Seasonal patterns of soil nitrogen availability in moist acidic tundra

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Abstract

Our ability to predict effects of changing soil nitrogen (N) in Arctic tundra has been limited by our poor understanding of the intra-annual variability of soil N in this strongly seasonal ecosystem. Studies have shown microbial biomass declines in spring accompanied by peaks in inorganic nutrients. However, subsequent to this early pulse, there are few high temporal resolution measurements during the growing season. We hypothesized that: (1) low N would be maintained throughout the growing season; (2) peaks of total free primary amines (TFPA), ammonium (NH_4^+), and nitrate (NO_3^-) would follow a sequential pattern driven by mineralization and nitrification; (3) a peak in soil N would occur as plants senesce. We conducted weekly measurements of TFPA, NH_4^+ and NO_3^- in two tundra sites, from soil thaw in spring to freeze in fall. At each site, NH_4^+ peaks were followed by smaller peaks in NO_3^-, supporting the hypothesis that excess NH_4^+ would be nitrified. Furthermore, peaks in NH_4^+ were observed both shortly after leaf expansion and also at plant senescence. The variation in timing between sites and the peaks in NH_4^+ subsequent to thaw indicate that nutrient limitation in these ecosystems is more dynamic and spatially variable than previously thought.

Keywords: Nitrogen availability; Nitrogen mineralization**;** Seasonality; moist acidic tundra; Total Free Primary Amines

Introduction

As a key limiting nutrient to primary productivity in the Arctic, soil nitrogen (N) is an essential control on the carbon (C) balance of tundra ecosystems. One of the key challenges to understanding tundra soil N availability is that it is strongly seasonal, corresponding with fluctuations in intra-annual environmental conditions (Buckeridge and Grogan 2010; Buckeridge et al. 2013; Darrouzet-Nardi and Weintraub 2014; Edwards and Jefferies 2013). However, these intra-annual patterns in soil nutrient availability are not well quantified. Determining seasonal patterns of soil N availability requires sampling at a high temporal resolution, as pulses in N may be short lived. The few studies that have sampled with high temporal frequency focused their sampling at the winter-spring transition (Buckeridge et al. 2013; Edwards and Jefferies 2013; Larsen et al. 2007), with fewer studies including sampling throughout the growing season (Darrouzet-Nardi and Weintraub 2014; Giblin et al. 1991; Weintraub and Schimel 2005). As the timing and variability of seasonal weather events in the Arctic are predicted to change with global warming (Anisimov et al. 2007), potential changes in snowpack depth, timing of spring thaw and fall freeze, as well as winter and growing season soil temperatures may influence both the timing and magnitude of Arctic soil nutrient availability. Understanding intra-annual nutrient dynamics is essential for predicting effects of changing seasonal weather patterns on processes controlled by soil N availability, including primary productivity (Gough et al. 2012) and microbial decomposition processes (Sistla et al. 2012), ultimately affecting C storage in Arctic ecosystems.

Pulses in soil available N in early spring have been observed and characterized in Arctic, subarctic and alpine tundra ecosystems. N availability at thaw appears to be controlled by strong seasonal dynamics in soil microbial biomass. Microbial biomass has consistently been shown to be high over the winter months when soil is frozen and snow-covered, and then drop substantially during the spring thaw (Buckeridge et al. 2013; Edwards and Jefferies 2013; Larsen et al. 2007; Larsen et al. 2012 Sistla and Schimel 2013). Although these early season crashes in microbial biomass have led to predictions of

subsequent peaks in inorganic N released from lysed cells (Schmidt and Lipson 2004), this has not been consistently observed. High levels of soil inorganic N have been observed shortly before (Edwards et al. 2006) and simultaneously (Buckeridge and Grogan 2010) with soil microbial biomass declines, while others have found no corresponding peak in inorganic nutrients (Brooks et al. 1998; Larsen et al. 2007) or peaks only in some years (Edwards and Jefferies 2013).

Schmidt et al. (2007) proposed a conceptual model of seasonal fluctuations in soil N in alpine tundra, describing transitions in both microbial community structure and soil N availability, particularly focusing on the winter-spring transition. This model proposes a microbial buildup (primarily of fungi rather than bacteria (Buckeridge and Grogan 2008)) and immobilization of N into microbial cells during the winter, followed by a crash in microbial biomass and release of dissolved N at snow melt due to a limitation of microbial resources and lysis induced by temperatures above $0^{o}C$ (Edwards et al. 2006). They further predict a high turnover of microbial biomass during the summer, driven by cycles of root exudate release and increased predation on microbes, which in turn releases N. This N is likely either immediately immobilized again by microbes, taken up by plants, or lost to leaching. We are not aware of a tundra model that extends predictions of soil N availability through the rest of the growing season, likely because of the paucity of data with high enough temporal resolution to characterize fluctuations during the short and dynamic tundra growing season.

Critically missing is high resolution sampling during the growing season, and as a result we do not know if the observed peaks in soil nutrients at soil thaw are atypical of the rest of the growing season, especially if later peaks are of short duration. Changes in the rates of microbial turnover, or shifts between plant phenological stages associated with differences in nutrient uptake may also result in changes in soil nutrient availability. A study in Arctic sedge meadows indicated consistently low soil inorganic N availability throughout the summer, especially when compared with winter values, but had relatively infrequent measurements throughout the growing season (Edwards and Jefferies 2013). In

78 contrast, soil extractions every two weeks suggest peaks in extractable TFPA and/or NH_4^+ in moist acidic,
79 shrub, and wet sedge tundra soils following increases in air and soil surface temperature in late June,
80 barely detectable levels in July, and then increases in August in some ecosystems (Weintraub and
81 Schimel 2005). Regular sampling of soil pore water in moist acidic tundra suggests that N availability
82 peaks of varying size and duration might also be observed at times other than thaw during the summer
83 growing season (Darrouzet-Nardi and Weintraub 2014; Ström et al. 2012). Substantial variation
84 observed within and among plant communities suggests that differences in microsite conditions and
85 plant phenological patterns can result in contrasting soil N dynamics between even nearby sites. Data
86 from more locations and with greater temporal resolution are required to tease apart these interacting
87 controls on tundra soil N availability.
88 Thus, the first objective for this study was to use N availability in soil pore water measured
89 frequently (weekly to every two weeks) throughout the summer growing season at two different Arctic
90 tundra sites to determine seasonal patterns of soil N availability with high resolution. A second objective
91 of this study was to expand on the soil N availability component of the model proposed by Schmidt et al.
92 (2007) to include seasonal patterns of different forms of soil N throughout the growing season in moist
93 acidic tundra. In particular, peaks in soil NO_3^-, which is less frequently adsorbed to soil due to the rarity
94 of anion exchange sites (Sposito 2008), may indicate a high possibility for leaching at particular points in
95 the season. We use a preliminary conceptual model (Fig. 1) to establish predictions of potential variation
96 in soil N during the growing season in Arctic tundra, including the post-thaw peak in available N
97 predicted by Schmidt et al. (2007) resulting from decreases in microbial biomass at thaw described
98 above, and predicted low and stable soil N during the growing season.
99 Hypothesis 1: We predict that soil solution N will stay low throughout the most active part of the
100 growing season (between deciduous leaf expansion and the onset of senescence) due to high plant
101 and microbial demand for N, as reported by Giblin *et al* (1991).

Hypothesis 2: We predict that any peaks in soil N will have sequential timing in different N forms, with an initial peak in TFPA, which are then mineralized, resulting in a peak in NH_4^+, followed by NO_3^- as the NH_4^+ is nitrified.

Hypothesis 3: We predict a late-season peak in soil solution N with the onset of plant senescence as plant nutrient uptake is reduced and leaching from dropped foliar litter and senesced root litter increases, which then decline again due to a combination of leaching and microbial uptake. Support for this hypothesis comes from late season increases in NH_4^+, NO_3^-, and TFPA in an earlier study in the same region (Weintraub and Schimel 2005).

Methods

We sampled soil pore water from two sites ("Toolik" and "Imnavait") near the Toolik Field Station in the northern foothills of the Brooks Range in Alaska (68° 38'N, 149° 43'W, elevation 760 m). Both sites are in moist acidic tundra (MAT) where the vegetation is characterized by a nearly equal abundance of graminoids (including *Eriophorum vaginatum),* deciduous shrubs (including *Betula nana*), evergreen shrubs and mosses (Gough et al. 2012). *E. vaginatum* grows in dense tussocks which cover ca. 20-25% of the ground surface area, whereas the remainder of the surface area is classified as 'intertussock' and is composed of moss that is well colonized by evergreen and deciduous shrubs at both sites. Soils from both sites have a well-developed organic layer of ca. 10 cm depth, underlain by a silty mineral layer, and an active layer (the layer of soil above permafrost which thaws each summer) of less than 50 cm. Rooting depth typically follows the thawed soil layer downward over the growing season (Chapin et al 1979) but even by the end of the growing season > 75% of root biomass for *E. vaginatum* (Chapin et al. 1979) and the MAT community (Sullivan et al. 2007) are found within the surface 20 cm of the soil. Soils are acidic (Toolik average pH 4.3, Imnavait pH 4.4), with high soil moisture (ca. 400% dry weight soil), and a total N content of about 1% (Toolik: Organic layer mean C is 43% and mean N is 1.2%. Imnavait:

Organic layer mean C is 45% and mean N is 0.8%). Gravimetric soil moisture content remains relatively stable at these sites throughout the summer (~0.8 g water g^{-1} wet soil; Darrouzet-Nardi et al. 2014, Weintraub and Schimel 2005).

At the first site (Toolik) we sampled in the intertussock spaces from the unmanipulated control plots of a series of replicated experiments maintained by the Arctic Long-Term Ecological Research project; three control plots from each of three experimental areas were sampled, for a total of nine plots. All nine plots are within 100 m of one another, and contain similar vegetation, so the plots are considered replicates in this analysis. At the second site ("Imnavait") we likewise sampled from five unmanipulated control plots of an early snowmelt × warming experiment. Plots at Imnavait were separated by less than 50 m. Samples at that site were collected in microsites both inside *Eriophorum vaginatum* tussocks and in the intertussocks spaces (spaces between tussocks with little to no *E. vaginatum*). Data from the tussock samples are presented in a separate study comparing lysimetry with soil core extractions (Darrouzet-Nardi and Weintraub 2014). Although samples at the Imnavait site were collected for an unrelated study, they parallel the samples collected from the Toolik site in sampling year, frequency and location (intertussock). Thus, here we use the previously unpublished intertussock data for the purposes of comparison with the Toolik site data to increase the breadth of potential conclusions made from these sites.

At both sites, in each plot, a single 10 cm long microlysimeter (Rhizon soil moisture sampler, Eijkelkamp Soil & Water, The Netherlands) was inserted perpendicularly into the soil until flush with the soil surface in the intertussock area when thaw depth reached at least 10 cm (June 9, 2011 for Toolik and May 21, 2011 for Imnavait) and left in place throughout the growing season. The necessity of soil thawing to the depth of the microlysimeter prior to insertion meant that the soil water could not be sampled between snow melt and the very early stages of thaw. Although installation dates were different between the two sites, soil temperatures and plant phenology data (see below) indicate that

150 the timing of seasonal events was likely similar between the two sites. Approximately 1-6 mL of soil
151 solution was sampled weekly using a sealed 6-ml tube under vacuum (Greiner Vacuette No. 456089)
152 attached to each lysimeter from June 11 to September 16, 2011 at the Toolik site, and about twice
153 weekly from May 21, 2011 to September 17, 2011 at the Imnavait site. New vacuettes were attached
154 each week, and soil water was only collected on the sampling day and not left to accumulate between
155 samplings. We concluded seasonal sampling when the top 10 cm of soil was frozen at both sites and soil
156 water could no longer be collected.
157 Samples were frozen upon collection until analysis. NH_4^+, NO_3^-, and TFPA were analyzed using
158 colorimetric (NO_3^- and NH_4^+) or fluorometric (TFPA) microplate assays. NH_4^+ was determined using a
159 modified Berlethot reaction (Rhine et al. 1998) and NO_3^- using a modified Griess reaction (Doane and
160 Horwath 2003), which involves the reduction of nitrate to nitrite, followed by colorimetric
161 determination of nitrite. TFPA, which is primarily amino acids but may also contain some amino sugars
162 and other monomeric primary amines, was measured by fluorescence of samples in microplates with *o*-
163 phthaldialdehyde and β-mercaptoethanol (Darrouzet-Nardi et al. 2013; Jones et al. 2002). At the Toolik
164 site only we also analyzed phosphate (PO_4^{-3}) using the malachite green assay (D'Angelo 2001), and then
165 read colorimetrically. Absorbance and fluorescence values were determined on a Bio-Tek Synergy HT
166 microplate reader (Bio-Tek Inc., Winooski, VT).
167 Soil temperatures were recorded every 4 hours throughout the 2011 growing season using
168 iButtons (Maxim, San Jose, CA), waterproofed with parafilm and deployed 5 cm below the soil surface in
169 each plot in fall 2010. Soil temperature data were collected continuously through the soil solution
170 sampling period at Toolik, but ibuttons were removed from Imnavait plots during the growing season
171 and data for July 22 through August 19 are missing for Imnavait. Vegetative plant phenology data are
172 presented for *B. nana* using a combination of data collected by the Toolik Field Station Environmental
173 Data Center (2011 data from http://toolik.alaska.edu/edc/; leaf expansion and color change dates are

both recorded for the first date of each event for each species) and a visual assessment of experimental plots (last leaf drop, date when there were fewer than 10 remaining leaves on the majority of individuals). Vegetation phenology data are not available for the Imnavait site for most stages except first leaf expansion, which was one day later (June 4) than at the Toolik site (Darrouzet-Nardi, unpublished data). *B. nana* was chosen as a representative species as it is co-dominant in the moist acidic tundra and has phenological stages that are relatively easy to determine. Other co-dominant species are evergreen and do not lose their leaves or undergo distinct fall color changes, or, in the case of *E. vaginatum,* may pass through several phenological stages, including flowering, while still under snow cover.

We calculated the normalised difference vegetation index (NDVI) as (NIR – R) / (NIR + R), where NIR indicates mean reflectance at near-infared wavelengths (841 - 876 nm) and R mean reflectance at visible red wavelengths (620 - 670 nm). For Toolik, spectral radiance measurements were collected by the Arctic LTER using a hand-held dual channel spectrophotometer (Unispec DC, PP Systems, Amesbury, MA, USA) (Shaver & Gough 2015). Radiance measurements were taken throughout the summer of 2011 on multiple dates for each plot. On each date, five replicate scans were taken 1m apart along a 5-meter transect located ca. 0.5 m from the edge of each plot. NDVI at the Imnavait site was monitored using radiation sensors mounted at ~50 cm height, recording a circular area of ~0.75 m2, using a technique described in Sweet et al. (2015). All spectral measurements were converted to reflectance values and vegetation indices calculated. NDVI, in combination with the phenology measures, shows the temporal trend for the timing of leaf out, greening, and leaf senescence. NDVI measurements are difficult to compare between sites, however, given the different sensors and spatial area measured for the two different sites.

Statistical hypothesis tests were not conducted on these data sets, though standard errors are presented as indicators of variability on each measurement date. Our analyses focus on the presence of

peaks in concentrations throughout the time series and these features are clearly visible by examining the means and standard errors alone. We note cases in which high variation precludes identification of clearly visible peaks in concentrations.

Results

At Toolik, average concentrations of TFPA were very low (most <50 μg L^{-1}) compared with the inorganic components of soil N, and we observed the highest levels during the first sampling (120 ± 12 μg L^{-1}, mean ± SE), immediately after leaf emergence (Fig. 2a). NH_4^+ in soil solution peaked shortly following TFPA at 1280 ± 600 μg L^{-1} (Fig. 2b) and then dropped to much lower levels (<150 μg L^{-1}) for the rest of the snow free season. This was accompanied by a similar peak in PO_4^{-3} at Toolik (supplementary figure S1). NO_3^- concentrations in soil solution increased later, approximately two weeks after the peak in NH_4^+, but did not form a distinct peak (Fig. 2c). The increase in NO_3^-, to a level of 210 ± 100 μg L^{-1} on Aug 27 for example, was less than half of the NH_4^+ peak earlier in the season, and NO_3^- levels remained at or close to these values throughout the rest of the growing season. There was a subtle decrease in NO_3^- late in the growing season, about one week before leaves dropped. NDVI increases after leaf emergence until peak growing season – measurements did not continue late into the season and thus did not capture senescence.

At the Imnavait site, TFPA concentrations were similarly low (most <50 μg L^{-1}), and again we observed the highest concentrations during the first sampling (120 ± 20 μg L^{-1}), though the timing of the first sampling at Imnavait was directly after thaw instead of after leaf emergence. While NH_4^+ was the most common labile N constituent of the three we measured, we did not see as large of an early season peak in NH_4^+ at Imnavait as at Toolik. Instead, NH_4^+ peaked to a maximum of 660 ± 200 μg L^{-1} around the time of plant senescence in the first half of August. This peak was followed by several sampling dates with higher NO_3^- concentrations, with a peak NO_3^- of 360 ± 20 μg L^{-1}. Until that time, NO_3^- was variable, but generally lower (<150 μg L^{-1}) in concentration. The NO_3^- peaks in turn were followed by a slight rise

222 in TFPA concentrations, though those concentrations were still relatively low, overall. On the last
223 sampling dates, NH_4^+, NO_3^-, and TFPA all returned to low concentrations. NDVI increases gradually
224 throughout the season until leaf senescence and then decreases until measurements were ceased in
225 late August.
226
227 **Discussion**
228 We found some, but not complete support for the predictions of our conceptual model of seasonal soil
229 N dynamics (Fig. 1). We predicted that soil N would remain low throughout the growing season because
230 of high microbial and plant demand for N (Hypothesis 1). In contrast, we found surprising variability in
231 both soil NH_4^+ and NO_3^- during the portions of the season that plants are most active and suggest that
232 more soil N data collected at a high temporal resolution will need to be collected to improve our
233 conceptual model of seasonal soil N dynamics in Arctic tundra.
234 At both of our study sites, we found early growing season peaks in multiple soil nutrient pools. At
235 both sites there was an early-season peak in TFPA. However, this peak only occurred in the first
236 sampling after lysimeters were placed, raising the possibility of an insertion effect. Only at Toolik was
237 this TFPA peak followed by peaks in other nutrients including large and distinct peaks in NH_4^+ and PO_4^{3-}
238 (PO_4^{3-} was not measured at Imnavait), a pattern also seen in alpine tundra (Lipson et al. 1999). The early
239 growing season NH_4^+ peak at Toolik was immediately followed by an increase in NO_3^-, suggesting that a
240 significant proportion of the NH_4^+ was nitrified in concordance with our model, and Hypothesis 2.
241 Although at Imnavait we did not see this early season peak in NH_4^+, Darrouzet-Nardi & Weintraub (2014)
242 reported both high TFPA and NH_4^+ in adjacent tussock soils (rather than the intertussock data presented
243 here). Thus, early growing season N dynamics were similar in tussock and intertussock soils at Imnavait,
244 but the early season peak in pore water TFPA did not carry over into the NH_4^+ pool in intertussock soil,
245 suggesting greater N limitation. After the rapid early growing season declines in dissolved N, we found

246 that there were no subsequent peaks in inorganic N until late in the growing season, likely through a
247 combination of plant uptake and microbial immobilization, in accordance with Hypothesis 1.
248 We suggest that the timing of these early season peaks indicate that they may be distinct from the
249 peaks in soil available N that are often predicted following a crash in microbial biomass at thaw (Schmidt
250 et al. 2007; Schmidt and Lipson 2004). Our sampling was focused on soil available N during the growing
251 season and began after soil had thawed to 10 cm and allowed insertion of the lysimeters into the soil.
252 Plant phenology and soil temperature data indicated that these early season peaks we detected at
253 Toolik only occurred well after we would typically expect a 'thaw peak' in N. Our measured peaks in soil
254 N occurred after spring was well in progress: the soil temperatures at 10 cm depth had consistently
255 reached a daily minimum above 0 °C, and bud break in *Betula nana*, one of the dominant species in this
256 ecosystem, had already occurred. The combination of these data sets provides support for the
257 possibility of a second early growing-season peak in TFPA and NH_4^+ which occurs after a post-thaw
258 related pulse of N released by microbial turnover. As we do not have TFPA and NH_4^+ values immediately
259 after soil thaw, however, we cannot exclude the possibility that the early season pulse of TFPA and NH_4^+
260 is the tail end of a peak in nutrients resulting from a crash in microbial biomass. Also, because peaks in
261 NH_4^+ were found only in specific microsites at Imnavait (tussock but not intertussock soils), the site-
262 specific degree of N-limitation may also determine the presence of these early season peaks in soil N.
263 A number of potential mechanisms could be responsible for the observed second peak in soil N
264 early in the growing season. The peak in TFPA and NH_4^+ could be a result of increases in microbial
265 mineralization resulting from increases in soil temperature. Although microbes can remain active below
266 freezing (Brooks et al. 1996; Larsen et al. 2007), activity at these temperatures is often limited by
267 availability of unfrozen water (Jefferies et al. 2010), which becomes much more abundant when soil
268 temperatures remain above freezing and allows for increased microbial activity and possibly N
269 mineralization (Mikan et al. 2002, Schmidt et al. 1999)). The timing of this soil N peak at Toolik also

coincides with bud break in birch, which may result in increases in labile C from root exudates stimulating microbial growth. However, exudates contain very little N (Hutsch et al. 2002) and to make use of the exudate C subsidy, microbes must acquire N from other sources. If this N is acquired from polymeric sources such as proteins, their breakdown could possibly result in an increase in soil N mineralization rates (Weintraub et al. 2007).

Of the four studies we cite that describe seasonal patterns in growing season N availability in the Arctic (Darrouzet-Nardi and Weintraub 2014; Giblin et al. 1991; Ström et al. 2012; Weintraub and Schimel 2005), only Darrouzet-Nardi & Weintraub (2014), who also frequently sampled soil water, describe similar soil N variability to that described here. Other studies that do not show these patterns either sampled less frequently (Edwards and Jefferies 2013), or sampled organic, rather than inorganic soil N (Ström et al. 2012). Using K_2S0_4 extractions, Weintraub and Schimel (2005) describe high early season soil NH_4^+ but this likely represents a pool of extractable N that is greater than that available in soil pore water samples (Darrouzet-Nardi and Weintraub 2014). Interannual variability in patterns of soil N availability are likely to be highly dependent on annual weather conditions, timing of snowmelt, and even on growing conditions in the previous year which may determine over-winter nutrient and soil microbial biomass quantities. Nevertheless, the accumulated evidence suggests that growing season inorganic soil N may be more variable than previously thought and that fluctuations in inorganic nutrients may be missed because sampling at such short time intervals is rare during the growing season.

At senescence, we and others (Weintraub and Schimel 2005) predicted an increase in soil available N with the decrease in plant uptake (Hypothesis 3). At Imnavait, NH_4^+ increased in late July, and remained at higher concentrations for multiple weeks. Levels of NO_3^- were also elevated through leaf color change, although there was a slight decrease in NO_3^- during and after senescence at both Imnavait and Toolik. Although the increase in NH_4^+ and elevated NO_3^- occurs before senescence for most species

in this system, peak growing season NDVI had passed (Steltzer, Darrouzet-Nardi and Weintraub, unpublished) and decreases in nutrient uptake may have already occurred.

Possible reasons for the late season decrease in NO_3^- include N uptake by plants; *E. vaginatum* continues N uptake late into fall (Shaver et al. 1986) and maintained uptake of N by plants beyond the active growing season has been observed in alpine meadows (Xu et al 2011). Also, increases in immobilization rates may occur if plant senescence results in a flush of available C from annual root turnover. High concentrations of $NO3^-$ after fertilization in these plots suggest that denitrification may also be an important source of $NO3^-$ loss (Mack et al. 2004). Because there is relatively little adsorption of NO_3^- onto soils (Sposito 2008) and decreased root biomass at depth (Sullivan et al. 2007), leaching, which could increase with decreasing plant N demand late in the growing season, may also be responsible for late season decreases in NO_3^- with the potential for NO_3^- to be transported to local streams and lakes (Giblin et al. 1991). Relatively little sampling of streams and lakes on the North Slope of Alaska has occurred into the fall months. However, McNamara *et al*. (2008) reported increases in North Slope Alaska stream NO_3^-in early fall in some, but not all years, with similar seasonal timing as our reported decrease in soil NO_3^-. Water sampled from the outlet of Toolik Lake continuously into early September in 2006, did not show a similar peak in NO_3^- (Snyder and Bowden 2014), although in 2011 local stream NO_3^- increased in mid-September (Bowden, Pers. Comm.), coinciding with the described decrease in soil NO_3^- in this study. Local stream NO_3^- has not peaked in subsequent years, however, and NO_3^- concentration may be dependent on stream discharge rather than directly dependent on inputs from terrestrial systems (Bowden, Pers. Comm.). In an Arctic soil incubation experiment, Treat et al (2016) estimate that one third of yearly N loss may occur through fall leaching, and found that NO_3^- leaching in particular was highest in fall. A substantial portion of the N input in Imnavait Creek has been reported to be N resulting from N-fixation (Hobara et al. 2006). We did not measure seasonal trends in N-fixation and cannot rule out increases in N-fixation as the mechanism driving the late season pulse in

318 NO_3^-, although Hobara et al. (2006) have suggested little seasonal variation in N-fixation rates in this
319 ecosystem. Further, in other low-Arctic ecosystems N-fixation has been reported to be highest during
320 peak growing season rather than at plant senescence (Stewart et al. 2011). Nevertheless, the limited
321 evidence available suggests the hypothesis that late season decreases in soil NO_3^- may represent a
322 leaching of NO_3^- into the aquatic ecosystem. Simultaneous sampling of terrestrial and nearby aquatic
323 ecosystems at a high temporal resolution will be required to support this conclusion.
324 We described a conceptual model of seasonal soil N fluctuations in Arctic tundra (Fig. 1), expanding
325 on an earlier model from alpine tundra by Schmidt et al. (2007), and suggested that data from the
326 growing season required to expand this model post-thaw was lacking. Our results, and those presented
327 recently in Darrouzet-Nardi and Weintraub (2014), suggest the need for increased awareness that soil N
328 availability is dynamic; pulses of N after budbreak indicate that N may not be limiting through the
329 entirety of the Arctic tundra growing season, and NO_3^- may continue to be available after other
330 dissolved N forms are depleted. These data highlight the potential for generalization of soil N patterns if
331 future investigators sample soil nutrients at a higher temporal resolution to determine the potential
332 variability in growing season soil N that may occur after the well-described post-thaw peak.
333 *Summary and Future Directions*
334 Using weekly sampling of soil pore water at two tundra sites in northern Alaska we demonstrate
335 that soil N availability during the growing season may be more variable than previously described. We
336 observed an early growing season peak in soil N, which may represent an increase in mineralization
337 activity with the onset of plant growth. These results provide more support for a late season peak in
338 inorganic N which may represent the decrease in plant uptake with plant senescence. The subsequent
339 decreases in inorganic N may represent NO_3^- leaching into surrounding streams, as suggested by parallel
340 reported increases in stream NO_3^-. There is currently little seasonal soil N availability data outside of the
341 North Slope of Alaska, or from other years, and even our repeated sampling at two sites does not show

consistent patterns. We suggest a growing need for seasonal soil N availability data from multiple sites, ecosystem types, and across multiple years. Understanding the seasonal nature of the forms of this limiting nutrient in Arctic ecosystems, as well as the potential drivers of soil N variability, is essential to predict patterns in the processes controlled by soil N availability, such as plant productivity (Gough et al. 2012; Shaver and Chapin 1980, 1995) and organic matter decomposition (Sistla et al. 2012), and ultimately to understand feedbacks on atmospheric C levels.

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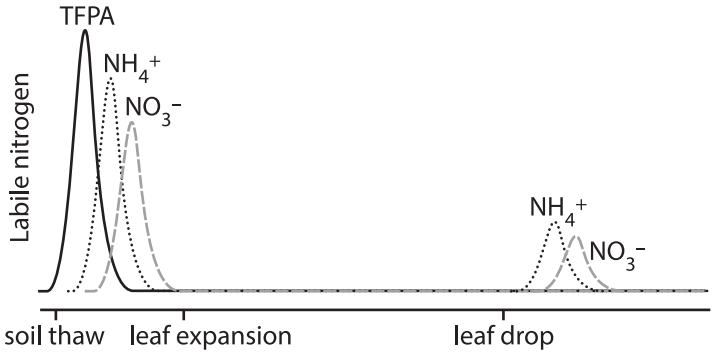
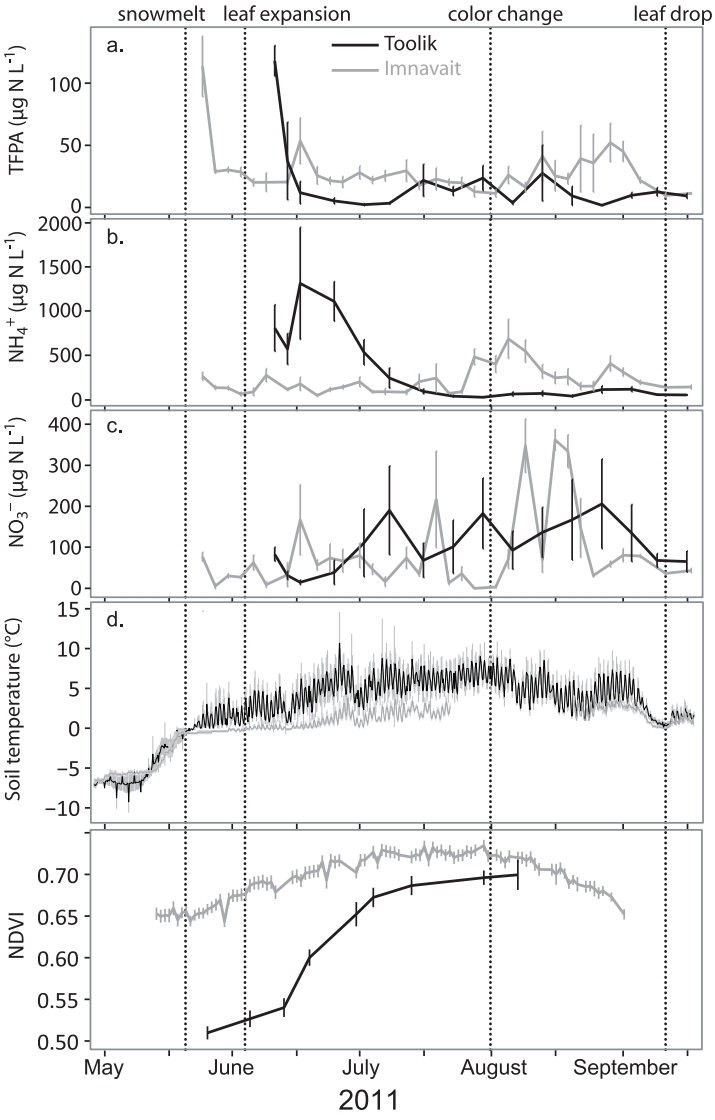
480 **Figure Captions**

481 **Fig. 1** Conceptual diagram of seasonal soil dissolved inorganic nitrogen (NH_4^+ and NO_3^-, dotted and
482 dashed lines respectively) and total free primary amines (TFPA, solid lines) patterns during the snow free
483 season in moist acidic tundra. All soil N types are presented on the same graph, but magnitudes of
484 variation in N are only intended to be compared within an N-type. We suggest that initial peaks in TFPA
485 produced with microbial turnover at thaw will be deaminated to produce NH_4^+, which will subsequently
486 be nitrified to NO_3^-. Consistently low levels of N during the active growing season are the result of high
487 plant and microbial demand for N. Finally, late season peaks in soil solution N are the result of
488 decreased plant uptake with subsequent declines due to leaching or microbial uptake.

489 **Fig. 2** Mean concentrations (± SE) of total free primary amines (a), ammonium (b) and nitrate (c) in soil
490 water collected weekly from moist acidic tundra between June 9 and September 16, 2011; d) soil
491 temperatures at 10 cm (± SE) and e) NDVI (± SE). Arrows represent the mean date for phenological
492 events of *Betula nana*. Note that due to slight differences in the microsites in which the iButtons were
493 placed, the seemingly large difference in soil temperature between the sites may not be indicative of
494 true site differences. NDVI was measured using different sensors at the two sites which have a different
495 measurement surface area. The snowfree date for Imnavait (May 21) is shown since it was more
496 carefully recorded, but the snow free date at Toolik was approx. 4 days later


TFPA
NH_4^+
NO_3^-
Labile nitrogen
NH_4^+
NO_3^-
soil thaw
leaf expansion
leaf drop


snowmelt
leaf expansion
color change
leaf drop
a.
Toolik
Imnavait
TFPA (µg N L-1)
b.
NH4+ (µg N L-1)
c.
NO3− (µg N L-1)
d.
Soil temperature (°C)
NDVI
May
June
July
August
September
2011