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N A. Startsev

V.J. Lieffers

S M. Landhausser

A Velazquez-Martinez

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## N-transfer through aspen litter and feather moss layers after fertilization with ammonium nitrate and urea

N. A. Startsev · V. J. Lieffers · S. M. Landhäusser ·  
A. Velazquez-Martinez

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**Abstract** When fertilizer is broadcast in boreal forest stands, the applied nutrients must pass through a thick layer of either feather moss or leaf litter which covers the forest floor. In a growth chamber experiment we tested the transfer of N through living feather moss or aspen litter when fertilized with urea ((NH<sub>2</sub>)<sub>2</sub>CO) or NH<sub>4</sub>NO<sub>3</sub> at a rate of 100 kg ha<sup>-1</sup> and under different watering regimes. When these organic substrates were frequently watered to excess they allowed the highest transfer of nutrients through, although 72% of the applied fertilizer was captured in the substrates. In a field experiment we also fertilized moss and aspen litter with urea ((NH<sub>2</sub>)<sub>2</sub>CO) or NH<sub>4</sub>NO<sub>3</sub> at a more operationally relevant rate of 330 kg ha<sup>-1</sup>. We captured the NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> by ion exchange resin at the substrate–mineral soil interface. In contrast to the growth chamber experiment, this fertilizer rate killed the moss and there was no detectable increase in

nutrient levels in the aspen litter or feather moss layers. Instead, the urea was more likely transferred into the mineral soil; mineral soil of the urea treatment had 1.6 times as much extractable N compared to the NH<sub>4</sub>NO<sub>3</sub> treatment. This difference between the growth chamber and field studies was attributed to observed fertilizer-damage to the living moss and possibly damage to the litter microflora due to the higher rate of fertilization in the field. In addition, the early and substantial rainfall after fertilization in the field experiment produced conditions for rapid leaching of N through the organic layers into the mineral soil. In the field, only 8% of the urea-N that was applied was captured by the ion exchange resin, while 34% was captured in for the NH<sub>4</sub>NO<sub>3</sub> fertilization. Thus, the conditions for rapid leaching in the field moved much of the N in the form of urea through the organic layers and into the mineral soil before it was hydrolyzed.

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N. A. Startsev · V. J. Lieffers (✉) · S. M. Landhäusser  
Department of Renewable Resource,  
Centre for Enhanced Forest Management,  
University of Alberta,  
4-42 ESB,  
Edmonton, AB T6G 2H1, Canada  
e-mail: Victor.Lieffers@ualberta.ca

A. Velazquez-Martinez  
Colegio de Postgraduados,  
Montecillo,  
Mexico City 56230, Mexico

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### Introduction

In the boreal and montane forests of Canada, there is interest in using fertilization to increase forest productivity (Mugasha et al. 1999; Yang 1998; Weetman et al. 1995; Brockley 2006). There is particular interest in fertilization of middle aged stands and those approaching maturity to minimize

the time between application and harvest, ensuring the best economic use of the fertilizer. Fertilization rates ranging from 79 to 540 kg ha<sup>-1</sup> (Yang et al. 1988; Mugasha et al. 1999) but rates of ~300 kg ha<sup>-1</sup> are commonly applied (Brockley 2006) when there is a single application of fertilizer. The recovery of fertilizer by the trees in forest stands is often very low; as little as 2–10% of the applied N may actually be taken up by trees (Preston and Mead 1994). While there have been discussions of possible reasons for variation in response, there have been few studies investigating the movement of fertilizers in boreal forest sites.

The very low recovery of fertilizer by the trees in forest stands is puzzling considering that up to 80% of the applied N in agricultural fields may be taken up by the crops (Bouwman et al. 2005). A critical difference in the method of fertilization between agricultural and forestry systems is that in agricultural applications, the fertilizers are usually applied directly on or into the mineral soil while in forest applications, the fertilizer is broadcast onto the forest floor. This is particularly important in boreal forests where the mineral soil may be covered by a thick organic forest floor, which is capable of absorbing large amounts of moisture and potentially holding nutrients for some time (Weber and Van Cleve 1984). Therefore, when fertilizer is applied in these forests, it must pass through the forest floor before it can be taken up by the tree roots. In boreal forests, forest floor may be derived from deciduous broadleaf litter (typically moder humus types) or from feather mosses and slowly decomposing conifer litter (predominant mor humus types). The living layer of feather moss adds another level of complexity, as mosses can actively take up N into its tissues (Carleton and Read 1991; Startsev and Lieffers 2006). There is a need to understand the fate of the applied N following fertilization. It is not known how much of the N is intercepted by these different layers and more importantly how much passes through these layers to the rooting zone below, to be available for uptake by the trees (Cabrera et al. 2005).

N can be added in the form of mineral fertilizer, such as ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) or in the organic form of urea ((NH<sub>2</sub>)<sub>2</sub>CO). Preston and Mead (1994) reported that efficiency of both forms of fertilizer was limited in the forest applications, and attributed observed losses to leaching and denitrifica-

tion. Urea in particular, is noted to have lower rates of transfer to the trees than the mineral forms of N (Nõmmik et al. 1994) but it is commonly used in forestry operations because of its lower costs, safety, and ease of transport. In order to be available for plant uptake, urea has to undergo enzymatic hydrolysis to NH<sub>4</sub><sup>+</sup> but this form of N is susceptible to volatilization losses in the form of NH<sub>3</sub> (Black et al. 1987). Only a few studies have examined how forest floor quality and type of fertilizer will affect the N transfer through the upper forest floor layers into the rooting zone in boreal forest ecosystems (Weber and Van Cleve 1984).

The rate of transfer may also be affected by timing of rainfall after fertilization (Kissel et al. 2004) but this has not been studied in boreal systems which have much thicker layers of organic matter than most other forests. A heavy rain storm soon after application of the fertilizer can saturate the organic layer and efficiently leach nutrients into the rooting zone of the trees (Black et al. 1987) while the same amount of precipitation delivered in a sequence of small events over a number of days would keep the organic layer moist but little water would be moved through to the mineral soil (Carrier and Bernier 1971). Extended retention of fertilizer in the organic layer may also promote volatilization of ammonia if fertilizer is applied in the form of urea (Kissel et al. 2004).

A growth chamber and field experiment were conducted to assess the efficiency of transfer of N either in the form of ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) or urea ((NH<sub>2</sub>)<sub>2</sub>CO) through two different types of organic substrates (aspen litter and feather moss) of the forest floor. In the growth chamber experiment we also tested the effect of different watering regimes on N-transfer through the organic substrates. We hypothesized that organic substrates, especially living layers of feather moss, would take up a large proportion of the fertilizer and that rapid flushing of these substrates would tend to move the fertilizer through the organic layers before this uptake could occur.

## Methods

### Growth chamber experiment

In this experiment our objective was to test the movement of N through live feather moss and aspen

substrates without the interfering uptake by tree roots. For this growth chamber experiment, the organic forest floor layers (L, F, and most of the H horizon) were collected in May 2006 in seven closed-canopy aspen (*Populus tremuloides*) stands and the feather moss layer in seven closed-canopy lodgepole pine (*Pinus contorta*) stands. Aspen stands were selected along a 10 km stretch of road 25 km south of Fox Creek, Alberta (N 54°05', W 116°45') and the pine stands were selected in an area 55 km west of Fox Creek (N 54°23', W 117°41'). All stands were in the Lower Foothills Natural Subregion (Beckingham et al. 1996). Feather moss layers were dominated by *Pleurozium schreberi* with lesser amounts of *Hylocomium splendens* and *Ptilium crista-castrensis*. Intact layers of forest floor (substrate) were carefully collected in continuous mats placed on large flat trays, thereby avoiding the mixing and disturbing of the organic horizons. In the laboratory, the substrate on each tray was cut into nine circular sections and placed into double-bottom pots. These pots had a false bottom—an attached pan that allowed accumulation of drainage water, while allowing complete drainage of the main pot.

All pots were placed in a growth chamber with 16 h of light ( $50 \mu\text{mol m}^{-2} \text{s}^{-1}$  at the pot surface), with 20°C during day time and 16°C night time with a relative humidity of 60%. The study design was a  $2 \times 3 \times 3$  factorial design (two substrates, three fertilizer regimes, and three watering treatments). The seven sampling locations served as replicates in the experiment, resulting in a total of 126 pots. The nine pots from each substrate and sampling location were randomly assigned to one of the three fertilization regimes (control,  $\text{NH}_4\text{NO}_3$ , or  $(\text{NH}_2)_2\text{CO}$ ) and three water regimes (continuously moist, flushing, or wet/dry) treatment combinations. The fertilizer treatment consisted of an unfertilized control, and  $0.177 \text{ g pot}^{-1}$  of N in the form of chemical grade  $\text{NH}_4\text{NO}_3$  and  $(\text{NH}_2)_2\text{CO}$ , which is an equivalent application of  $100 \text{ kg ha}^{-1}$  N. We originally started the experiment with  $330 \text{ kg ha}^{-1}$  N but shortly after application the moss layer died, so we repeated the experiment with a lower dose of  $100 \text{ kg ha}^{-1}$  N that feather mosses are known to tolerate (Kellner and Marshagen 1991). In order to prevent volatilization losses of N from the leachate collected in the false bottom of the pots, 0.25 g of copper sulphate ( $\text{CuSO}_4$ ) was added to the false bottom to stop hydrolysis and 5.0 g of boric acid

( $\text{H}_3\text{BO}_3$ ) was added to maintain a low pH to prevent conversion of existing  $\text{NH}_4^+$  to  $\text{NH}_3$  gas. The three watering regimes were applied as: (1) a continuously moist regime (daily misted with distilled water at a rate that did not cause run-through)—hereafter referred to as continuously moist; (2) a daily simulated small rain events equivalent to  $\sim 2.0 \text{ mm/day}$  (daily misting resulting in water collection in the false bottom of the pots)—referred to as flushing and (3) a repeated rewetting and desiccation treatment (forest floor was permitted to dry naturally and was re-moistened twice during the experiment)—referred to as wet-dry. To control evaporation, pots were all covered with a clear rigid plastic cover, with a 7 mm diameter hole in the centre. In a pilot study we found that at this size of opening, the tips of the mosses showed only minor desiccation over a period of 24 h. Pots were moved weekly to different areas of the bench to minimize spatial effects in the growth chamber.

After 28 days, the amount of leachate collected in the bottom reservoir of the pot in the flushing treatment was approximately 250 ml. At this time the other treatments were also watered to excess to accumulate 250 ml of leachate in the bottom reservoir of the pot. This was achieved by sprinkling  $\sim 2.0 \text{ mm}$  of water over the surface of organic layer every 10–15 min over a period of 60 min. Leachates, including any suspended material, were collected, weighed, and total dissolved N concentration was analyzed using a carbon and nitrogen analyzer (Shimadzu Corp., Kyoto, Japan). The remaining substrate in the pots was dried at 68°C, weighed, ball-ground and analyzed for total N concentration using the Dumas method of high temperature combustion (Costech elemental analyzer (ESC 4010) (AOAC Official Methods of Analysis 2000). For both leachate and substrate N was expressed as mass of total N.

### Field experiment

In summer of 2006, a field experiment was conducted to test the movement of N through moss and aspen litter layers at a more operationally-relevant rate of fertilization and under field conditions. Ten sites were located along a 10 km transect, 20–30 km southeast of Hinton, Alberta (N 53°09', W 117°20') in the Lower Foothills Natural Subregion (the same subregion as used in the above study). This Subregion has

an average 464 mm annual precipitation and 12.8°C mean temperature from May to August and -7.8°C mean temperature from November to February (Beckingham et al. 1996). The ten sites were at least 200 m apart and were located in second-growth 30 to 45-year-old stands regenerated naturally following logging and drag scarification. Stands were dominated by lodgepole pine with interspersed patches of aspen. Thus, feather moss and aspen litter layers were found on the same sites. Stands were fully-stocked and ranged from 12 to 16 m tall. Soils across the sites ranged from Orthic Dystric Brunisol to Gleyed Dystric Brunisol (Canadian Soil Classification 1998) with textures ranging from sandy loam to loam. pH of the top mineral horizons (10 cm) under the pine canopy were on average 4.0 and under an aspen canopy 4.7. In each site, three plots were selected in areas with a uniform overstory of pine and a continuous layer of feather moss (dominated by *Pleurozium schreberi*) 7 to 9 cm thick and three plots were selected in nearby areas dominated by aspen which had a continuous LFH layer dominated by aspen litter (6–7 cm thick). Therefore, each site had six plots for a total of 60 plots in this study. Each plot was 30×60 cm in size and was assigned to one of three fertilization treatments: control (no fertilizer), ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>), and urea ((NH<sub>2</sub>)<sub>2</sub>CO).

Two flat resin bags were inserted horizontally at the organic–mineral soil interface, at both ends of the longest axis of each plot. The centre of each plot was thus preserved for soil sampling at the end of the experiment. The resin bags were made as follows: A rigid polyethylene screen (1 mm mesh) that was cut in a 10 cm diameter disc to maintain a flat shape and consistent area. Discs were inserted in a stretchable nylon mesh bag. Exchange resin (25 g of mixed bead Exchange Resin, IONAC® (TJ Baker Phillipsburg NJ) NM-60, H<sup>+</sup>/OH<sup>-</sup> form, <1 mm bead was washed with NaCl, HCl and rinsed with distilled water (Thiffault et al. 2000) and placed into the bag. The bag was then stretched over the rigid screen, tied tightly and the resin was evenly spread over the surface of the screen, before inserting it horizontally into the soil at the organic–mineral interface. The resin was sufficient to cover the disc and the disc defined the size of the exchange surface for the ions moving through the disc. In the middle of July, fertilizer treatments were applied evenly over the plot at a rate of 6 g N plot<sup>-1</sup> which is equivalent to a rate

of ca. 330 kg N ha<sup>-1</sup>. After 17 days, resin bags were collected and a sample of the litter layer and of the mineral soil (0–5 cm depth) was collected in the middle of the plot. It was assumed that 17 days was sufficient time for full hydrolysis of urea (Foster et al. 1985); leaving the experiment longer would have increased the error because of uptake of the N by plant roots. Soil samples were brought to the lab, dried at 68°C and ground to pass a 2 mm mesh.

Extractable mineral N in the soil sample (5 g dry weight) as well as mineral N in resin bags was extracted with 100 ml of 2 N KCl. Extracts of both mineral soil and resin were analyzed for NO<sub>3</sub><sup>-</sup> or NH<sub>4</sub><sup>+</sup> colorimetrically using a Technicon™ autoanalyzer. Results for soil mineral N were expressed per weight of dry soil; results for the mineral N extracted from resin bags were expressed per unit surface area (square centimeter) of the flat resin bag.

At the time of fertilization, the forest floors were moist and there was ca. 10 mm of precipitation the next day. During the experimental period, total precipitation was measured in each of the ten sites using two rain-gauges installed under either the aspen or pine in each stand. Over the time of the experiment, there was a mean of 27±1.8 mm (SD) of precipitation recorded and there was no difference between the pine or aspen plots ( $p=0.338$ ). According to records from the nearby Hinton airport (ca. 30 km away) during the 17 day measurement period there were 15 days with precipitation and of those, 3 days with over 10 mm of precipitation.

### Statistical analysis

The growth chamber experiment was a fully factorial 2×3×3 design with two substrates (moss and aspen litter), three fertilizations (control, NH<sub>4</sub>NO<sub>3</sub>, (NH<sub>2</sub>)<sub>2</sub>CO), and three watering regimes (continuously moist, flushing, wet/dry) as the treatment factors. Response variables were total N in substrate and N in the leachate. All response variables conformed to the assumptions of normality and equality of variance.

The field experiment was a fully randomized 2×3 block design with sites as the block factor and substrate (moss and aspen litter) and fertilization (control, NH<sub>4</sub>NO<sub>3</sub>, (NH<sub>2</sub>)<sub>2</sub>CO) as the treatment factors. Response variables measured were extractable NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> in mineral soil and resin and the ratio of NO<sub>3</sub><sup>-</sup>/NH<sub>4</sub><sup>+</sup> in the soil and the resin. All data of

extractable N were  $\ln(x+1)$  transformed to conform to the assumptions of normality and equality of variance.

To test for treatment effects, ANOVA procedures using the general linear model in release 8.1 of SAS® (SAS Institute Inc. Cary, NC) were performed. Multiple comparisons were made with the LSD test and a significance level of  $\alpha=0.05$  was used for all response variables.

## Results

### Growth chamber experiment

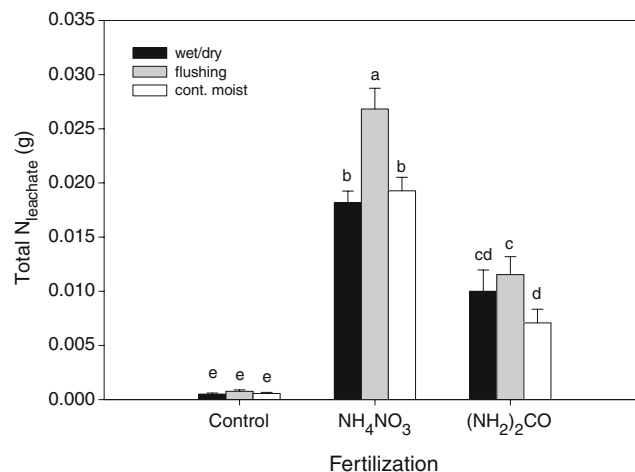
Urea fertilization caused some browning of the feather moss shoots, while after fertilization with  $\text{NH}_4\text{NO}_3$  there was a shift from dark green to a lighter green. In both cases, however, the moss still appeared to be alive at the end of the experiment and the color changes occurred regardless of watering regimes.

There were no significant three-way interactions between substrate type, moisture regime, and fertilization for both total N in the substrate ( $p=0.630$ ) and total N in the leachate ( $p=0.745$ ). Fertilizer treatments significantly increased N in both substrates by an average of 0.128 g over the control (no fertilizer applied) ( $p<0.001$ ) but there were no differences between the two fertilizer types ( $p=0.802$ ) and the

three watering regimes ( $p=0.227$ ). In the control, aspen litter contained an average of 0.52 g of N compared with 0.40 g N in the moss ( $p<0.001$ ).

Total N in the leachate, was significantly affected by the main effects of substrate, fertilizer type, and watering regime (all  $p<0.001$ ). In terms of main effects, on average there was 0.009 g N in the leachate of the moss compared 0.012 g N in the aspen litter (data not shown). The repeated flushing treatment moved more of the N into the leachate while the wet/dry cycle had the least effect on total N concentration in the leachate (Fig. 1). There was nearly twice as much N in the leachate after fertilization with  $\text{NH}_4\text{NO}_3$ , and more N was transferred to the leachate by the aspen litter than by the feather moss (Fig. 1) There was, a significant two-way interaction for total N in the leachate between fertilization and the watering regime ( $p=0.029$ ) (Fig. 1). While total N in the leachate was little affected by the watering regime in the control treatment, significantly more N was leached through the substrates in the flushing treatment, especially in the  $\text{NH}_4\text{NO}_3$  treatment compared to the urea treatment (Fig. 1).

By the end of the experiment, across all treatments 72% of the applied N, regardless of substrate, fertilizer type, and watering regime (all  $p>0.272$ ) was retained in the substrate and 9% was captured in



**Fig. 1** Growth chamber experiment: total N in the leachate (AVG  $\pm$  SE) of both substrates combined, at three different fertilizer treatments and watering regimes; there was a significant fertilizer by watering regime interaction (see “Results” for further explanation). Watering regimes were wet/dry—two cycles of wet–dry; flushing—daily simulated rainfall (with

small amounts of run-through) equivalent to  $\sim 2 \text{ mm day}^{-1}$ ; continually moist—daily misted but with no run-through. At the end of the experiment the wet/dry and continuously moist treatments were watered to have total leaching equal to that of the flushing treatment (see “Methods”). Bars with different letters were significantly different at  $\alpha<0.05$  ( $n=7$ )



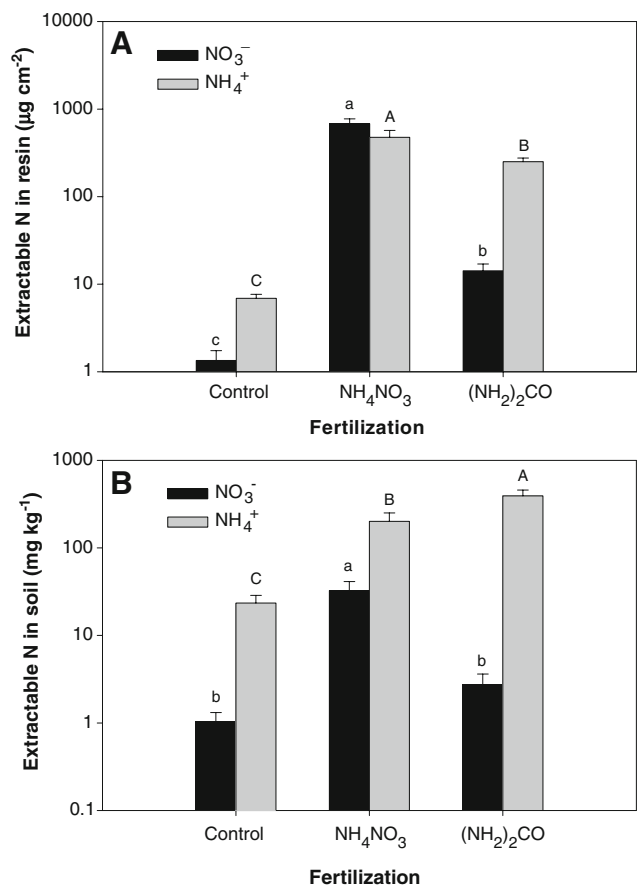
the leachate (but there was variation in N in leachates among treatments—see above). Overall, 19% of added N was not accounted for by these analyses.

### Field experiment

Both urea and  $\text{NH}_4\text{NO}_3$  fertilizer application caused severe damage to the feather moss. Before fertilization aspen litter had 24% higher N than the moss substrate ( $p=0.013$ ) (data not shown). After fertilization there was no increase in N concentration of the fertilized substrate relative to the controls ( $p=0.416$ ) and there was no substrate by fertilizer interaction ( $p=0.637$ ).

Substrate type had no significant effect on any of the measured variable in either the resin or the mineral soil. The fertilizer treatments significantly affected the amount of extractable  $\text{NO}_3^-$  and  $\text{NH}_4^+$  and their ratios ( $\text{NO}_3^-/\text{NH}_4^+$ ) in both the resin (Fig. 2a) and the mineral soil (Fig. 2b) (all  $p<0.001$ ).

**Fig. 2** Field experiment: extractable N from **a** resin bags and **b** mineral soil (0–5 cm), below the litter layer dominated by either feather moss or aspen litter, after three fertilization treatments. Data for both substrates (feather moss and aspen litter) were combined as there was no significant differences between substrates ( $p>0.209$ ) and their interactions with the fertilizer regimes ( $p>0.349$ ).  $\text{NH}_4^+$  and  $\text{NO}_3^-$  were analyzed separately and *different letters* indicate significant differences at  $\alpha<0.05$  ( $n=10$ )



The interception of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  by the resin was higher in the  $\text{NH}_4\text{NO}_3$  treatment than in the urea treatment (both  $p<0.05$  for  $\text{NH}_4^+$  and  $\text{NO}_3^-$ ; Fig. 2a). On an area basis, the resin intercepted 34% of the total applied N in the  $\text{NH}_4\text{NO}_3$  treatment in the form of  $\text{NH}_4^+$  and  $\text{NO}_3^-$ , compared to 8% capture in the urea treatment (Fig. 2a). The ratio of  $\text{NO}_3^-/\text{NH}_4^+$  collected in the resin was 1.70 for the  $\text{NH}_4\text{NO}_3$  fertilization treatment compared to 0.41 for the urea and 0.08 for the control treatments ( $p<0.05$ ). However, the ratios between the control and the urea treatment were not statistically different ( $p>0.05$ ).

In the mineral soil the  $\text{NO}_3^-$  concentration was higher in the  $\text{NH}_4\text{NO}_3$  treatment compared to the urea treatment ( $p<0.05$ ); the amounts of  $\text{NO}_3^-$  were  $52.2 \text{ mg kg}^{-1}$  in the  $\text{NH}_4\text{NO}_3$  treatment compared to  $2.7 \text{ mg kg}^{-1}$  in the urea and  $1.04 \text{ mg kg}^{-1}$  in the control treatments. These concentrations of  $\text{NO}_3^-$  were small relative to the  $\text{NH}_4^+$  concentrations in

the mineral soil (Fig. 2b), thus, the  $\text{NO}_3^-$  to  $\text{NH}_4^+$  ratios in the mineral soil were much lower than in the resin. The  $\text{NO}_3^-$  to  $\text{NH}_4^+$  ratio was the highest in the  $\text{NH}_4\text{NO}_3$  treatment (0.39) compared to the control (0.09) and the urea treatment (0.009) ( $p < 0.001$ ). In terms of total extractable N in the mineral soil, the urea treatment had the highest total N concentration with approximately 400 mg of N  $\text{kg}^{-1}$ , compared to 250 mg of N  $\text{kg}^{-1}$  for the  $\text{NH}_4\text{NO}_3$  treatment and 24 mg of N  $\text{kg}^{-1}$  in the control ( $p < 0.001$ ).

## Discussion

Results from the growth chamber experiment indicate that under low rates of application (100 kg  $\text{ha}^{-1}$ ) much of the fertilizer (72%) was retained in the aspen litter and moss substrates and very little of the N was actually moved through these substrates, regardless of the type of fertilizer; this was so even with the large final flushing with water. These results agree with a previous study where small additions of labeled  $\text{NH}_4^+$  or  $\text{NO}_3^-$  were retained in feather moss in Alaska (Weber and Van Cleve 1984). In moderate doses, N additions inhibit enzymatic activity in the forest floor and this process was suggested to increase N retention in the organic horizons (Kang and Lee 2005). On the other hand, adding 330 kg  $\text{ha}^{-1}$  of N in the field experiment resulted in relatively little of the added N being retained by the feather moss or aspen litter layer regardless of the type of fertilizer. This may be related to the heavy rainfall shortly after application of the fertilizer in the field flushing the fertilizer through the organic substrates. Secondly, it might also relate to the osmotic stress from the higher levels of N in the field experiment damaging or killing the feather moss and likely the microflora in both substrates (Söderström et al. 1983; Parrent and Vilgalys 2007; Demoling et al. 2008) thereby inhibiting their capacity to take up the fertilizer (Startsev and Lieffers 2006). This allowed nutrients to be moved unimpeded down through the forest floor layers either to the resin or the mineral soil. This may explain the need for relatively high doses of fertilizer applied to organic substrates before there is a growth effect from single doses of fertilization of boreal forests (Nõmmik and Möller 1981; Farnden and Herring 2002).

In terms of N transfer through the feather moss or aspen litter substrates, in the field experiment, there

was relatively little difference between the two substrates. This was unexpected as we anticipated that the living moss would take up more nutrients than the aspen litter, but given that the moss layer was damaged, it apparently behaved similar to decomposing organic materials such as the leaf litter. In the growth chamber experiment where the moss remained alive because of the lower rates of fertilization, there was less of the N transferred to the leachate than in the aspen litter. This may relate to the greater potential for uptake by the moss but more likely it was related to the inherently higher level of N in the litter compared to the moss.

In terms of the type of fertilizer, in the field experiment, a greater proportion of the N from the urea treatment was transported through the organic layers, while in the growth chamber experiment the  $\text{NH}_4\text{NO}_3$  treatment had higher levels of N leached through. This was unexpected as there are examples of less N being moved to the rooting zone with urea fertilization than with  $\text{NH}_4\text{NO}_3$  fertilization (e.g. Nõmmik and Möller 1981). The reason for this was likely the significant rainfall events over the 17 days of the field experiment, which resulted in considerable flow of water through the substrates. Heavy precipitation soon after application likely moved the urea through the organic layer in its organic form. This speculation is supported by the relatively poor interception of N by the ion-exchange resin in the urea fertilization treatment (Fig. 2a) and the fact that the resin under the organic substrates captured more than 4 times as much N in the  $\text{NH}_4\text{NO}_3$  compared to the urea treatment. Secondly, while the urea treatment did not have an elevated concentration of N in the organic substrates, it had the highest levels of total extractable N in the mineral soil layer (Fig. 2b), indicating that the fertilizer was transferred to the mineral soil. In contrast, the urea treatment in the growth chamber experiment resulted in less N passing through the substrate than in the  $\text{NH}_4\text{NO}_3$  treatment. The lack of transfer of N may be related to the warmer temperatures of the substrates in the growth chamber experiment that allowed for faster hydrolysis of the urea within the substrate compared to the field study. Once hydrolyzed, the elevated pH following urea hydrolysis (Malhi et al. 1992) likely created conditions that promoted some volatilization of  $\text{NH}_3$  from substrates with inherently high pH or low buffering capability (Fan and Mackenzie 1993).



Volatilization of  $\text{NH}_3$  or denitrification (Startsev and Lieffers 2007) may explain the 19% of added N that could not be accounted for in either the substrate or leachate in the growth chamber experiment.

In terms of watering regime, the growth chamber experiment indicated that the best transfer of N through the substrates occurred when there was daily excess of moisture. While in the growth chamber experiment, the 2 mm of water per day was not a heavy level of flushing, the trends are similar to the rainy conditions that were observed during the field study. This reinforces the notion of the importance of applying urea immediately before a heavy rainfall (Kissel et al. 2004). In the growth chamber experiment, wet/dry conditions transmitted less of the N to the leachate. If the pots were allowed to dry out and then be rewetted the rate of urea hydrolysis was likely reduced during the periods of drying (Klose and Tabatabai 1999). It is also possible that fracturing of the substrate during handling might have increased water channeling through the litter, thereby make the leaching less effective during the larger watering events. Continuously moist conditions may have increased the nutrients immobilization rate in the substrate (Compton and Boone 2002) or promoted denitrification (Startsev and Lieffers 2007) as under those conditions N transfer to the leachate was low.

Results of the field experiment suggest that various N-transformation processes take place in the organic layers of the forest. The urea treatment resulted in increased levels of  $\text{NO}_3^-$  captured by the resin than the control treatment (Fig. 2a). Since the endpoint of urea hydrolysis is  $\text{NH}_4^+$  (Malhi et al. 1992) these data indicate that some nitrification occurred in the litter layer. Typically it is thought that a low pH in boreal forest litter is an obstacle for nitrification (Rudebeck and Persson 1998); however, since urea hydrolysis elevates the substrate pH, it may have temporarily created conditions conducive to nitrification. In the  $\text{NH}_4\text{NO}_3$  treatment exchange resin captured more  $\text{NO}_3^-$  relative to  $\text{NH}_4^+$ ; assuming that the resin has an equal efficiency of capture of  $\text{NO}_3^-$  and  $\text{NH}_4^+$  (Kjønnaas 1999), the greater rate of transfer of  $\text{NO}_3^-$  to the resin was likely related to the greater mobility of  $\text{NO}_3^-$  ions compared with  $\text{NH}_4^+$ . The  $\text{NH}_4^+$ -N was more likely to reside in the substrate for a longer period of time (Vitousek et al. 1982). In soil, however, the  $\text{NO}_3^-$  to  $\text{NH}_4^+$  ratio was less than 1 even in the  $\text{NH}_4\text{NO}_3$  treatment. This could be the result of rapid

$\text{NO}_3^-$  uptake by roots and the microflora in the soil (Forde and Clarkson 1999) or of processes related to assimilative nitrate reduction which occur at a greater rate than nitrification in forest soils (Westbrook and Devito 2004).

Overall, these experiments indicate that the organic substrates are effective in retaining the applied fertilizer when application rates were low. However, retention by the organic layers was virtually stopped when fertilization rates were high enough to damage microflora and moss tissues (Demoling et al. 2008), in combination with enough rain to leach nutrients through the organic layer. Second, under moist (leaching) and cool conditions of the field site, it is likely that much of the urea was transferred through the organic substrates before hydrolysis. This supports the idea that urea is best-applied immediately before significant rainfall. Third, it is notable that in the urea treatment some of  $\text{NH}_4^+$  was converted to  $\text{NO}_3^-$  while still in the organic layer, likely because of elevated pH after hydrolysis.

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