$0°C, whereas dry meadow soils would immobilize N at temperatures $\geq$0°C (Miller et al. 2007). Both predictions appeared to be borne out to some degree by our results from the field, where even winter soil temperatures remained >0°C. Wet meadow soils mineralized N year-round, particularly as soils reached temperatures $\geq$5°C in the late spring through early fall. Dry meadow soils immobilized N on most sampling dates.

Our field measurements indicated that N_2O release may represent an additional pathway for N loss, particularly in the late spring and early summer, when soils are still saturated but are well above 0°C. Estimated annual N_2O release from the wet meadow site was approximately 8.9 mg N m^{-2} $year^{-1}$, or roughly 3% of mean annual N loading (3 kg ha^{-1} $year^{-1}$; Sickman et al. 2001), with the highest N_2O fluxes recorded before sites were entirely snow-free. If, as we observed, fall storm events largely control annual N dynamics in the dry meadow, earlier or more frequent fall storms could promote greater N release from that site. Earlier snowmelt dates could enhance annual N production in the wet meadow by increasing the length of the snow-free season while presumably maintaining microbial activity during snowmelt. A prolonged snowmelt season, e.g., in response to deeper snowpack, could conceivably result in both greater net N production and greater N_2O release.

Conclusions

The relationship between short-term fates of nitrogen and longer-term patterns of N retention is determined by the recycling of N among plant, microbial, and soil organic matter pools in the ecosystem. The seasonality of these processes, paired with the capacity for N uptake at different times of the year, will determine the fate of N in the short-term. Longer-term ecosystem N retention will depend not only on the flux of nitrogen from rapidly cycling (e.g., microbial) to slowly cycling (e.g., soil organic matter) pools of N, but also the timing and duration of these turnover events. Our results showed pronounced differences between the subalpine wet and alpine dry meadows in terms of overall N availability and seasonal N dynamics. Wet meadow soils showed characteristic increases in N availability associated with declines in microbial biomass, whereas N availability in dry meadow soils was relatively insensitive to seasonal fluctuations in microbial N. Whether soils become a net source or sink for N will likely depend upon the interaction between environmental factors (e.g., temperature, moisture, substrate availability) and microbial population dynamics during seasonal transitions. Enhanced net N production during the spring and fall could offset N uptake, and thus seemingly incremental changes in soil temperature and moisture could have wide-reaching effects on annual N budgets.

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