

1 Running Head: N Dynamics in an Arctic Watershed

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8 **Nitrogen Dynamics in a Small Arctic Watershed – Retention and Downhill**
9 **Movement of ¹⁵N**

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1 ABSTRACT

2 We examined short- and long-term nitrogen (N) dynamics and availability along an arctic
3 hillslope in Alaska, USA, using stable isotope of nitrogen (^{15}N), as a tracer. Tracer levels of
4 $^{15}\text{NH}_4^+$ were sprayed once onto the tundra at six sites in four tundra types; heath (crest), tussock
5 with high and low water flux (mid- and foot-slope), and wet sedge (riparian). ^{15}N in vegetation
6 and soil was monitored to estimate retention and loss over a 3-yr period.

7 Nearly all $^{15}\text{NH}_4^+$ was immediately retained in the surface moss-detritus-plant layer and >
8 57 % of the ^{15}N added remained in this layer at the end of the second year. Organic soil was the
9 second largest ^{15}N sink. By the end of the third growing season, the moss-detritus-plant layer
10 and organic soil combined retained ≥ 87 % of the ^{15}N added except at the mid-slope site with
11 high water flux, where recovery declined to 68 %. At all sites, non-extractable and non-labile-N
12 pools were the principal sinks for added ^{15}N in the organic soil.

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

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- 1 *Key words:* $^{15}\text{NH}_4^+$; arctic tundra watershed; total dissolved N; downhill transport of N;
- 2 hydrolysable amino acids; hydrolysable amino sugars; mosses; N dynamics; N immobilization;
- 3 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 CO₂ 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

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

3 We added an enriched $^{15}\text{NH}_4^+$ tracer at several sites along a hillslope at Imnavait Creek to
4 better understand: 1) short- and long-term fate of inorganic N deposited on the tundra surface,
5 and 2) spatial and seasonal movement of N along the hillslope via leaching. We chose NH_4^+
6 over NO_3^- to minimize rapid ^{15}N losses via denitrification. NH_4 is also a product of N
7 fixation, which accounts for more than two thirds of total annual N inputs. Internal N cycling
8 was examined by following the ^{15}N tracer into plants and various soil-N pools over three
9 growing seasons at the same locations as ^{15}N addition. Nitrogen leaching down the hillslope
10 was examined by following ^{15}N into downslope-flow water at and farther downslope of the
11 point of ^{15}N addition.

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

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

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

4 **METHODS**

5 *Study area and experimental sites*

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

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

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

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

14 ^{15}N addition

15 On 5 July 2003, we added ($^{15}\text{NH}_4$) $_2\text{SO}_4$ dissolved in Imnavait Creek water to each plot at
16 3.92 mmol/m^2 using backpack sprayers. This amount was $< 0.006 \%$ of the annual N-
17 requirement for plant uptake in tussock tundra (Shaver and Chapin III 1991). The total nitrogen
18 concentration in creek water was very low (total dissolved N $< 23 \mu\text{M}$) so no corrections for the
19 ^{15}N from creek water were made. Each ^{15}N -addition plot served as an experimental unit for ^{15}N
20 recovery in the vegetation, soil, soil-extracts, and soil-pore water. For water flowing downhill,
21 each experimental unit included the area directly below the plot (Figure 1-B, “sampling transects
22 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., *Eriophorum 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 50°C.

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

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

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

8 Within 12 hrs of collection, soil samples were weighed, homogenized, and live roots and
9 rocks hand-picked; the rocks were weighed to determine rock mass. Subsamples were dried at
10 50 °C to determine moisture content. To assess recovery of ^{15}N in water-extractable N pool over
11 time, the remaining homogenized soils were combined by plot and by layer, further
12 homogenized, and then subsamples (approx. 50–100 g, wet weight) were taken from the 1st and
13 2nd Organic Layers. Within 5 hrs, subsamples were added to deionized (DI) water and extracted
14 for 8 hr at 20 °C on a shaker table. In 2003, the ratio of wet soil-to-DI water was 1:10 (w/w) for
15 the 1st Organic Layer, but was 1:5 for 2nd Organic Layer to ensure a sufficiently high N
16 concentration for later analyses. A soil-to-water ratio of 1:10 was used for both layers in 2004
17 and 2005. The extractants were filtered through ashed GF/F glassfiber filters and frozen.

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

1 collection into salt (0.5 N K₂SO₄)-extractable dissolved N (SEDN), chloroform-fumigation
2 extractable N (CFN), and residual N (N_{res}) using the method of Brookes et al. (1985). We
3 determined ¹⁵N in these fractions using alkaline persulfate digestion (Cabrera and Beare 1993)
4 followed by alkaline diffusion (Brooks et al. 1989). Total ¹⁵N recovery in the bulk soil was
5 determined in ground subsamples. Because we were not certain whether the extractability
6 coefficient (*Kn*) used in other studies would apply to our study site, we report CFN instead of an
7 adjusted microbial N value.

8 We assume changes in ¹⁵N distribution across CFN fractions are minimal based on
9 previous ¹⁵N labeling studies. Redistribution of N among soil-N pools following the initial
10 assimilation was little when ¹⁵NH₄⁺ was added to temperate forest soils (Perakis and Hedin 2001)
11 or to tundra soils (Schimel and Chapin 1996). Therefore, to obtain more complete picture of N
12 dynamics at Imnavait Creek, we took additional subsamples of the 1st Organic Layer in year 2
13 and fractionated N by acid hydrolysis rather than chloroform-fumigation. To obtain natural ¹⁵N
14 abundance for these fractions, six additional soil cores were collected from outside the plots and
15 processed as described above.

16 *Sampling soil pore water*

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

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

7 *Sampling snow and downslope-flow water*

8 *Snow* – To determine snow N concentration, we collected snow cores 1–3 wk prior to the
9 start of slush-flow; the bottom ~ 1 cm that had contact with Green Layer was removed. The snow
10 was thawed, filtered, refrozen at the field station, and shipped to MBL for chemical analyses. In
11 2004, 5 snow cores each were collected from 5 evenly-spaced topolocations along the hillslope
12 (total 25 cores) and the samples were analyzed separately. Based on the small variability among
13 individual samples in 2004, one core each was collected from the five topolocations (total 5
14 cores) in 2006 and all samples were combined for analysis.

15 *Downslope-flow water during the spring melt* – In May 2004 and 2006, we collected
16 spring melt water to determine ^{15}N movement. Prior to snowmelt, six 6m sampling transects
17 were established for each labeled plot. The transects ran perpendicular to the hill slope, located
18 1–5 m above the labeled plots and 1, 2, 5, 10, or 15 m below the labeled plots (Fig 1-B). At
19 Riparian, where negative grades were found toward the creek and downstream, two sets of two
20 transects were established around the plots, one perpendicular to the creek and the other parallel
21 to the creek (Figure 1-B). All transects at Riparian were 1 m from the plots. The two Riparian
22 transects that were elevated (those on the crest and upstream sides) relative to the other two were
23 considered as a reference, and the two below the plot were considered to be “below” the plot.

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

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

18 *Downslope-flow water during the growing season* – Downslope-flow water during the
19 summer was collected at the bottom of the thawed active layer in the same manner as the
20 collection of downslope-flow water during the spring melt. In 2004, samples were collected 1 d,
21 1 wk, 1 mo, and 1 yr after ^{15}N addition. Soil moisture was too low to collect water samples after
22 long periods without rain, especially during the growing season and at the Crest site.

Chemical analysis

1
2 ¹⁵N recovery in hydrolysable N pools – We measured the pool size and ¹⁵N recovery into
3 three hydrolysable labile-N pools; hydrolysable NH₄⁺ (H₂NH₄⁺), amino acids (HAA), and amino
4 sugars (HAS). Soils were thawed and five replicates (2–10 g) of ground samples were
5 hydrolyzed and the hydrolysates were neutralized with NaOH (Mulvaney and Khan 2001).
6 Hydrolysable NH₄⁺ was determined by a hypochlorite-alkaline phenol method. To determine
7 ¹⁵N recovery in this pool, aliquots (~10 μmol-N) of the hydrolysates were diffused in a 450-mL
8 Mason jar with MgO at 50 °C for 6 hr onto an acid trap according to Mulvaney and Khan (2001),
9 but replacing their “wet” acid trap (a beaker containing 5 mL of 4% H₃BO₃) with a “dry” acid
10 trap (an acidified GF/D filter disc encapsulated in Teflon tape) so that N on the trap could be
11 directly analyzed for ¹⁵N by isotope ratio mass spectrometry.

12 Concentration and ¹⁵N recovery for hydrolysable amino sugar and amino acid were
13 determined by a sequential diffusion of the neutralized hydrolysates. For concentration, the
14 hydrolysate was diffused for 8 hrs into a wet acid trap, then re-diffused with a new wet acid trap
15 for 6 hrs after converting amino acids to NH₄⁺ by a ninhydrin reaction under an acidic condition
16 (Mulvaney and Khan 2001). Both first (HAS) and second (HAA) acid traps were analyzed for
17 NH₄⁺ concentration as for hydrolysable ammonium. To determine ¹⁵N recovery in the HAS and
18 HAA pools, aliquots of the hydrolysate (~10 μmol-N) were sequentially diffused with dry acid
19 traps in place of wet traps. Because total soil N is a sum of hydrolysable and non-hydrolysable
20 N (i.e, total soil-N = H₂NH₄⁺ + HAS + HAA + hydrolysable-unknown N + non-hydrolysable N),
21 pool sizes and δ¹⁵N signatures of hydrolysable-unknown N and non-hydrolysable N fractions
22 combined, or non-labile N fraction (non-LN), were calculated by differences in δ¹⁵N and mass of
23 known N pools (total soil N, H₂NH₄⁺, HAS, and HAA). We are aware that some of the

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

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

11 *DIN & DON* – In solution samples, NH_4^+ -N was determined by the hypochlorite-alkaline
12 phenol method. A Cd-reduction method was used to determine NO_3^- -N concentrations in
13 samples collected in 2003-2005, whereas ion chromatography was used for samples collected in
14 2006. Nitrite in our samples was below the detection limit ($< 0.2 \mu\text{M}$). Total dissolved N (TDN)
15 was determined by a persulfate digestion (modified Solozano and Sharp 1980) followed by NO_3^-
16 analysis. For 2005 samples, a high-temperature combustion method was used. Cross
17 comparison of selected samples showed that the between-methods difference in measured
18 concentration was $< 5\%$. Dissolved organic N (DON) was calculated as the difference between
19 TDN and DIN.

20 *Data analysis*

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

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

5 The recovery of added ^{15}N in plants, Green Layer, and soil, was determined as percent
6 ^{15}N -gain relative to background ^{15}N . The enrichment of ^{15}N in the dissolved-N pool was
7 expressed as ^{15}N atom % excess, which is the excess percentage of ^{15}N atom % relative to the
8 reference level. A minimum detectable difference for ^{15}N analysis of dissolved-N was 5 ‰ in
9 $\delta^{15}\text{N}$ or 0.0018 ‰ in ^{15}N atom % excess.

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

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

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

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

14 RESULTS

15 *Short-term dynamics*

16 *^{15}N Recovery in the soil and Green layer* – More than 99% of the added ^{15}N was recovered
17 in the soil and Green Layers immediately (1 d) after addition, and recovery remained high
18 through the first growing season (Figure 2). The Green Layer retained > 63% of all recovered
19 ^{15}N in the ground at both 1 d and 1 mo. The 1st Organic Layer was the second largest sink for
20 ^{15}N at these dates, and this and Green Layers together retained > 86% of ^{15}N added.

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

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

3 *N in soil pore water and WEDN* – During the growing season, DON was the dominant form
4 of N ($\geq 90\%$ of TDN) in both lysimeter water and water-extractable dissolved N (WEDN; Table
5 3-A). The ^{15}N -atom % excess of pore water at 10 and 20 cm (approximately at the bottom of 1st
6 and 2nd Organic Layers, respectively) showed a sharp drop (9-fold on average) within the first
7 month (Figure 3).

8 ^{15}N -atom % excess of the WEDN pool was 2–7 times greater in the 1st Organic Layer
9 than in the 2nd Organic Layer (Figure 4), as observed for the bulk soil (Figure 2). Measured
10 ^{15}N -atom % excess of the WEDN pool within the 1st Organic Layer was significantly greater at
11 Crest than all other sites immediately after the addition (4 d), corresponding with the highest ^{15}N
12 recovery for this soil layer at Crest (Figure 2). Contrary to the decreasing trend within the first
13 month of ^{15}N addition observed for the pore water collected by lysimeters at 10 cm, mean ^{15}N -
14 atom % excess of the WEDN pool in the 1st Organic Layer increased by up to 5-fold on average
15 during the same time period for all but Crest sites. The highest increase in atom % excess of
16 WEDN was observed at NWT and Riparian sites.

17 *Long-term dynamics*

18 *^{15}N Recovery in the soil and Green layer* – The strong ^{15}N retention in the ground layers
19 during the first growing season persisted even after 2 yrs, with $\geq 92\%$ recovery of added ^{15}N at
20 most sites (Figure 2). Exceptions were Crest and Midslope watertrack (Midslope_WT) sites,
21 where total recovery tended to decrease over time with 89% (Crest) and 69% (Midslope_WT)
22 of the added ^{15}N recovered at the end of the 2nd year. For Crest the decline was due mostly to
23 decreased recovery in the soil (Organic and Mineral) layers, whereas for Midslope_WT recovery

1 declined in both Green Layer and soil layers. Extremely high total recovery (as high as >180 %,
2 much more than was added) was found only at footslope non-watertrack (Footslope_NWT) and
3 Riparian sites and was due to very high ^{15}N recovery in one sample of the 1st Organic Layer (2–
4 3 times greater than the average of all sites, Figure 2).

5 Throughout the first 2 yrs, the Green Layer was the largest sink for added ^{15}N ; at both year 1
6 and 2 this layer still retained ~60 % or more of added ^{15}N . Combined with the second largest
7 ^{15}N -sink (1st Organic Layer), the upper two ground layers retained 66 % of added ^{15}N at
8 Midslope_WT site, 84 % at Crest and ≥ 92 % at all other sites at the end of second year. The
9 Mineral Layer contributed little to ^{15}N retention, except at the Crest, where the mineral soil
10 (average thickness above rocks = 4 cm) accounted for up to half of the overall recovery in the
11 soil (Figure 2).

12 *Hydrolysable and non-hydrolysable N pools* – Acid hydrolysis of the 1st Organic Layer
13 after 2 yrs of ^{15}N addition revealed that hydrolysable labile-N (HLN) pools (hydrolysable NH_4^+ ,
14 amino sugar, and amino acids) were an important sink for ^{15}N and were 15–39 % of ^{15}N retained
15 in the 1st Organic Layer (Table 4). The non-labile N pool, which is chemically less labile than
16 HLN, contained the rest of the ^{15}N (61–85 % of total ^{15}N). Within the HLN pool, hydrolysable
17 amino acid (HAA) was the dominant sink for added ^{15}N , containing on average 4 (non-Crest
18 sites) to 8 times (Crest) more ^{15}N than hydrolysable ammonium ($\text{H}\text{N}\text{H}_4^+$) and amino sugar
19 (HAS) pools combined.

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

1 wetter sites (i.e., two WT sites and Riparian), the contribution of HLN was intermediate (23–28
2 %).

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

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

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

1 the major long-term sinks for added $^{15}\text{NH}_4^+$ especially at the tussock tundra sites (Midslope and
2 Foothslopes). In contrast, the non-moss component, mostly plant detritus, was the dominant ^{15}N
3 sink at the Crest and Riparian tundra sites, accounting for 72–74% (Crest) and nearly 100 %
4 (Riparian) recovery within the Green layer.

5 *Chemistry and movement of dissolved N in downslope-flow waters*

6 *DIN vs. DON* – Downslope-flow waters were dominated by DON across all seasons.
7 Although NO_3^- was the dominant form (66 %) of TDN in the snow pack, unlike the soil waters
8 collected during the growing season (WEDN and lysimeter waters) (Table 3-A, B), this
9 dominance quickly disappeared and NO_3^- became < 1 % of TDN as soon as the melt water from
10 the snow pack made contact with the frozen ground and was collected as snowmelt water. The
11 concentration of NO_3^- stayed low for all water samples collected during the rest of the year. In
12 contrast, DON, which was a minor component of TDN in the snow pack (17 %), increased
13 rapidly as the melt event progressed, comprising 90–96 % of TDN in downslope-flow water
14 during snowmelt and 96–99 % during soil melt. DON remained a predominant fraction
15 throughout the growing season (95–96 % of TDN, Table 3-B).

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

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

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

5 At least some dissolved ^{15}N leached downslope in all seasons at Midslope sites. The
6 negative linear relationship between the natural-log of ^{15}N -atom % excess (Ln-APE) and the
7 distance from the plots for several sites indicates that enrichment of ^{15}N in downslope-flow water
8 declined exponentially as the water moved downhill (Figure 7). At Midslope sites the linear
9 relationship held though the entire spring melt (snowmelt and soil melt) of both 2004 and 2006
10 ($R^2 = 0.57$ to 0.93), whereas at Crest, a tight linear relationship was found only during the
11 snowmelt of normal snow year (2004, $R^2 = 0.95$). For the Riparian site, the decline of ^{15}N -atom
12 % excess with distance was greatest; thus at this site only 1 m was required to reduce ^{15}N in the
13 water by the same proportion as at 5–10 m below the other sites.

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

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

9 *Short-term dynamics*

10 Over 70 % of all the added $^{15}\text{NH}_4^+$ -N was immediately and tightly retained within the
11 Green Layer across all sites (Figure 9). The high average recovery during the first growing
12 season at Midslope and Footslope sites (73–117 %) was largely due to the mosses' ability to
13 retain incoming $^{15}\text{NH}_4^+$ at high efficiency, and because mosses dominated total biomass at these
14 sites (58–79 % of total biomass; Hahn et al. 1996). In contrast the non-moss component, mainly
15 plant detritus rather than live vascular plants, was the major sink for ^{15}N at Crest and Riparian
16 sites, where moss accounted for only 1–17 % of total biomass (Hahn et al. 1996). Uptake by
17 vascular plants within the Green Layer was unimportant at all sites, because even with our
18 highest estimate of ^{15}N recovery by vascular plants in the Green Layer we could explain only ~1
19 % of ^{15}N recovery.

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

1 cell walls (Clymo 1963), capturing atmospherically deposited NH_4^+ along with other cations. By
2 monitoring $^{15}\text{NH}_4^+$ added to the surface of *Hylocomium* species, Eckstein (2000) found that the
3 mosses had high nutrient recycling ability and that most added ^{15}N was allocated to new growth
4 in the following year rather than being released to the soil. Our results are consistent with other
5 studies: Kotanen (2002) found that mosses assimilated N more efficiently than higher plants, and
6 Li and Vitt (1997) found that nearly all ^{15}N added as atmospheric deposition was retained in the
7 moss layer in boreal peatlands.

8 Decomposing plant detritus can be also a significant sink for added NH_4^+ in northern
9 temperate forests (Currie et al. 1999, Nadelhoffer et al. 1999) and in laboratory sorption
10 experiments with peat, fulvic- and humic-acids (Thorn and Mikita 1992). These studies point to
11 non-biological fixation (e.g., with quinones) as one of the major mechanisms that retains added
12 NH_4^+ . Similarly, by tracing $^{15}\text{NH}_4^+$ injected into tussock and soil cores near our study site,
13 Schimel and Chapin (1996) found that the detritus was the dominant sink for ^{15}N (2.7-times
14 greater than live plants). They found that live graminoids (*Carex*) were the major sink for ^{15}N in
15 cores from a wet-meadow near Imnavait Creek, recovering 21-times more ^{15}N than plant
16 detritus, but this may have been because ^{15}N was injected into the rooting zone, where it would
17 be immediately available for uptake, rather than applied to the surface where it would most likely
18 be immobilized into litter and mosses.

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

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

8 Other short-term studies in tussock and wet-sedge tundras at nearby Toolik Lake have
9 reported much higher recovery of ^{15}N in the chloroform fumigation-extractable N (CFN) pool
10 than we found in this study (< 4 % of added ^{15}N). For example, Nordin et al. (2004) found that
11 20–28 % of ^{15}N injected to the tussock tundra (0–10 cm below the moss layer) in July was
12 recovered in the CFN pool after 4 hrs, and Schimel and Chapin (1996), who also injected the
13 label beneath the surface, found similar recovery for both tussock (39 %) and wet-meadow (22
14 %) tundras after 5 d incubation in August. (Note: to estimate CFN in these previous studies, the
15 reported microbial-N values were back-corrected for the extractability coefficient, K_n ; 0.45 for
16 Nordin et al. and 0.54 for Schimel and Chapin).

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

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

3 The relatively high ^{15}N -atom % excess values observed for the soil pore water 4 d after
4 ^{15}N addition indicate that a small fraction of ^{15}N applied to the tundra surface penetrated quickly
5 to as deep as 20 cm, presumably facilitated by the rain event the night after ^{15}N addition. The
6 sharp declines in ^{15}N -atom % excess between 4-d and 1-mo were likely caused by turnover of the
7 dissolved-N pool via uptake by microbes or plants, and by non-biological stabilization of
8 dissolved N followed by replenishment of the dissolved-N pool with natural-abundance level N.
9 In this ecosystem, microbes and plants quickly take up nearly all N in labile forms. The
10 dissolved-N pool would be subsequently replenished via slow dissimilation of soil organic N
11 (Shaver et al. 1992), but dissolved-N produced in this way would be less enriched with ^{15}N
12 because of discrimination against ^{15}N during dissimilation and export (Dijkstra et al. 2008). This
13 would lead to preferential stabilization of ^{15}N into microbial biomass and the recalcitrant soil-N
14 pool. For example, for wide range of soil types, vegetation, and climate, Dijkstra et al. (2006)
15 found that the microbial-N pool was 3.7 ‰ larger than the extractable-N pool and that microbial
16 ^{15}N enrichment and net N mineralization rate were positively correlated. Similarly, in Swedish
17 Lapland Clemmensen et al. (2008) found that most ^{15}N added as NH_4^+ , NO_3^- , or glycine was
18 retained in microbial biomass after 2 d but half of the immobilized ^{15}N was transferred to a non-
19 extractable-N pool within 26 days and that ^{15}N exported as dissolved-N was < 1 % during this
20 time. In our study we found the opposite short-term (≤ 1 mo) trends in ^{15}N -atom % excess, i.e.,
21 decreasing soil-pore-N and increasing WEDN over time (Figures 3 and 4). This suggests that
22 part of the ^{15}N initially dissolved in soil-pore water was removed over time and became more
23 stable WEDN, perhaps via assimilation to microbial biomass or abiotic sorption.

Long-term dynamics

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

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

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

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

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

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

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

Hydrology and Movement of dissolved N

1
2 A conceptual model of the movement of ^{15}N down the hillslope (Figure 10) indicates that
3 flux and flow path of water are a critical element that determines the magnitude of total ^{15}N
4 released from the Green Layer and soil in this tundra ecosystem. For example, when we
5 estimated ^{15}N released into downslope-flow water during the 2004 snowmelt (Figure 10), the
6 high ^{15}N enrichment of dissolved-N pools at the Crest site was offset by the low water flux,
7 whereas the smaller ^{15}N enrichment at the Riparian site was accompanied by high water flux,
8 resulting in an estimated total ^{15}N -release into the snowmelt water of as much as ~20 % of added
9 (Figure 8). Similarly, total ^{15}N release into downslope-flow water was greater during the
10 snowmelt than other seasons at all sites, it was greater at Riparian than other sites at all times,
11 and it was greater at WT than NWT sites (Figure 10). The high total ^{15}N release during
12 snowmelt occurs because: 1) the snowmelt water exclusively flows through the thawed Green
13 Layer where most ^{15}N is retained, because most soil underneath it is still frozen at this time; 2)
14 snowmelt water is highly concentrated with ions relative to rain water (up to three times higher
15 concentration, Everett et al. 1996), thus exchanging $^{15}\text{NH}_4^+$ that was retained on the cation-
16 exchange sites of mosses in the Green Layer more efficiently than rain water; 3) any soluble-N
17 compounds that have accumulated in Green Layer over winter are subject to leaching at
18 snowmelt; and 4) biological ^{15}N immobilization and non-biological sorption in the organic soil
19 would be negligible because most of the organic soil and rooting zone is still frozen, although
20 some microbial uptake within the thawed layer would be expected during the melt season
21 (Brooks et al. 1998). The difference in the pattern of ^{15}N enrichment of downslope-flow water
22 between 2004 and 2006 may be attributed to the unusually thin snowpack, and thus less
23 meltwater volume, in 2006.

1 ^{15}N enrichment in downslope-flow water declined with distance from the plots (Figure 10).
2 This decline is most likely caused by the addition of N from other sources, lowering ^{15}N -to- ^{14}N
3 ratio of dissolved-N pool. Potential N inputs include N-fixation, dissimilation, and N from the
4 snow pack and melt water itself. However, because virtually no added ^{15}N was lost from the
5 Riparian and the two NWT sites throughout the study period even though as much as 21 % of
6 added ^{15}N was released into downslope-flow water (Figure 8), subsequent immobilization of
7 released ^{15}N must also be very high. Loss of ^{15}N to deep soil via vertical leaching is unlikely at
8 all but Crest sites due to shallow thaw depth especially during snowmelt or soilmelt.

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

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

22 Based on the total ^{15}N recovered within the plot, the spatial and temporal variations of
23 $D_{0.95}$ and of total ^{15}N released into downslope-flow water, and differences in flow rates among

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

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

3 Slow flow rate allows more time for ^{15}N in the water to be immobilized, leading to little loss
4 of added ^{15}N . The small $D_{0.95}$ for Riparian can be explained partly by a slower downslope-flow
5 water because of the smaller topographic gradient at this site. Newbold et al. (1982) showed in
6 their conceptual model that flow rate and flux of stream water was one of the factors that
7 determine nutrient spiral length in stream ecosystems. In a simulation study, Rastetter et al.
8 (2004) found that N movement on hillslopes was very slow (over 98 % of added N taken up in
9 2–5 m), their estimated rate being equivalent to $D_{0.95} < 2\text{--}5$ m. However, their simulations
10 included only DIN, which was taken up rapidly by microbes and plants and was a minor
11 component of total dissolved N in downslope-flow water (Table 3). Our longer transport
12 distances ($D_{0.95}$) are probably due to inclusion of DON in our sampling. Although Rastetter et al.
13 (2004) used annual averages and did not account for the large transport during snow and soilmelt
14 periods, they did find that transport distances increased with the rate of water flowing downslope
15 in agreement with our findings.

16 *Conclusion & Implications*

17 Most $^{15}\text{NH}_4^+$ added to the surface of tundra was retained tightly in the Green Layer at all
18 sites. High ^{15}N retention was due to the high immobilization in mosses and to direct
19 immobilization into plant detritus. Once it reached the soil beneath the Green layer, ^{15}N was
20 incorporated quickly into less labile pools that are unavailable for uptake by vascular plants. The
21 Green Layer serves as the point of entry for most N inputs to this hillslope, as it efficiently
22 captures atmospherically-deposited NH_4^+ and it is the primary location of N fixation (0-3 cm,
23 Hobara et al. 2006). However, N cycling within the Green Layer is distinct from that of the soil

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

10 The N cycle in this arctic landscape may be greatly changed by the climatic warming that
11 has occurred in the Alaskan Arctic over the last several decades (Serreze et al., 2000), and is
12 expected to continue. In a warmer climate, dissimilation of recalcitrant N in the soil would be
13 faster and more complete (i.e., the end products would be DIN and smaller organic compounds),
14 resulting in higher N availability in the soil. Warmer climate has also extended the length of
15 snow-free season in Alaska (Stone et al. 2002). This extended snow-free season and resulting
16 deeper depth of thaw would allow more frequent vertical infiltration of water (because more
17 precipitation events would be rain rather than snow), and may result in a greater flux of relatively
18 labile N from the Green Layer to soil. The increased abundance of deciduous shrub species in
19 non-shrub tundras in recent decades (Sturm et al. 2001) may be one of the best indications of
20 increased N availability in the soil as shrubs respond strongly to fertilizer addition (Chapin et al.
21 1995). A longer snow-free season and higher summer temperature would also create more
22 favorable conditions for wildfires, heretofore rare events North of the Brooks Range (Racine and
23 Jandt, *unpublished manuscript*). Fire may remove the functionally unique Green Layer and

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

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