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Nitrogen Dynamics in a Small Arctic Watershed – Retention and Downhill Movement of ^{15}N

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ABSTRACT

We examined short- and long-term nitrogen (N) dynamics and availability along an arctic hillslope in Alaska, USA, using stable isotope of nitrogen (^{15}N), as a tracer. Tracer levels of $^{15}NH_4^+$ were sprayed once onto the tundra at six sites in four tundra types; heath (crest), tussock with high and low water flux (mid- and foot-slope), and wet sedge (riparian). ^{15}N in vegetation and soil was monitored to estimate retention and loss over a 3-yr period.

Nearly all $^{15}NH_4^+$ was immediately retained in the surface moss-detritus-plant layer and > 57 % of the ^{15}N added remained in this layer at the end of the second year. Organic soil was the second largest ^{15}N sink. By the end of the third growing season, the moss-detritus-plant layer and organic soil combined retained ≥ 87 % of the ^{15}N added except at the mid-slope site with high water flux, where recovery declined to 68 %. At all sites, non-extractable and non-labile-N pools were the principal sinks for added ^{15}N in the organic soil.

Hydrology played an important role in downslope movement of dissolved ^{15}N. Crest and mid-slope with high water flux sites were most susceptible to ^{15}N losses via leaching perhaps because of deep permeable mineral soil (crest) and high water flow (mid-slope with high water flux). Late spring melt-season also resulted in downslope dissolved-^{15}N losses, perhaps because of an asynchrony between N release into melt water and soil immobilization capacity. We conclude that separation of the rooting zone from the strong sink for incoming N in the moss-detritus-plant layer, rapid incorporation of new N into relatively recalcitrant soil-N pools within the rooting zone, and leaching loss from the upper hillslope would all contribute to the strong N-limitation of this ecosystem. An extended snow-free season and deeper depth of thaw under warmer climate may significantly alter current N dynamics in this arctic ecosystem.

Key words: $^{15}NH_4^+$; arctic tundra watershed; total dissolved N; downhill transport of N; hydrolysable amino acids; hydrolysable amino sugars; mosses; N dynamics; N immobilization; N leaching; N limitation; snowmelt

INTRODUCTION

Productivity of arctic ecosystems is strongly nitrogen (N) limited despite very large stocks of organic N in soils (Jonasson et al. 1999, Shaver et al. 2001). Many past studies have indicated that the overall N limitation of productivity in these systems is due to slow N turnover in soils (e.g., Weintraub and Schimel 2005). These studies consistently find that net N mineralization during the growing season is lower than the annual plant N uptake requirement in Alaskan tundra ecosystems (e.g., Nadelhoffer et al. 1991, Marion et al. 1987, Giblin et al. 1991, Schmidt et al. 2002). Nitrogen fixation may account for as much as 76–90 % of total annual N inputs in arctic tundra ecosystems (Hobara et al. 2006), but it is not sufficient to fill this apparent gap between plant requirement and mineralization.

The seasonal downslope movement of dissolved N in relation to the timing of plant uptake has received little attention. The importance of dissolved-N losses is increasingly recognized for many terrestrial ecosystems (Kawahigashi et al. 2004, Rastetter et al. 2005, 2004, Perakis and Hedin 2002, Stepanauskas et al. 2000, Vitousek 1998). Rastetter et al. (2004) simulated downslope N movement in soil water in tundra and found that it influenced the timing and spatial pattern of responses to changes in CO_2 and climate. However, few studies have demonstrated the links among the temporal and spatial variations in soil N cycling (e.g., immobilization, mineralization), hydrology, and plant uptake along a hill slope of an arctic watershed.

Because arctic tundras are generally underlain by continuous permafrost, there is no deep leaching, and soil water must move laterally down hillslopes near the soil surface. The largest hydrological event in these ecosystems is the spring snowmelt when the largest solute losses, including N, occur (Everett et al. 1989). However, because the soil is still frozen, N released

into snowmelt water may bypass uptake by microbes and plants as well as non-biological sorption in soil, resulting in large N losses from the system.

We added an enriched $^{15}NH_4^+$ tracer at several sites along a hillslope at Imnavait Creek to better understand: 1) short- and long-term fate of inorganic N deposited on the tundra surface, and 2) spatial and seasonal movement of N along the hillslope via leaching. We chose NH_4^+ over NO_3^- to minimize rapid ^{15}N losses via denitirification. NH_4 is also a product of N fixation, which accounts for more than two thirds of total annual N inputs. Internal N cycling was examined by following the ^{15}N tracer into plants and various soil-N pools over three growing seasons at the same locations as ^{15}N addition. Nitrogen leaching down the hillslope was examined by following ^{15}N into downslope-flow water at and farther downslope of the point of ^{15}N addition.

We hypothesized that, within the first growing season, nearly all the added N would be immobilized into soils by microbes, as previously observed (Clemmensen et al. 2008, Schimel and Chapin 1996). Because turnover of microbial biomass can stabilize N in soil organic matter (Kramer et al. 2003, Knicker et al. 1997), we anticipated that most of the added N would be incorporated in organic matter and would remain unavailable to plants for several years. Additionally, because microbial cell walls are the single largest source of amino sugars (Kerley and Read 1997, Myrold 1998), we expected a large increase in ^{15}N content of hydrolysable amino sugar.

In the long term, we hypothesized that the major losses of the ^{15}N from the tundra would occur via leaching during snowmelt each year at all sites along the hillslope. We expected little leaching of ^{15}N during the growing season when plant and microbial uptake is high and the soil volume available for chemical sorption is high. One exception would be within

"watertracks" (channels of subsurface water drainage; Chapin et al. 1988) where soil water flows preferentially during the snow-free season. We expected leaching loss of ^{15}N to be significant at these sites during the snowmelt and throughout the growing season.

METHODS

Study area and experimental sites

The study was conducted on the east-facing slope of the Imnavait Creek watershed (2.2 km^2; 68º 37' N, 149º 18' W), a small arctic watershed located ~11 km east of Toolik Lake, on the North Slope of the Brooks Range in Alaska, USA (Figure 1-A). Annual mean temperature is about -7ºC, and the soil is underlain by continuous permafrost in summer and completely frozen in winter (November – April, Hinzman et al. 1996). The maximum depth of thaw is greatest at crest and valley bottom, where it may exceed 1 m. Elsewhere depth of thaw rarely exceeds 0.5 m. Annual mean precipitation is 350 mm, a third of which falls as snow (Hinzman et al. 1996). The watershed has a distinct spring snowmelt period, characterized by saturation of the snowpack followed by flow of a slushy mass of snow (slush-flow) and by discharge of melted snow. Once the snow pack disappears, a major source of stream water is thawing of frozen soil (soilmelt). Nutrient discharge in streams peaks during snowmelt and approaches baseline levels during soilmelt; plant uptake is minimal until the soil thaws. Historically, snowmelt occurs between early May and late June (Hinzman et al. 1996).

The vegetation and soils along the hillslope follow a typical North Slope toposequence (Walker and Walker 1996), including a dry, well-drained heath tundra at the crest, relatively well-drained but mesic tussock tundra interspersed with wet watertracks on the hillslope, and a wet-sedge tundra at the riparian zone (Figure 1-B). Watertracks are visually recognizable areas of greater soil water flux with higher density of shrub species and intermittent surface

water flow. Detailed description of watertracks is found in (Chapin III et al., 1988). On a hillslope ~1 km south (upstream) of a gauging station on Imnavait Creek, six experimental sites were established, each site containing 2 treatment plots. They were: heath tundra on crest (Crest), non-water track and watertrack tussock tundra on midslope (Midslope_NWT, Midslope_WT), non-water track and watertrack tussock tundra on footslope (Footslope_NWT, Footslope_WT), and wet-sedge tundra on riparian zone (Riparian) (Figure 1-B). There were two replicate treatment plots ($n = 2$) on each site (i.e., 2 x 6 = total of twelve plots), and each plot was 3 m x 6 m in area.

Nitrogen fixation, which may account for as much as 76–90 % of total annual N inputs, was significant only near the soil surface (0–3 cm) and fixation rates within this watershed were similar across the toposequence at ~80 mg m^{-2} yr^{-1} (Hobara et al. 2006). The activity of denitrification-enzyme was higher for soil cores collected from the riparian zone than from the crest (Alexander-Ozinskas 2008).

^{15}N addition

On 5 July 2003, we added $(^{15}NH_4)_2SO_4$ dissolved in Imnavait Creek water to each plot at 3.92 mmol/m^2 using backpack sprayers. This amount was < 0.006 % of the annual N-requirement for plant uptake in tussock tundra (Shaver and Chapin III 1991). The total nitrogen concentration in creek water was very low (total dissolved N < 23 μM) so no corrections for the ^{15}N from creek water were made. Each ^{15}N-addition plot served as an experimental unit for ^{15}N recovery in the vegetation, soil, soil-extracts, and soil-pore water. For water flowing downhill, each experimental unit included the area directly below the plot (Figure 1-B, "sampling transects for downslope-flow water").

Vegetation sampling

Prior to ^{15}N addition, green leaves and other plant parts of the dominant species were collected from areas adjacent to the plots. The species collected included deciduous shrubs (*Betula nana*, *Salix* spp.), evergreens (*Vaccinium vitis-idaea*), sedges (*Carex* spp., *Eriophrum vagenatum*), and mosses (*Hylocomium* spp., *Sphagnum* spp.). After the ^{15}N addition, the same tissues were collected from within the plots 1 and 2 yr later. Within 12 hrs of collection, plant samples were separated by species and tissue type and were dried at 50ºC.

Soil sampling and extraction

Five randomly-located soil cores (diameter range: 5-6cm) were collected from each plot immediately before and 1 d, 6 d, 1 mo, and 1 yr after ^{15}N addition (4, 6 & 11 Jul, and 5 Aug 2003; 12 & 14 Jul 2004), and four cores were collected after 2 yr (16 & 20 Jul 2005). At each collection, cores were taken to the maximum possible depth, defined as either the full core length (40 cm), the bottom of the seasonally thawed "active layer", or to a rock (at Crest only). Upon collection, the length of the core was measured and the core was separated in the field into the following layers by cutting with a knife on a clean plastic sheet:

(1) Green Layer, the surface live moss-detritus-plant layer including lichens, graminoid foliage < 2 cm above the ground, and groundcover species (e.g., *Vaccinium vitis-idaea*, *Empetrum nigrum*, forbs).

(2) 1st Organic Layer, the upper 10 cm of organic soil or, if the organic layer was < 10 cm thick, the entire layer (e.g., there was never > 4 cm of organic soil at Crest);

(3) 2nd Organic Layer, organic soil below 10 cm, ranging from 0 cm (Crest) to 17 cm (Riparian) thick; and

(4) Mineral Layer, the soil beneath the Organic Layers ranging from 0 cm (Riparian; organic soil extended beyond the depth of thaw) to 7 cm (Midslope_NWT).

The depth of soil thaw increases through the season, reaching a maximum in late July or early August. Because thaw depth is less variable spatially than the thickness of Oe and Oa horizons and our interest was in sampling water moving along the surface of the frozen soil, dividing soil cores by depth was more appropriate than by taxonomic horizons. Average thickness of each layer and their properties are listed in the Appendix A.

Within 12 hrs of collection, soil samples were weighed, homogenized, and live roots and rocks hand-picked; the rocks were weighed to determine rock mass. Subsamples were dried at 50 ºC to determine moisture content. To assess recovery of ^{15}N in water-extractable N pool over time, the remaining homogenized soils were combined by plot and by layer, further homogenized, and then subsamples (approx. 50–100 g, wet weight) were taken from the 1st and 2nd Organic Layers. Within 5 hrs, subsamples were added to deionized (DI) water and extracted for 8 hr at 20 ºC on a shaker table. In 2003, the ratio of wet soil-to-DI water was 1:10 (w/w) for the 1st Organic Layer, but was 1:5 for 2nd Organic Layer to ensure a sufficiently high N concentration for later analyses. A soil-to-water ratio of 1:10 was used for both layers in 2004 and 2005. The extractants were filtered through ashed GF/F glassfiber filters and frozen.

We examined ^{15}N distribution across chemically-fractionated N pools twice during our study. At 1.5 mo after ^{15}N addition in 2003, three random samples of the 1st Organic Layer were collected, pooled, and processed as described above. We chose this layer because we expected to find a larger fraction of added ^{15}N and greater microbial activity because of higher temperatures and a longer thaw period than deeper soil layers. Within 2 d the wet soils were transferred on ice to the University of Michigan; there the soils were fractionated within 5 d of

collection into salt (0.5 N K_2SO_4)-extractable dissolved N (SEDN), chloroform-fumigation extractable N (CFN), and residual N (N_{res}) using the method of Brookes et al. (1985). We determined ^{15}N in these fractions using alkaline persulfate digestion (Cabrera and Beare 1993) followed by alkaline diffusion (Brooks et al. 1989). Total ^{15}N recovery in the bulk soil was determined in ground subsamples. Because we were not certain whether the extractability coefficient (*K*n) used in other studies would apply to our study site, we report CFN instead of an adjusted microbial N value.

We assume changes in ^{15}N distribution across CFN fractions are minimal based on previous ^{15}N labeling studies. Redistribution of N among soil-N pools following the initial assimilation was little when $^{15}NH_4^+$ was added to temperate forest soils (Perakis and Hedin 2001) or to tundra soils (Schimel and Chapin 1996). Therefore, to obtain more complete picture of N dynamics at Imnavait Creek, we took additional subsamples of the 1st Organic Layer in year 2 and fractionated N by acid hydrolysis rather than chloroform-fumigation. To obtain natural ^{15}N abundance for these fractions, six additional soil cores were collected from outside the plots and processed as described above.

Sampling soil pore water

Seven to eight days prior to ^{15}N addition, ten tension micro-lysimeters were installed at each plot by excavating five holes (diam ~20 cm) and inserting lysimeters on the uphill-side wall of the hole at 10 cm and 20 cm depth, leaving the soil column above the lysimeters intact. The holes were backfilled and marked. Each lysimeter was individually connected to a 500-mL HDPE Erlenmeyer flask, and soil water was collected as described by Lajtha (1999). In a preliminary study, we installed micro-lysimeters at various depths (0-33 cm) and topolocations near the study site and found no installation effect on the concentration of dissolved N after 3, 4,

10, 15, and 23 d (data not shown). Therefore, we purged the micro-lysimeters and allowed them to equilibrate for ≥ 5 d prior to the first sampling. Sample collections were made on the day before ^{15}N addition and 4 d, 1 mo, and 1 yr after addition. Because the void space of a micro-lysimeter was negligible relative to sample volume collected (< 1 %), we did not purge the lysimeters prior to each sampling. However, at the beginning of each growing season, all lysimeters were purged to remove any over-winter water.

Sampling snow and downslope-flow water

Snow – To determine snow N concentration, we collected snow cores 1–3 wk prior to the start of slush-flow; the bottom ~1cm that had contact with Green Layer was removed. The snow was thawed, filtered, refrozen at the field station, and shipped to MBL for chemical analyses. In 2004, 5 snow cores each were collected from 5 evenly-spaced topolocations along the hillslope (total 25 cores) and the samples were analyzed separately. Based on the small variability among individual samples in 2004, one core each was collected from the five topolocations (total 5 cores) in 2006 and all samples were combined for analysis.

Downslope-flow water during the spring melt – In May 2004 and 2006, we collected spring melt water to determine ^{15}N movement. Prior to snowmelt, six 6m sampling transects were established for each labeled plot. The transects ran perpendicular to the hill slope, located 1–5 m above the labeled plots and 1, 2, 5, 10, or 15 m below the labeled plots (Fig 1-B). At Riparian, where negative grades were found toward the creek and downstream, two sets of two transects were established around the plots, one perpendicular to the creek and the other parallel to the creek (Figure 1-B). All transects at Riparian were 1 m from the plots. The two Riparian transects that were elevated (those on the crest and upstream sides) relative to the other two were considered as a reference, and the two below the plot were considered to be “below” the plot.

Water samples were collected using a 60-mL syringe connected by Tygon tubing to a thin, 30-cm long stainless steel tube with small holes drilled within 5 cm from the closed tip. These samples were collected from the bottom of the thawed layer, from 3–5 random locations within the ^{15}N-addition plot and on each transect. To collect within an extremely shallow thawed layer, the sampling needle was inserted horizontally to the surface of the frozen layer. These were pooled by plot or transect in the field to make a similar final volume and processed as for other water samples.

Water collected while snow existed on the plots was called "snowmelt", and water collected after all snow on the plot had disappeared was called "soilmelt". Snowmelt collection became possible only ~2 d prior to slush-flow at the gauging station, and the snow cover disappeared completely from the entire hillslope within one (2006) or two (2004) weeks after the slush-flow. In 2004, we collected snowmelt samples twice, (18 or 19 May and 22 May), and soilmelt samples once (31 May). In 2006, snowmelt samples were collected on 16, 17, and 19 May and soilmelt samples on 25 May. The winter before the 2004 snowmelt was typical, whereas the winter before the 2006 snowmelt was one of the driest winters for this watershed, which has been studied since 1976. Nonetheless, the major slush-flow at the gauging station occurred on 18 May for both years (Rob Giek, *personal communication*).

Downslope-flow water during the growing season – Downslope-flow water during the summer was collected at the bottom of the thawed active layer in the same manner as the collection of downslope-flow water during the spring melt. In 2004, samples were collected 1 d, 1 wk, 1 mo, and 1 yr after ^{15}N addition. Soil moisture was too low to collect water samples after long periods without rain, especially during the growing season and at the Crest site.

Chemical analysis

^{15}N recovery in hydrolysable N pools – We measured the pool size and ^{15}N recovery into three hydrolysable labile-N pools; hydrolysable NH_4^+ (HNH_4^+), amino acids (HAA), and amino sugars (HAS). Soils were thawed and five replicates (2–10 g) of ground samples were hydrolyzed and the hydrolysates were neutralized with NaOH (Mulvaney and Khan 2001). Hydrolysable NH_4^+ was determined by a hypochlorite-alkaline phenol method. To determine ^{15}N recovery in this pool, aliquots (~10 μmol-N) of the hydrolysates were diffused in a 450-mL Mason jar with MgO at 50 °C for 6 hr onto an acid trap according to Mulvaney and Khan (2001), but replacing their "wet" acid trap (a beaker containing 5 mL of 4% H_3BO_3) with a "dry" acid trap (an acidified GF/D filter disc encapsulated in Teflon tape) so that N on the trap could be directly analyzed for ^{15}N by isotope ratio mass spectrometry.

Concentration and ^{15}N recovery for hydrolysable amino sugar and amino acid were determined by a sequential diffusion of the neutralized hydrolysates. For concentration, the hydrolysate was diffused for 8 hrs into a wet acid trap, then re-diffused with a new wet acid trap for 6 hrs after converting amino acids to NH_4^+ by a ninhydrin reaction under an acidic condition (Mulvaney and Khan 2001). Both first (HAS) and second (HAA) acid traps were analyzed for NH_4^+concentration as for hydrolysable ammonium. To determine ^{15}N recovery in the HAS and HAA pools, aliquots of the hydrolysate (~10 μmol-N) were sequentially diffused with dry acid traps in place of wet traps. Because total soil N is a sum of hydrolysable and non-hydrolysable N (i.e, total soil-N = HNH_4^+ + HAS + HAA + hydrolysable-unknown N + non-hydrolysable N), pool sizes and $\delta^{15}N$ signatures of hydrolysable-unknown N and non-hydrolysable N fractions combined, or non-labile N fraction (non-LN), were calculated by differences in $\delta^{15}N$ and mass of known N pools (total soil N, HNH_4^+, HAS, and HAA). We are aware that some of the

proteinaceous-N in soil may not be hydrolyzed by hot 6 N HCl because of a physical protection of N compounds by non-hydrolysable components, such as humic substances (Friedel and Scheller 2002, Zang et al. 2000). We assumed that HAA determined in this study is a fraction of peptidic N in soil that was more susceptible to degradation by extracellular enzymes in the soil than the non-hydrolysable fraction.

^{15}N analysis – Following alkaline-persulfate digestion (Cabrera and Beare 1993), all solution samples were diffused onto dry traps by the method of Sigman et al. (1997) for $\delta^{15}N$ analysis. Dried vegetation and soil samples were ground to pass a 0.15-mm screen for total C, N, and $\delta^{15}N$ analyses. All ground and diffused samples were analyzed by isotope ratio mass spectrometry at the MBL Stable Isotope Laboratory, unless noted otherwise.

DIN & DON – In solution samples, NH_4^+-N was determined by the hypochlorite-alkaline phenol method. A Cd-reduction method was used to determine NO_3^--N concentrations in samples collected in 2003-2005, whereas ion chromatography was used for samples collected in 2006. Nitrite in our samples was below the detection limit (< 0.2 μM). Total dissolved N (TDN) was determined by a persulfate digestion (modified Solozano and Sharp 1980) followed by NO_3^- analysis. For 2005 samples, a high-temperature combustion method was used. Cross comparison of selected samples showed that the between-methods difference in measured concentration was < 5%. Dissolved organic N (DON) was calculated as the difference between TDN and DIN.

Data analysis

The physico-chemical properties of each layer were determined separately, and the mean for each layer was calculated by plot. For 1- and 2-yr Green Layer samples, we estimated the partitioning of ^{15}N between live mosses and non-moss compartments. First, using the $\delta^{15}N$

values of mosses within the enriched plots and the moss biomass estimates by Hahn et al. (1996) and Hastings et al. (1989) we estimated the total recovery of ^{15}N in mosses. The non-moss component, mainly the detritus trapped in the mosses, was then calculated as a difference between total recovery in Green Layer and recovery in mosses.

The recovery of added ^{15}N in plants, Green Layer, and soil, was determined as percent ^{15}N-gain relative to background ^{15}N. The enrichment of ^{15}N in the dissolved-N pool was expressed as ^{15}N atom % excess, which is the excess percentage of ^{15}N atom % relative to the reference level. A minimum detectable difference for ^{15}N analysis of dissolved-N was 5 ‰ in $\delta^{15}N$ or 0.0018 % in ^{15}N atom % excess.

To evaluate rates of changes in mobilized ^{15}N (i.e., dissolved ^{15}N) along the hillslope, we estimated for downslope-flow waters the distance required to reduce ^{15}N-atom % excess in the water by 50 % and 95 % of the level in downslope-flow water within the plots. To do so, we used linear regression to estimate the relationship between the natural-log of ^{15}N-atom % excess (Ln-APE) and distance from the plots for each site, then used the regression equation to calculate the distance for 50 % and 95 % reduction.

To estimate ^{15}N transport along the hillslope during snowmelt in 2004, we used our ^{15}N-atom % excess data and snowpack-water equivalents (Table 1), which were calculated from data reported for the years 1985–1987 and 1989–1990 by Hinzman et al. (1996), assuming that the snowpack water equivalents during our study were similar to 1985–1990 average. We assumed that each site received all melt water in the snowpack directly above it because the frozen ground would prevent penetration of water and because evapotranspiration would be negligible. We did not include data from 2006, because the 2006 winter was unusually dry with an unusually thin snowpack.

Statistical analysis was performed using SYSTAT 11.0 (2004). We tested the effect of sites on N concentrations, ^{15}N recovery, and ^{15}N-atom % excess at each time point or for specific time period using analysis of variance (ANOVA), followed by multiple comparisons using a least significant difference (LSD) test and a significance level of $P < 0.05$. When necessary, data were natural-log or square-root transformed prior to statistical analysis to obtain similar distribution and variance across sites.

Our attempt to accomplish a detailed characterization of temporal as well as spatial N dynamics along the complete hillslope required some compromises on sample replication and on the suite of analyses conducted simultaneously. Low replication ($n = 2$) constrained our choice of statistical analysis to simple analyses such as one-way ANOVA, t-test, or regression analysis for samples collected on the same day, and no statistical significance could be reported for ^{15}N level across different points in time. Nonetheless, the synthesis of the information across time and space does provide insights into N dynamics within a N-limited watershed.

RESULTS

Short-term dynamics

^{15}N Recovery in the soil and Green layer – More than 99% of the added ^{15}N was recovered in the soil and Green Layers immediately (1 d) after addition, and recovery remained high through the first growing season (Figure 2). The Green Layer retained > 63% of all recovered ^{15}N in the ground at both 1 d and 1 mo. The 1st Organic Layer was the second largest sink for ^{15}N at these dates, and this and Green Layers together retained > 86% of ^{15}N added.

Chloroform-fumigation and salt-extractable N vs. residual N – Only 1.5 mo after addition, most (89–97 %) of the ^{15}N in the 1st Organic Layer was in the non-extractable pool (N_{res}) at all sites, whereas ≤ 3 % of recovered ^{15}N in this soil layer was found in salt (K_2SO_4)-extractable

dissolved N (SEDN) and ≤ 9 % in chloroform-fumigation-extractable N (CFN) (Table 2). The SEDN and CFN pools together accounted for < 4 % of total added ^{15}N in all sites.

N in soil pore water and WEDN – During the growing season, DON was the dominant form of N (≥ 90% of TDN) in both lysimeter water and water-extractable dissolved N (WEDN; Table 3-A). The ^{15}N-atom % excess of pore water at 10 and 20 cm (approximately at the bottom of 1st and 2nd Organic Layers, respectively) showed a sharp drop (9-fold on average) within the first month (Figure 3).

^{15}N-atom % excess of the WEDN pool was 2–7 times greater in the 1st Organic Layer than in the 2nd Organic Layer (Figure 4), as observed for the bulk soil (Figure 2). Measured ^{15}N-atom % excess of the WEDN pool within the 1st Organic Layer was significantly greater at Crest than all other sites immediately after the addition (4 d), corresponding with the highest ^{15}N recovery for this soil layer at Crest (Figure 2). Contrary to the decreasing trend within the first month of ^{15}N addition observed for the pore water collected by lysimeters at 10 cm, mean ^{15}N-atom % excess of the WEDN pool in the 1st Organic Layer increased by up to 5-fold on average during the same time period for all but Crest sites. The highest increase in atom % excess of WEDN was observed at NWT and Riparian sites.

Long-tem dynamics

^{15}N Recovery in the soil and Green layer – The strong ^{15}N retention in the ground layers during the first growing season persisted even after 2 yrs, with ≥ 92 % recovery of added ^{15}N at most sites (Figure 2). Exceptions were Crest and Midslope watertrack (Midslope_WT) sites, where total recovery tended to decrease over time with 89 % (Crest) and 69 % (Midslope_WT) of the added ^{15}N recovered at the end of the 2nd year. For Crest the decline was due mostly to decreased recovery in the soil (Organic and Mineral) layers, whereas for Midslope_WT recovery

declined in both Green Layer and soil layers. Extremely high total recovery (as high as >180 %, much more than was added) was found only at footslope non-watertrack (Footslope_NWT) and Riparian sites and was due to very high ^{15}N recovery in one sample of the 1st Organic Layer (2–3 times greater than the average of all sites, Figure 2).

Throughout the first 2 yrs, the Green Layer was the largest sink for added ^{15}N; at both year 1 and 2 this layer still retained ~60 % or more of added ^{15}N. Combined with the second largest ^{15}N-sink (1st Organic Layer), the upper two ground layers retained 66 % of added ^{15}N at Midslope_WT site, 84 % at Crest and ≥ 92 % at all other sites at the end of second year. The Mineral Layer contributed little to ^{15}N retention, except at the Crest, where the mineral soil (average thickness above rocks = 4 cm) accounted for up to half of the overall recovery in the soil (Figure 2).

Hydrolysable and non-hydrolysable N pools – Acid hydrolysis of the 1st Organic Layer after 2 yrs of ^{15}N addition revealed that hydrolysable labile-N (HLN) pools (hydrolysable NH_4^+, amino sugar, and amino acids) were an important sink for ^{15}N and were 15–39 % of ^{15}N retained in the 1st Organic Layer (Table 4). The non-labile N pool, which is chemically less labile than HLN, contained the rest of the ^{15}N (61–85 % of total ^{15}N). Within the HLN pool, hydrolysable amino acid (HAA) was the dominant sink for added ^{15}N, containing on average 4 (non-Crest sites) to 8 times (Crest) more ^{15}N than hydrolysable ammonium (HNH_4^+) and amino sugar (HAS) pools combined.

Of the ^{15}N recovered in the 1st Organic Layer, the contribution of various N pools differed across sites. The HLN pool contributed most to ^{15}N recovery at the Crest (39 %), whereas the non-labile N pool was more important at the two NWT sites (85 %, Table 4). At the

wetter sites (i.e., two WT sites and Riparian), the contribution of HLN was intermediate (23–28 %).

N in soil pore water and WEDN – The ^{15}N-atom % excess of soil pore water collected at 10 and 20 cm by lysimeters after 1 yr was low, similar to the amount at 1 mo, except at the Crest site (Figure 3). For the entire study period, ^{15}N-atom % excess of the WEDN in the 1st Organic Layer generally was higher than the level observed immediately after (6 d) ^{15}N addition (Figure 4). In contrast, in the 2nd Organic Layer, ^{15}N-atom % excess of the WEDN disappeared entirely after the first year except in NWT sites. During the first 2 years, ^{15}N recovered in the WEDN pool in the soil was only < 1 % of added ^{15}N.

Recovery in the vegetation – Combining the ^{15}N results in this study with the biomass data from the same sites by Hahn et al. (1996) and Hastings et al. (1989), we found that ^{15}N recovery by mosses was much greater than by vascular plants at all but the Riparian site, and this trend was magnified in year 2 for the tussock tundra sites (Midslope and Footslope, Table 5). At 1 year, mosses contained 20–50 % of added ^{15}N at all sites but the Riparian, whereas the entire aboveground biomass of the vascular plants contained only 6 % at Riparian and 1–3 % at all other sites. Retention by the vascular plants declined over time and by year 2, vascular plants accounted for ≤ 1 % of added ^{15}N at all sites. In contrast, ^{15}N recovery in the mosses increased from year 1 to year 2 at the tussock tundra sites (Midslope and Footslope), becoming the predominant ^{15}N sink (Table 5, Figure 2). If we make the unlikely assumption that 10 % of the entire aboveground vascular biomass was found in the Green Layer, vascular plants could explain a maximum of ~1 % of the added ^{15}N.

For both 1- and 2-yr samples, the partitioning of ^{15}N between live mosses and non-moss components within the Green Layer differed across the sites (Figure 2). The mosses were one of

the major long-term sinks for added $^{15}NH_4^+$ especially at the tussock tundra sites (Midslope and Footslope). In contrast, the non-moss component, mostly plant detritus, was the dominant ^{15}N sink at the Crest and Riparian tundra sites, accounting for 72–74% (Crest) and nearly 100 % (Riparian) recovery within the Green layer.

Chemistry and movement of dissolved N in downslope-flow waters

DIN vs. DON – Downslope-flow waters were dominated by DON across all seasons. Although NO_3^- was the dominant form (66 %) of TDN in the snow pack, unlike the soil waters collected during the growing season (WEDN and lysimeter waters) (Table 3-A, B), this dominance quickly disappeared and NO^{3-} became < 1 % of TDN as soon as the melt water from the snow pack made contact with the frozen ground and was collected as snowmelt water. The concentration of NO_3^- stayed low for all water samples collected during the rest of the year. In contrast, DON, which was a minor component of TDN in the snow pack (17 %), increased rapidly as the melt event progressed, comprising 90–96 % of TDN in downslope-flow water during snowmelt and 96–99 % during soilmelt. DON remained a predominant fraction throughout the growing season (95–96 % of TDN, Table 3-B).

Mobility and transport of ^{15}N-TDN – The ^{15}N enrichment in downslope-flow water within the ^{15}N-treated plots was measured as ^{15}N-atom % excess of TDN. For the snowmelt period, the enrichment differed by site for both 2004 and 2006, and was roughly 2–3-fold greater at Crest than at other sites (Figure 5), whereas the enrichment was more similar among sites during the soilmelt.

Through the 2004 season, ^{15}N-atom % excess of downslope-flow waters within the plots was generally larger during snowmelt than during soilmelt or the growing season (Figure 6). Even though ^{15}N-atom % excess of downslope-flow water declined with increasing distance

from the plots, at Midslope sites the atom % excess below the treatment plots was still elevated ($P < 0.05$) relative to the reference location above the plots at all sampling dates. In contrast, the atom % excess of water below the treatment plots was not elevated significantly relative to the reference location at Footslope and Riparian sites ($P > 0.05$).

At least some dissolved ^{15}N leached downslope in all seasons at Midslope sites. The negative linear relationship between the natural-log of ^{15}N-atom % excess (Ln-APE) and the distance from the plots for several sites indicates that enrichment of ^{15}N in downslope-flow water declined exponentially as the water moved downhill (Figure 7). At Midslope sites the linear relationship held though the entire spring melt (snowmelt and soilmelt) of both 2004 and 2006 ($R^2 = 0.57$ to 0.93), whereas at Crest, a tight linear relationship was found only during the snowmelt of normal snow year (2004, $R^2 = 0.95$). For the Riparian site, the decline of ^{15}N-atom % excess with distance was greatest; thus at this site only 1 m was required to reduce ^{15}N in the water by the same proportion as at 5–10 m below the other sites.

The loss of ^{15}N atom % excess in the downslope-flow water varied both temporally and spatially. Regardless of the initial enrichment, the distance required to reduce ^{15}N-atom % excess by 95 % ($D_{0.95}$) at Midslope was greatest during the soilmelt compared to other seasons (Table 6). During the soilmelt of 2004, the $D_{0.95}$ at Midslope was 29–51 m, approximately 2–4 times greater than during the snowmelt and up to 4 times farther than during the growing season (Table 6). Although smaller in magnitude, the $D_{0.95}$ at Midslope for the soilmelt of 2006 was also greater than for snowmelt (1.1–1.2 times). The $D_{0.95}$ at more steeply sloping sites (Crest and Midslope) was on average twice as great as on less steep sites (Footslope and Riparian), with Riparian sites having the shortest $D_{0.95}$; 1 m (2004) and 3 m (2006).

We estimated the total amount of ^{15}N mobilized from soil during the snowmelt of 2004, a year of near-normal snowpack, using ^{15}N-atom % excess of TDN and the estimated volume of downslope-flow water during snowmelt (Table 1). When the volume of the downslope-flow water was taken into account, the largest total ^{15}N release from the plots into the water occurred at the Riparian site (21 % of initial ^{15}N added), which had roughly 600-times more surface water flow (based on upslope drainage area) than the Crest (Figure 8). In contrast, although the ^{15}N enrichment at the Crest was the highest of all sites (Figure 5), the total ^{15}N release during the snowmelt accounted for the smallest proportion of ^{15}N among all sites (0.04 % of the added ^{15}N; Figure 8). As observed for ^{15}N-atom % excess, nearly all (96 %) of the ^{15}N released into the snowmelt water at the Riparian site disappeared within 1 m of the plots.

DISCUSSION

At Imnavait Creek, $^{15}NH_4^+$-N deposited on the tundra surface followed two alternative pathways during the first growing season: it either remained relatively labile in the Green Layer (> 70 % of added ^{15}N) or it became stabilized into a recalcitrant soil-N pool below the Green Layer (< 30 %), presumably via turnover of microbial biomass (Figure 9). The strong N-sink within the Green Layer suggests that this thin surface layer plays a key role in N cycling in this tundra ecosystem.

Added ^{15}N was tightly retained, with very little loss over 3 years from all but Crest and Midslope_WT sites, where ^{15}N was likely lost in vertical or horizontal leaching (Figure 9). The strong and persistent retention of ^{15}N within Green Layer and relatively recalcitrant soil-N pool contributes significantly to the chronic N limitation in this ecosystem.

A conceptual model of the downhill movement of ^{15}N along the Imnavait Creek hillslope (Figure 10) suggests that hydrology played the single most important role in mobilizing the

retained ^{15}N, resulting in a greater ^{15}N release into downslope-flow water at WT and Riparian sites than at NWT, and during snowmelt than other times of the year. Losses of ^{15}N were driven by a combination of total^{15}N released in gravitational-flow water, the N-immobilization capacity of the system, and the flow rate of the water. Thus we hypothesize that ^{15}N retained in the ground is most vulnerable to losses at Midslope_WT during soilmelt, mainly because of asynchrony between the timing of the release and biological and non-biological immobilization. In contrast, N losses at Crest occur mainly by vertical leaching to deep mineral soil. Together, these leaching losses further intensify N limitation this ecosystem.

Short-term dynamics

Over 70 % of all the added $^{15}NH_4^+$-N was immediately and tightly retained within the Green Layer across all sites (Figure 9). The high average recovery during the first growing season at Midslope and Footslope sites (73–117 %) was largely due to the mosses' ability to retain incoming $^{15}NH_4^+$ at high efficiency, and because mosses dominated total biomass at these sites (58–79 % of total biomass; Hahn et al. 1996). In contrast the non-moss component, mainly plant detritus rather than live vascular plants, was the major sink for ^{15}N at Crest and Riparian sites, where moss accounted for only 1–17 % of total biomass (Hahn et al. 1996). Uptake by vascular plants within the Green Layer was unimportant at all sites, because even with our highest estimate of ^{15}N recovery by vascular plants in the Green Layer we could explain only ~1 % of ^{15}N recovery.

Fast and strong retention of NH_4^+ has been reported for both mosses and plant detritus. Many moss species can take up water and nutrients over their entire surface (Turetsky 2003), and thus efficiently scavenge both NH_4^+ and NO_3^- at low concentrations (Press and Lee 1982 cited in Turesky 2003). Additionally, some *Sphagnum* species possess cation-exchange capacity on their

cell walls (Clymo 1963), capturing atmospherically deposited NH_4^+ along with other cations. By monitoring $^{15}NH_4^+$ added to the surface of *Hylocomium* species, Eckstein (2000) found that the mosses had high nutrient recycling ability and that most added ^{15}N was allocated to new growth in the following year rather than being released to the soil. Our results are consistent with other studies: Kotanen (2002) found that mosses assimilated N more efficiently than higher plants, and Li and Vitt (1997) found that nearly all ^{15}N added as atmospheric deposition was retained in the moss layer in boreal peatlands.

Decomposing plant detritus can be also a significant sink for added NH_4^+ in northern temperate forests (Currie et al. 1999, Nadelhoffer et al. 1999) and in laboratory sorption experiments with peat, fulvic- and humic-acids (Thorn and Mikita 1992). These studies point to non-biological fixation (e.g., with quinones) as one of the major mechanisms that retains added NH_4^+. Similarly, by tracing $^{15}NH_4^+$ injected into tussock and soil cores near our study site, Schimel and Chapin (1996) found that the detritus was the dominant sink for ^{15}N (2.7-times greater than live plants). They found that live graminoids (*Carex*) were the major sink for ^{15}N in cores from a wet-meadow near Imnavait Creek, recovering 21-times more ^{15}N than plant detritus, but this may have been because ^{15}N was injected into the rooting zone, where it would be immediately available for uptake, rather than applied to the surface where it would most likely be immobilized into litter and mosses.

Upon entering the 1st Organic Layer, ^{15}N was quickly converted into recalcitrant forms of N within the first growing season (N_{res} > 89% of ^{15}N recovered in the 1st Organic Layer). The small ^{15}N recovery in the CFN pool relative to N_{res} pool may be an indication of rapid turnover of microbial biomass (Table 2). Alternatively, non-biological immobilization of ^{15}N into recalcitrant soil organic matter, by such as NH_4^+ fixation with quinones, may explain the greater

recovery in N_{res}. Transfer of undecomposed necromass of ^{15}N-enriched mosses to the 1st Organic Layer would not likely explain the high recovery of ^{15}N in the N_{res} pool (Table 2), because the life-span of *Sphagnum* and *Hylocomium* shoots is at least 2–3 yr (Aerts et al. 1999, Eckstein 2000) and because if the necromass of mosses from the first growing season accounts for 22–31 % of added ^{15}N in the N_{res} pool at Midslope and Footslope sites (the N_{res} pool explained 89–97 % of recovered ^{15}N within the 1st Organic Layer, Table 2), we would see a large decline of ^{15}N recovery in the Green Layer by the end of the third growing season.

Other short-term studies in tussock and wet-sedge tundras at nearby Toolik Lake have reported much higher recovery of ^{15}N in the chloroform fumigation-extractable N (CFN) pool than we found in this study (< 4 % of added ^{15}N). For example, Nordin et al. (2004) found that 20–28 % of ^{15}N injected to the tussock tundra (0–10 cm below the moss layer) in July was recovered in the CFN pool after 4 hrs, and Schimel and Chapin (1996), who also injected the label beneath the surface, found similar recovery for both tussock (39 %) and wet-meadow (22 %) tundras after 5 d incubation in August. (Note: to estimate CFN in these previous studies, the reported microbial-N values were back-corrected for the extractability coefficient, *K*n; 0.45 for Nordin et al. and 0.54 for Schimel and Chapin).

The much higher recovery in CFN in these previous studies can be attributed principally to the injection method, which bypasses the Green Layer that was the principal location of NH_4^+ retention in our study. The short duration between labeling and sampling (4 hrs and 5 d) in the previous studies compared to ours (1.5 mo) might also have resulted in greater recovery of ^{15}N in microbial biomass. However, Schimel and Chapin (1996) found that N partitioning into different N pools within the first 5 d and 1 mo was similar. A comparison of our study with these previous studies also suggests that the dominant sink for NH_4^+ produced in the organic soil

(e.g., by mineralization) is microbial biomass, whereas NH_4^+ deposited on the surface is initially retained in the Green Layer.

The relatively high ^{15}N-atom % excess values observed for the soil pore water 4 d after ^{15}N addition indicate that a small fraction of ^{15}N applied to the tundra surface penetrated quickly to as deep as 20 cm, presumably facilitated by the rain event the night after ^{15}N addition. The sharp declines in ^{15}N-atom % excess between 4-d and 1-mo were likely caused by turnover of the dissolved-N pool via uptake by microbes or plants, and by non-biological stabilization of dissolved N followed by replenishment of the dissolved-N pool with natural-abundance level N. In this ecosystem, microbes and plants quickly take up nearly all N in labile forms. The dissolved-N pool would be subsequently replenished via slow dissimilation of soil organic N (Shaver et al. 1992), but dissolved-N produced in this way would be less enriched with ^{15}N because of discrimination against ^{15}N during dissimilation and export (Dijkstra et al. 2008). This would lead to preferential stabilization of ^{15}N into microbial biomass and the recalcitrant soil-N pool. For example, for wide range of soil types, vegetation, and climate, Dijkstra et al. (2006) found that the microbial-N pool was 3.7 ‰ larger than the extractable-N pool and that microbial ^{15}N enrichment and net N mineralization rate were positively correlated. Similarly, in Swedish Lapland Clemmensen et al. (2008) found that most ^{15}N added as NH_4^+, NO_3^-, or glycine was retained in microbial biomass after 2 d but half of the immobilized ^{15}N was transferred to a non-extractable-N pool within 26 days and that ^{15}N exported as dissolved-N was < 1 % during this time. In our study we found the opposite short-term (≤ 1 mo) trends in ^{15}N-atom % excess, i.e., decreasing soil-pore-N and increasing WEDN over time (Figures 3 and 4). This suggests that part of the ^{15}N initially dissolved in soil-pore water was removed over time and became more stable WEDN, perhaps via assimilation to microbial biomass or abiotic sorption.

Long-term dynamics

The loss of ^{15}N over 2 yrs at Crest and Midslope_WT sites may be attributed to leaching (Figure 9). At the Crest, the highly permeable mineral soil (rock content = 52 % (w/w), Appendix A) and deep thaw beneath the thin (~4 cm) organic mat facilitated a vertical leaching of ^{15}N at significant rain events. Thaw depth in the heath tundra of the Crest in this study area exceeds 1 m (Everett et al. 1989). Thus, it is possible that ^{15}N not recovered in our samples was retained in the mineral soil deeper than our sampling-depth limit (8cm because of obstruction by rocks). Gravitational downslope-flow water movement would also explain the decline of ^{15}N recovery during our study period for Midslope_WT (Figure 9). Although there was no difference in the depth of thaw or any clear gradient between NWT and WT (Yano et al., *unpublished data*), we frequently observed a flow of water through and under the Green Layer in gaps between tussocks within the Midslope_WT, whereas no such flow was visible at Midslope_NWT. Thus, at Midslope_WT, greater flux of downslope-flow water within the Green and thawed Organic Layers during the snow-free season may have resulted in the loss of added ^{15}N from the plots. At the Footslope_WT and Riparian, both horizontal and vertical movement of water is slower because of the smaller gradient of the slope and the shallower water table, leading to the small change over time in ^{15}N recovery at Footslope and Riparian sites. Although some N could be lost via denitrification in the Riparian site, as suggested by the higher denitrification-enzyme activity in Riparian than Crest soils (Alexander-Ozinskas 2008), little overall losses of ^{15}N at the Riparian site suggest that neither denitrification nor leaching losses are large there.

Nitrogen fixation could reduce the enrichment of ^{15}N in the ground-N pool by dilution. However, this is not likely the reason for the decline of ^{15}N at Crest and Midslope_WT sites,

because the rate of N fixation near these sites (~0.08 g m^{-2} yr^{-1}; Hobara et al. 2006) was too low to account for the observed changes in the ^{15}N atom % of the ground-N pool (165-860 g/m^2). Furthermore, if dilution by N fixation were important, it should be observed at other sites, given the similar N-fixation rate down the entire hillslope (Hobara et al. 2006).

The few measurements of extremely high ^{15}N recovery at Footslope_NWT and Riparian (i.e., recovery of more than 100 % of the amount added; Figure 2) were probably due to uneven distribution of ^{15}N within the 1st Organic Layer as a result of the application method. This unevenness may have been amplified by disruption of the connectivity of soil water as the tundra progressively dried out in mid-late growing season. In 2003, the study area continued to dry through the early and mid-season until a rain event on 10 August (Shaver et al., *unpublished data*). The summer of 2005 was one of the driest years in the 30-year history of research at Imnavait Creek, and by the July sampling date all surface water had disappeared, except for a few patches at Riparian. Because of the slight topographic gradient and lack of a watertrack at Footslope_NWT and Riparian sites, water in the soil would be expected to move little during dry periods, creating hot spots for ^{15}N in the soil. The exceptionally high recoveries in the 1st Organic Layer at these sites were generally driven by only one of the 4–5 cores taken per plot, and removing these outliers from the analysis gave us more reasonable recovery (dotted lines in Figure 2).

Nearly all ^{15}N that remained in the plots between years 1 and 2 was found in the ground (Green Layer and soil). As observed for short-term dynamics, the relatively recalcitrant soil-N pool (i.e., non-labile N pool, or hydrolysable-unknown N and non-hydrolysable N combined) accounted for > 60 % of ^{15}N recovered in the 1st Organic Layer after 2 yrs (Figure 9, Table 4). This combined with the very small recovery in the vascular plants (< 6% of added ^{15}N in year 1

and < 1 % in year 2, Table 5) indicates that very little of the added ^{15}N had become available for uptake by vascular plants during the study period. The strong ^{15}N retention by the Green Layer, which is above the rooting zone, and the incorporation of new NH_4^+-N into the relatively recalcitrant soil-N pool in the rooting zone may play a significant role limiting N availability to vascular plants in this ecosystem.

The greater contribution of non-labile N and HAA pools to ^{15}N recovery relative to hydrolysable NH_4^+ pool (Table 4) indicates turnover of microbial biomass and/or non-biological stabilization as processes that incorporate ^{15}N into recalcitrant soil-N. In a laboratory study, Knicker et al. (1997) found that NH_4^+ added to organic residues was quickly assimilated into microbial biomass as peptides and amides and that these compounds were the major forms of recalcitrant soil-N. Because proteinaceous compounds are generally chemically reactive, they react with soil organic matter to form recalcitrant N. In contrast, a number of studies have suggested that non-biological condensation of phenolic or quinone structures with NH_4^+, amino acids, and proteins is one of the major mechanisms that form recalcitrant N in soils (studies summarized in Knicker, 2004). Contrary to our expectations, the recovery of added ^{15}N in the HAS pool was low (Table 4). This may indicate that in our study non-biological immobilization is more important than turnover of microbial biomass. Alternatively, the low recovery in the HAS pool may be a result of relatively fast turnover of this pool. One recent study found a decline in HAS upon changes in land-use (Zhang et al. 1999); in this study the cultivation of native grassland for > 80 yrs reduced HAS concentration by 6 %, suggesting faster turnover of HAS pool relative to other N-compounds in the soil.

Hydrology and Movement of dissolved N

A conceptual model of the movement of ^{15}N down the hillslope (Figure 10) indicates that flux and flow path of water are a critical element that determines the magnitude of total ^{15}N released from the Green Layer and soil in this tundra ecosystem. For example, when we estimated ^{15}N released into downslope-flow water during the 2004 snowmelt (Figure 10), the high ^{15}N enrichment of dissolved-N pools at the Crest site was offset by the low water flux, whereas the smaller ^{15}N enrichment at the Riparian site was accompanied by high water flux, resulting in an estimated total ^{15}N-release into the snowmelt water of as much as ~20 % of added (Figure 8). Similarly, total ^{15}N release into downslope-flow water was greater during the snowmelt than other seasons at all sites, it was greater at Riparian than other sites at all times, and it was greater at WT than NWT sites (Figure 10). The high total ^{15}N release during snowmelt occurs because: 1) the snowmelt water exclusively flows through the thawed Green Layer where most ^{15}N is retained, because most soil underneath it is still frozen at this time; 2) snowmelt water is highly concentrated with ions relative to rain water (up to three times higher concentration, Everett et al. 1996), thus exchanging $^{15}NH_4^+$ that was retained on the cation-exchange sites of mosses in the Green Layer more efficiently than rain water; 3) any soluble-N compounds that have accumulated in Green Layer over winter are subject to leaching at snowmelt; and 4) biological ^{15}N immobilization and non-biological sorption in the organic soil would be negligible because most of the organic soil and rooting zone is still frozen, although some microbial uptake within the thawed layer would be expected during the melt season (Brooks et al. 1998). The difference in the pattern of ^{15}N enrichment of downslope-flow water between 2004 and 2006 may be attributed to the unusually thin snowpack, and thus less meltwater volume, in 2006.

^{15}N enrichment in dowslope-flow water declined with distance from the plots (Figure 10). This decline is most likely caused by the addition of N from other sources, lowering ^{15}N-to-^{14}N ratio of dissolved-N pool. Potential N inputs include N-fixation, dissimilation, and N from the snow pack and melt water itself. However, because virtually no added ^{15}N was lost from the Riparian and the two NWT sites throughout the study period even though as much as 21 % of added ^{15}N was released into downslope-flow water (Figure 8), subsequent immobilization of released ^{15}N must also be very high. Loss of ^{15}N to deep soil via vertical leaching is unlikely at all but Crest sites due to shallow thaw depth especially during snowmelt or soilmelt.

We suspect that rapid biological and/or non-biological immobilization combined with dilution of the label by the replenishment of the dissolved-N pool from dissimilation is most responsible for the decline of ^{15}N enrichment in the downslope-flow water. As discussed above ("*Short-term dynamics*"), immobilization would remove both ^{14}N and ^{15}N proportionally, whereas dissimilation/export process would preferentially release ^{14}N. During snowmelt, dilution by incoming N at natural-abundance levels may have also contributed to the reduced ^{15}N enrichment of the dowslope-flow water.

Nitrogen fixation is unlikely to be very important in the decline of ^{15}N enrichment in downslope-flow water, for the reasons discussed above ("*Long-term dynamics*"). Furthermore, the laboratory experiments of Hobara et al. (2006) suggest that fixation would be higher later in the year, during soilmelt not snowmelt. If N fixation is important to ^{15}N enrichment and the rates are higher during soilment, this would be inconsistent with the longer 95 % depletion distance ($D_{0.95}$) during soilmelt than snowmelt (Table 6).

Based on the total ^{15}N recovered within the plot, the spatial and temporal variations of $D_{0.95}$ and of total ^{15}N released into downslope-flow water, and differences in flow rates among

topolocations and dates, the primary factors that determine leaching losses of added ^{15}N are: the magnitude of total ^{15}N released into downslope-flow water, N-immobilization capacity of the site, and flow rate of downslope-flow water. During snowmelt, mosses and detritus in the Green Layer may serve as both source and sink for dissolved ^{15}N, because at that time downslope-flow water flows through the Green Layer over frozen soil. Little loss of ^{15}N from Riparian and two NWT sites despite high ^{15}N release into downslope-flow water (Figure 8) are possible because of the strong immobilization capacity of the Green Layer. In contrast, the long $D_{0.95}$ (Figure 7 and 10) observed during soilmelt suggests the lack of strong N sinks or slower dissimilation/export processes during this season than other seasons, or both. Downslope-flow water during soilmelt lacks full contact with the Green Layer, because the water flows below it within the upper soil, yet the depth of thaw is still shallow. Furthermore, the soilmelt period may correspond to transition from microbial-based N retention to plant-based retention (by root uptake). Brooks et al. (1998) found that in the alpine soils of Colorado, USA, the microbial biomass-N pool peaked during the early stages of snowmelt, and declined rapidly during the later melt season. Similarly, by year-round monitoring of alpine tundra soils for changes in soil-N pools as well as microbial biomass and activity, Lipson and Monson (1998) concluded that N was mostly immobilized in microbial biomass in early spring, whereas the plants were a stronger sink for N during the growing season. These asynchronies between the timing of N released into dissolved-N pool and the ability of the plants to take up N may contribute to the N limitation of plant production (Shaver et al. 1992). By characterizing DON of northern European boreal streams Stepanauskas et al. (2000) found that up to 55 % of DON leached from the terrestrial system during the spring flood was labile DON (urea and hydrolysable amino acids), which can be taken up directly by some plants (Neff et al. 2003), whereas only < 28 % was labile at post-spring baseflow. These

studies combined with our results suggest that terrestrial N in arctic and boreal regions may be susceptible to loss via leaching during spring melt.

Slow flow rate allows more time for ^{15}N in the water to be immobilized, leading to little loss of added ^{15}N. The small $D_{0.95}$ for Riparian can be explained partly by a slower downslope-flow water because of the smaller topographic gradient at this site. Newbold et al. (1982) showed in their conceptual model that flow rate and flux of stream water was one of the factors that determine nutrient spiral length in stream ecosystems. In a simulation study, Rastetter et al. (2004) found that N movement on hillslopes was very slow (over 98 % of added N taken up in 2–5 m), their estimated rate being equivalent to $D_{0.95}$ < 2–5 m. However, their simulations included only DIN, which was taken up rapidly by microbes and plants and was a minor component of total dissolved N in downslope-flow water (Table 3). Our longer transport distances ($D_{0.95}$) are probably due to inclusion of DON in our sampling. Although Rastetter et al. (2004) used annual averages and did not account for the large transport during snow and soilmelt periods, they did find that transport distances increased with the rate of water flowing downslope in agreement with our findings.

Conclusion & Implications

Most $^{15}NH_4^+$ added to the surface of tundra was retained tightly in the Green Layer at all sites. High ^{15}N retention was due to the high immobilization in mosses and to direct immobilization into plant detritus. Once it reached the soil beneath the Green layer, ^{15}N was incorporated quickly into less labile pools that are unavailable for uptake by vascular plants. The Green Layer serves as the point of entry for most N inputs to this hillslope, as it efficiently captures atmospherically-deposited NH_4^+ and it is the primary location of N fixation (0-3 cm, Hobara et al. 2006). However, N cycling within the Green Layer is distinct from that of the soil

beneath it, differing in both the forms and timing of N turnover, uptake, and immobilization. The N cycles in Green Layer and in the soil beneath it are connected by vertical transport of dissolved N in infiltrating water only upon significant rain events during growing season. However, limited precipitation (~20 cm) during the growing season likely constrains the connection between the two N cycles and may contribute further to the N limitation in this system. The N-release from the Green Layer (and from the system as a whole) occurs during snowmelt when the most soil is still frozen and thus uptake by vascular plants is negligible. This asynchrony may lead to losses of dissolved N during late spring melt (soilmelt), contributing significantly to persistent N limitation in this arctic tundra ecosystem.

The N cycle in this arctic landscape may be greatly changed by the climatic warming that has occurred in the Alaskan Arctic over the last several decades (Serreze et al., 2000), and is expected to continue. In a warmer climate, dissimilation of recalcitrant N in the soil would be faster and more complete (i.e., the end products would be DIN and smaller organic compounds), resulting in higher N availability in the soil. Warmer climate has also extended the length of snow-free season in Alaska (Stone et al. 2002). This extended snow-free season and resulting deeper depth of thaw would allow more frequent vertical infiltration of water (because more precipitation events would be rain rather than snow), and may result in a greater flux of relatively labile N from the Green Layer to soil. The increased abundance of deciduous shrub species in non-shrub tundras in recent decades (Sturm et al. 2001) may be one of the best indications of increased N availability in the soil as shrubs respond strongly to fertilizer addition (Chapin et al. 1995). A longer snow-free season and higher summer temperature would also create more favorable conditions for wildfires, heretofore rare events North of the Brooks Range (Racine and Jandt, *unpublished manuscript*). Fire may remove the functionally unique Green Layer and

dramatically alter N dynamics as well as surface microclimate and energy and C balance. Understanding the impacts of these expected changes in N dynamics and N availability in arctic tundra landscapes on global biogeochemical cycling remains a significant challenges for future research.

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