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THE FATE OF ^{15}N-LABELED AMMONIUM AND NITRATE APPLIED ON TREES
CANOPY IN A MATURE BALSAM-FIR STAND, QUÉBEC.

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ABSTRACT

Canopy uptake of atmospherically deposited nitrogen (N) in N-limited boreal forests may represent an important source of N for growth. A tracer study was conducted by repeated applications of artificial precipitation with ambient inorganic N concentration (two treatments: ^{15}N-NH$_4$NO$_3$ and NH$_4$^{15}N-NO$_3$, 98 at.% with three trees per treatment) misted directly on the crown of individual mature balsam fir trees (Québec, Canada) during two growing seasons. Control trees were used to determine natural ^{15}N abundance values. At the end of the experiment, mature trees' needles and twigs, epiphytic lichens growing on their stem and branches, understory balsam fir seedlings' needles and branches, and litter layer (L), showed significant ^{15}N enrichments for both treatments as compared to control trees, while there were no differences for understory balsam fir seedlings' stem and roots, soil FH and B horizons. Following NH$_4$ and NO$_3$-tracer addition, except for mature trees' needles that showed significantly higher enrichment for ^{15}NH$_4$$^+$ relative to ^{15}NO$_3^-$, in all other compartments enrichment was similar for both ions. Because of the way the tracer was added, i.e. as a fine mist on an individual tree basis, a significant amount was lost. When taking into consideration total N biomass of each compartment, total tracer recovery was estimated to be between 25.8 and 53.2% for ^{15}NH$_4$$^+$ and 39.6 and 81.5% for ^{15}NO$_3^-$. Calculating the tracer repartition only for the amount recovered showed that most of the added ^{15}NH$_4$$^+$ and ^{15}NO$_3^-$ was found in aboveground vegetation (64.3 and 70.7%, respectively) as compared to the litter layer (L) and soil organic horizons (FH) (35.7 and 29.4%, respectively). Overall, twigs showed the highest recovery for both ions (49.9 and 63.0%, respectively). Contrary to studies where N is added directly to the soils, these data show that aboveground biomass may capture N in amounts comparable to or higher than the soil when the tracer is applied *in situ* directly on the canopy in a way that reproduces natural N deposition processes.

Keywords: ^{15}N-tracer, wet deposition, vegetation retention, ammonium, nitrate, *Abies balsamea*

ACKNOWLEDGMENTS

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDIXES

1. INTRODUCTION

Boreal forests growing in young postglacial landscapes are generally nitrogen (N) limited (Lambers et al. 2008; Tukey 1970; Vitousek and Howarth 1991). Increased deposition of this essential nutrient worldwide has raised biochemical and ecological questions (Galloway et al. 2004; Galloway et al. 2008; Grantz et al. 2003; Hogberg 2007). For instance, a growing number of studies suggest that increases in tree growth over recent decades in some areas of the northern hemisphere might be linked with higher N deposition (Magnani et al. 2007; Nave et al. 2009; Reich et al. 2006; Sievering et al. 2000; Thomas et al. 2010). However, the fate of N deposited on forest ecosystems is still largely unknown, particularly for forests receiving low rates of N deposition.

Labeling studies using the heavy stable isotope of N (i.e. ^{15}N) have been conducted to address some aspects of N cycling and retention in forests, under controlled as well as natural conditions. Until now, the soil, rather than the vegetation, has been considered the principal sink for N wet deposition in N-limited or N-saturated systems (Templer et al. 2012). However, this conclusion is somewhat in disagreement with throughfall and stemflow observational studies and experiments, where forest canopy is an important sink for inorganic N, especially in N-limited systems (Cape et al. 2001; Chiwa et al. 2004; Gaige et al. 2007; Houle et al. 1999). This discrepancy could be explained by two factors. In most experiments, ^{15}N was added directly to the forest floor bypassing potential interactions with aboveground compartments (as suggested by Jenkinson et al. 1999;

Sievering 1999). Secondly, when ^{15}N tracers were applied to the aboveground vegetation, either in a plantation (Adriaenssens et al. 2011; Bowden et al. 1989; Chavez-Aguilar et al. 2006; Eilers et al. 1992; Wilson and Tiley 1998), in a greenhouse (Lumme 1994; Lumme and Smolander 1996; Macklon et al. 1996), or directly in the field (Boyce et al. 1996; Garten et al. 1998; Nave and Curtis 2011; Vose and Swank 1990), it was done only partly on branches of mature trees (Boyce et al. 1996; Vose and Swank 1990) or on seedlings or saplings, which may not allow inferences to be made on processes by which the whole mature tree acquires N. Young versus mature trees are known to have important distinctive morphological, structural and physiological features that affect the chemistry and flux of throughfall and stemflow or directly influence N demand (see reviews in Olson et al. 1981; Wilson and Tiley 1998). In addition, most studies adding N either directly on the soil or below the aboveground vegetation have been conducted in areas receiving medium-to-high N deposition rates, which are known to alter the natural N cycling and demand in ecosystems (Galloway et al. 2003). Nitrogen inputs of less than 20 kg $ha^{-1} \cdot yr^{-1}$ are considered low while nitrogen inputs greater than 20 kg $ha^{-1} \cdot yr^{-1}$ are considered high (Feng et al. 2008). Nitrogen inputs above 40 kg N $ha^{-1} \cdot yr^{-1}$ are associated with harmful effects on vegetation (Mohren, 1986 in Sievering et al. 2000). Moreover, although relevant when trying to understand the effects of fertilizer N or simulating future elevated rates of atmospheric N deposition on ecosystem processes, many studies used N levels that are much higher than natural deposition rates.

Dail et al. (2009) are the firsts that have added N directly on the top of matures trees *in situ*. They distributed N isotopically labeled ((^{15}N-NH$_4$)$_2$SO$_4$ and Na^{15}N-NO$_3$, 10% enriched with NH$_4$NO$_3$) with a helicopter directly onto the canopy of a mature spruce-hemlock forest (five doses a year for a total of 19.8 kg·ha^{-1}·yr^{-1}). After three years of treatment, labeled-^{15}N ammonium (^{15}NH$_4^+$) and labeled-^{15}N nitrate (^{15}NO$_3^-$) recovery was 38 and 68% of the added ^{15}N, respectively. As opposed to other results available in the literature, the aboveground-parts of the trees were the principal sink for ^{15}N, with branches and stem bark being particularly efficient in retaining the applied ^{15}N, accounting together for 25 and 50% of the total added ^{15}NH$_4^+$ and ^{15}NO$_3^-$, respectively. Soil ^{15}NH$_4^+$ and ^{15}NO$_3^-$ recovery was 8.9 and 6.9% with no labeled N detected in the B horizon. However, only 1.5% of added ^{15}NH$_4^+$ and ^{15}NO$_3^-$ was recovered in live foliage and bole wood, suggesting that high retention by trees may have been through physical-chemical processes rather than by physiological uptake. Fine and coarse roots also recovered 3.2 and 2.1% of ^{15}NH$_4^+$ and ^{15}NO$_3^-$, respectively. It is not known at the moment if and how this N is redistributed in different tree parts over time.

Although the method used by Dail et al. (2009) allowed the fate of wet N inputs to be more closely approximated than in previous studies, they advocated that more frequent applications of smaller doses might have been preferable to better emulate ambient deposition and help overcome phenology-based uptake patterns. Each of their applications added more N than the total annual N load at their site. As stated earlier, the use of N quantities superior to the natural deposition rate is widespread in published ^{15}N studies and

appears to be a particularly important issue when one tries to simply trace N depositions. For example, it has been demonstrated that N-uptake by aboveground-parts increases with increasing N concentrations in the tracer solution (Chavez-Aguilar et al. 2006; Vose and Swank 1990). Other factors like duration of exposure and irrigation intensity (Eilers et al. 1992; Reiners and Olson 1984) or timing of N application during the year (Adriaenssens et al. 2011; Amponsah et al. 2004) may also affect N uptake by plants.

In addition to N uptake in the forest canopy and soil compartments that have been measured in the past, other forest compartments that have received less attention in ^{15}N studies may interact and intercept the downward flux of reactive N before it reaches the soil. Compartments such as lichens that proliferate in mature and old-growth boreal coniferous stands (Arseneau et al. 1998; Hauck and Meissner 2002; Lang et al. 1980) and that are particularly well-adapted to take up water and nutrients from atmospheric sources (Johansson et al. 2010; Lang et al. 1976; Nash 2008) may play an important role. Moreover, although there have been studies where N was added directly on seedlings and young trees, results are still scarce on natural retention rates of ^{15}N by seedlings of shade-tolerant species after applications on the mature trees' canopy (Garten et al. 1998). Seedlings experience severe resource limitations caused by competition with mature trees for belowground resources (Booth 2004; Coomes and Grubb 2000). In this context, access to N from precipitation may serve as a secondary source from which they can fulfill their demand for this element.

This study aimed at tracing the fate of N added by repeated applications of isotopically labeled $NH_4{}^{15}N\text{-}NO_3$ or $^{15}N\text{-}NH_4NO_3$ (98 at.%) below ambient rain concentration, to minimize fertilization effect. The tracer solutions were sprayed with sprinklers directly on the crown of individual mature trees throughout two growing seasons. Duration, intensity and chemistry of the sprayed solutions were similar to values of natural rain events at the site. The objectives were (1) to characterize retention patterns by the tree crown including lichens and foliage and twigs of different age classes and by the other compartments of the system such as the understory seedlings and different soil layers and (2) to determine if the ^{15}N interception of the compartments differed for $^{15}NH_4{}^+$ and $^{15}NO_3{}^-$. The main hypothesis tested was that more ^{15}N would be retained in aboveground vegetation than in the soil.

2. MATERIAL AND METHODS

2.1. Study area

The experiment took place in a 60-year-old even-aged stand regenerated from advance growth after cutting (800 m a.s.l) situated on the watershed of Lake Laflamme (41°17'N ; 71°14'W) in Québec, Canada. The stand cover consists of balsam fir (*Abies balsamea* (L.) Mill.; 80%) and white birch (*Betula papyrifera* Marsh.; 20%). During 1975-1984, the stand suffered serious defoliation (between 20 and 70% of the total foliage) following a spruce budworm (*Choristoneura fumiferana* Clem.) outbreak (Barry et al. 1988). Otherwise it is undisturbed. The understory vegetation is not abundant and is mostly composed of seedlings from the trees forming the canopy. Mosses, when present, consist of *Pleurizium schreberi* (Brid.) Mitt. and *Hylocomium splendens* (Hedw.) Schimp. Epiphytic green algal lichens are abundant on the crown and stem of the mature trees. The soil is a sandy loam Orthic Ferro-Humic Podzol developed on a glacial till (0.1-15 m) deposited on granitic bedrock. The underlying humus is classified as Mor. Maximum rooting depth is 0.60 m (Houle and Moore 2008). Average thickness for LFH layers and Ae, B and BC horizons are 20, 6, 60 and 20 cm, respectively (Ouimet and Duchesne 2005). There is a noticeable presence of woody debris on the soil.

Mean annual air temperature is -0.4 °C and annual precipitation is 1133 mm·yr⁻¹ (Houle and Moore 2008), of which 31.3% falls as snow (Houle and Carignan 1992). During the May-September periods in 2009 and 2010, the stand received 578 mm and 573 mm of

precipitation, respectively (Duchesne, unpublished data). The yearly wet N deposition rate $(N-NH_4^+ + N-NO_3^-)$ is $6 \: kg \cdot ha^{-1} \cdot year^{-1}$ (Houle and Moore 2008). The consumption (by roots and/or microbes) of NH_4^+ significantly exceeds its production while the consumption and production of NO_3^- are similar. Discrepancy between NH_4^+ production and consumption is indicative of a high NH_4^+ accumulation potential (Ste-Marie and Houle 2006). The mean residence time of the two forms of inorganic N in the forest floor varies from less than 1 day to 1 week (Houle and Moore 2008; Ste-Marie and Houle 2006). Fertilization experiments also show that this forest has a high retention rate for N (Houle and Moore 2008).

2.2. Labeled-^{15}N ammonium and nitrate additions

The spraying device consisted of a sprinkler installed at the top of a dominant tree in order to spray the tracer solution directly on the canopy of a co-dominant one (Figure 1). Each co-dominant tree represented an experimental unit. Six dominant (DBH of 232 ± 11 mm; height of 18.0 ± 0.6 m) and associated co-dominant trees (DBH of 228 ± 13 mm; height of 17.6 ± 0.8 m) were chosen and attributed randomly to two groups: two treatments (ammonium-labeled [$NH_4^{15}N-NO_3$] and nitrate-labeled [$^{15}N-NH_4NO_3$]; both 98 at.% ^{15}N) for a total of three replicates per group. Three additional trees were also chosen randomly as controls. No treatment was provided to those trees; they were only exposed to ambient N deposition. Average distance between each experimental unit was 13.28 ± 4.83 m. Each co-dominant tree was sprayed with a 75 L solution per application, which represents 1.35 mm of precipitation based on an average sprayed surface of 42 m^2. The solution was composed

of ambient ion concentrations to mimic the composition in rain (0.07 mg·L^{-1} Na$^+$; 0.15 mg·L^{-1} Ca$^+$; 0.03 mg·L^{-1} Mg$^+$; 0.04 mg·L^{-1} K$^+$; 0.02 mg·L^{-1} H$^+$; 0.11 mg·L^{-1} Cl$^-$; 1.64 mg·L^{-1} SO$_4^-$) so as to reproduce light rainfall events. Concentrations of NH$_4^+$ and NO$_3^-$ were 0.245 mg·L^{-1} and 0.845 mg·L^{-1}, respectively. The precipitations containing the ^{15}N tracers were applied from mid-June to mid-September during the 2009 and 2010 growing seasons. Spraying started only after the last spring frost when the site became accessible. However, the spraying period occurred during the most active period of cambial activity and tree growth (Deslauriers et al. 2003). The artificial precipitation lasted approximately 3h30. The treated trees were sprayed nine and fourteen times during the 2009 and 2010 seasons, respectively. The total amount of N and water added, based on an average sprayed surface of 42 m^2, corresponded to 1.1% (0.06 kg·ha^{-1} of which 0.03 kg·ha^{-1} was ^{15}N) and 1.7% (0.09 kg·ha^{-1} of which 0.05 kg·ha^{-1} was ^{15}N) of the yearly wet N-deposition rate in 2009 and 2010, respectively, and 2.1% and 3.3% of the natural rainfall during the 2009 and the 2010 May-September period, respectively.

Figure 1. Sprinkler installed at the top of a mature dominant tree spraying ^{15}N-tracer on the crown of a co-dominant one.

2.3. Sampling procedures

Samples were collected one week after the last application of the tracers in late-September of both years. This period is considered to be the more appropriate for sampling conifer foliage (Lavender and Carmichael 1966; White 1954). In 2009, current- and 1-year-old needles were sampled from three different locations on the upper third of each tree crown using a tree pruner. The needles taken on each tree were combined to form a composite sample. Foliose and fruticose epiphyte lichens were also collected at two opposite sides of the canopy (4.3 ± 0.5 m height) and from the stem (1.6 m height) of each tree. At the end of the 2010 growing season, current-, 1- and 2-year-old needles and twigs as well as ≥ 3-year-old twigs of each tree were sampled. Needles and twigs were also sampled in spring 2010 and during summer 2010. Five complete understory balsam-fir

seedlings (mean stem diameter of 7 ± 1 mm; mean height of 0.5 ± 0.2 m) were also sampled at a maximal distance of 1.59 m $\pm$ 0.22 mm from each treated and control mature tree. The seedlings were divided into four sub-compartments (needles, twigs, stems and roots). Finally, three soil cores were taken at a distance of one meter from each tree and were divided into four sub-compartments (litter (L) layer and H, F and top B horizons). Mosses occasionally present on the ground around the stem were not considered. Ae horizon, when present, was discarded. No attempts were made to separate fresh and older litter.

2.4. Elemental and isotopic analysis

Samples were air dried at 65 °C for 24 to 48 h and ground to a fine powder prior to analysis. Content analyses were then conducted at the organic and inorganic chemistry laboratory of *Direction de la recherche forestière* (*Ministère des Ressources naturelles et de la Faune du Québec*, QC, Canada) and N isotopic analyses at the GEOTOP research centre (*Université du Québec à Montréal*, QC, Canada). Nitrogen content ($g \cdot kg^{-1}$) was determined following Kjeldhal digestion (Kjeltec Tecator 1030). For, P, K, Ca and Mg content of needles, samples were digested with H_2SO_4 and concentrations determined by inductively coupled plasma-atomic emission spectroscopy. Nitrogen stable isotopes ($^{15}N/^{14}N$ or δ^{15}) measurements were made using an elemental analyzer (Elementar Vario Micro Cube^{TM}) coupled with a Micromass Isoprime^{TM} mass spectrometer in a continuous-flow mode and were reported in *per mil* (‰) against atmospheric air N_2 (AIR) standard

(Mariotti 1983). Except for some highly enriched twigs where it was higher, replicate $\delta^{15}N$

measurements from given samples yielded an overall analytical uncertainty of $\pm$ 0.2‰ (1σ).

2.5. ^{15}N enrichment and recovery

Enrichments of ^{15}N (‰) were calculated by subtracting the mean value of a particular N

pool in the control plots from the value of the same pool in each ^{15}N treated plot. The mass

recovery in each compartment is expressed as percent of total applied ^{15}N tracer.

Calculations were made using the mass balance equation (Nadelhoffer and Fry 1994):

$$^{15}N_{comp.} = \frac{m_{comp.}\,(at.\%^{15}N_{comp.} - at.\%^{15}N_{ref.})}{at.\%^{15}N_{tracer} - at.\%^{15}N_{ref.}} \qquad (1)$$

where $^{15}N_{comp.}$ is ^{15}N mass recovered in labeled ecosystem compartments (in g per tree for

needles and twigs or in $g \cdot m^{-2}$ for lichens, seedlings and soil); $m_{comp.}$ is N mass of the labeled

compartment ($g \cdot plot^{-1}$); $at.\%^{15}N_{comp.}$, $at.\%^{15}N_{ref.}$ and $at.\%^{15}\text{-}N_{tracer}$ are the atom percent ^{15}N

of the labeled compartment, the reference compartment (natural ^{15}N abundance) and the

applied ^{15}N tracer. With the $^{15}N_{comp.}$, calculation of % recovery from the fertilizer can be

performed by dividing it by the ^{15}N mass of the fertilizer. According to this equation, total

mass of experimental ^{15}N accumulated by an ecosystem compartment depends on its N

biomass and the proportion of N derived from the labeled tracer. Therefore, accumulation

of ^{15}N by compartments with relatively small N biomass is accompanied by a relatively

high $\delta^{15}N$ increase and accumulation by compartments with larger biomass is accompanied

by a lower increase. As more compartments were sampled and analyzed in 2010, only ^{15}N

recoveries at the end of this season are presented. $\delta^{15}N$ was converted to at.% ^{15}N excess notation using the following equation (Slater et al. 2001) :

$$\text{atom\% } ^{15}N = \frac{100}{\dfrac{1}{(\frac{\delta}{1000} + 1)R_{AIR}} + 1} \qquad (2)$$

where R_{AIR} is the isotope ratio of the atmospheric air N_2 standard $(3.6765 \cdot 10^{-3})$.

Local allometric equations relating needle and branch biomasses to tree diameter were used for calculating the biomass of each tree (Houle and Tremblay, unpublished). Proportion of needles of different ages (20% for each of the first years) is based on the work of Gilmore et al. (1995). Seedling biomasses were estimated using the sampling result from 2010 (data not shown). Lichens (641 kg·ha^{-1}, of which 18% on bole) and soil biomasses derive, respectively, from the works of Lang et al. (1980) and Houle (unpublished data). Finally 10% was added to N concentrations to overcome the underestimation associated with the Kjeldhal method (Houle, unpublished).

The surface effectively sprayed was highly variable and largely influenced by the wind conditions during each spraying event. Based on *in situ* measurements during the first spraying events of the first season (2009), the surface sprayed has been estimated to vary between 42 m^2 and 87 m^2. On certain days, during occasional wind drift, the spraying area could have been greater. If the wind was too strong spraying was delayed or annulled.

Nitrogen isotope results will be discussed in terms of 'retention'. Although the samples were not washed prior to analysis, the high number of experimental precipitation applications over a relatively long period of time suggests that the added ^{15}N was not only weakly retained by canopy surfaces because in this situation, it would have been easily washed off by natural rainfall over time. Retention of ^{15}N in this context includes (1) 'N absorption', i.e. the movement of fluid or a dissolved substance across the plasma membrane; (2) 'N assimilation' i.e. the utilization by a living organism of absorbed nutrients in the process of growth, reproduction, or repair; and (3) 'N adsorption' i.e. the formation of a layer of gas, liquid, or solid on a surface (Garten et al. 1998). N absorption and N assimilation represent the N uptake by the different compartments. In the present study, as soil surface was not cleared of ^{15}N tracer, i.e. tracer could reach the soil via throughfall and stemflow, and owing to the fact that in coniferous species N recycling between the different tree parts is high (Pang 1985), it is impossible to separate roots N uptake from that of the canopy compartments (Boyce et al. 1996; Chavez-Aguilar et al. 2006; Eilers et al. 1992; Lumme 1994; Lumme and Smolander 1996; Nave and Curtis 2011).

2.6. Statistical analysis

Since the sprayed surface area of each treated tree was considered as plot, seedlings (5 repetitions of each sub-compartment per tree) and soil (3 repetitions each sub-compartment per tree) data were pooled prior to analysis. Results from 2009 (preliminary sampling) and 2010 (broader sampling) were analyzed separately.

Analyses of variance (ANOVAs) were performed for each compartment. First, single-factor unreplicated repeated-measures ANOVAs were performed on $\delta^{15}N$, N, P, K, Ca and Mg content of needles and on $\delta^{15}N$ and N content of twigs, seedlings and soil of control plots (random), with sub-compartment as the repeated within-plot factor (fixed; variable levels). For, $\delta^{15}N$ and N content of lichens in those plots, two-factor unreplicated repeated-measures ANOVAs were performed with location (fixed; 2 levels) and functional group (fixed; 2 levels) both as repeated within-plot factors.

In order to compare treatments and control, separate ANOVAs were conducted for each compartment. For needles, twigs, seedlings and soil $\delta^{15}N$ and N contents, completely randomized (CR) split-plot (SP) ANOVAs were performed with treatment (fixed; 3 levels) as the between-plot factor, sprayed tree's surface area as the plot (random) and sub-compartment (fixed; variable levels) as the repeated within-plot factor. Needles' P, K, Ca, Mg and Mn contents were analyzed in a similar way. For lichens, completely randomized (CR) split-split-plot (SSP) ANOVAs were performed on $\delta^{15}N$ and N contents, with treatment (fixed; 3 levels) as the between-plot factor, sprayed tree's surface area as the plot (random), and location (fixed; 2 levels) and functional group (fixed; 2 levels) both as repeated within-plot factors. Analyses for ^{15}N enrichments and recoveries were done similarly but without the control treatment.

Homogeneity of variance was verified by visual analysis of residuals, and logarithmic transformations were performed when necessary to homogenize the variance. Significance of each factor and their interactions were tested. Differences were considered significant at $P < 0.05$. Tukey's HSD procedure for multiple comparisons was conducted when the hypothesis of equal means was rejected. Analyses were performed using the SAS MIXED procedure with the Kenward-Roger correction.

3. RESULTS

3.1. N content and ^{15}N abundance in control plots

3.1.1. N content

Among compartments, the litter (14.3 to 15.8 g·kg^{-1}) and soil organic layers (15.6 to 18.4 g·kg^{-1}) showed the highest N content, followed by the needles of mature firs (10.6 to 13.3 g·kg^{-1}) and seedlings (11.7 to 12.2 g·kg^{-1}), lichens (6.2 to 8.9 g·kg^{-1}), twigs of mature firs (3.9 to 11.3 g·kg^{-1}), branches of seedlings (6.3 to 6.4 g·kg^{-1}), stem and roots of seedlings (3.0 to 3.9 g·kg^{-1}), and top mineral soil (B) (1.9 to 2.0 g·kg^{-1}). There were no significant differences between needles from different age classes in the mean N content, after either the first (F = 0.07; P = 0.8163) or second year (F = 3.47; P = 0.1336). Similarly, lichens from different positions (F = 8.52; P = 0.1001) or functional groups (F = 7.86; P = 0.1072) presented similar mean N content (Interaction: F = 0.56; P = 0.5318). Twigs' N content decreased with age (F = 73.21; P < 0.0001), although not significantly between three- and four-year-old twigs. For seedlings, N content significantly decreased from needles to branches to stem (F = 218.08; P < 0.0001) but there were no significant differences between stem and root values. In the soil, N content decreased with depth (F = 212.47; P < 0.0001), although not significantly between F and H layers.

3.1.2. ^{15}N abundance

The control plots sampled in 2009 all showed negative natural ^{15}N abundance (-5.42 to -1.86‰). Lichens (-5.42 to -3.28‰) were the most depleted, followed by seedling sub-

compartments (-4.32 to -2.82‰) and needles of mature trees (-1.87 to -1.86‰). There were no significant differences in the mean $\delta^{15}N$ signature between needles from different age classes (F = 0.02; P = 0.8993), between lichens from different positions on the tree (F = 7.22; P = 0.1151) or different functional groups (F = 0.55; P = 0.5354), or between seedling sub-compartments (F = 0.72; P = 0.5388). No significant interactions were observed for lichens (F = 2.1; P = 0.2840). Seedlings' stem values included only two replicates and have not been included in the statistical analysis (Figure 2).

In 2010, all compartments of the control showed negative natural ^{15}N abundance (-4.05 to -0.35‰) except for the B mineral horizon (5.97‰), which was not analyzed in 2009. Lichens (-4.05 to -2.46‰) were the most depleted followed by seedling sub-compartments (-2.96 to -2.29‰), mature trees' twigs (-2.59 to -0.68‰) and needles (-2.19 to -1.78‰), litter (-1.47‰) and organic soil layers (-1.40 to -0.35‰). Similarly to the results obtained in 2009, there were no significant differences in the mean $\delta^{15}N$ signature between needles (F = 2.93; P = 0.1648) or twigs (F = 3.65; P = 0.0830) from different age classes, between lichens from different functional groups (F = 0.06; P = 0.8333) or between seedlings' sub-compartments (F = 0.72; P = 0.5770). However, contrary to the preliminary results obtained in 2009, lichens sampled on the tree stems showed higher values than those of the lower canopy (F = 72.50; P = 0.0135). Interaction between lichens position and functional groups was again not significant (F = 0.11; P = 0.7759). In the soil, $\delta^{15}N$ values increased with depth but only significantly between the H and B horizons (F = 69.99; P < 0.0001) (Figure 2).
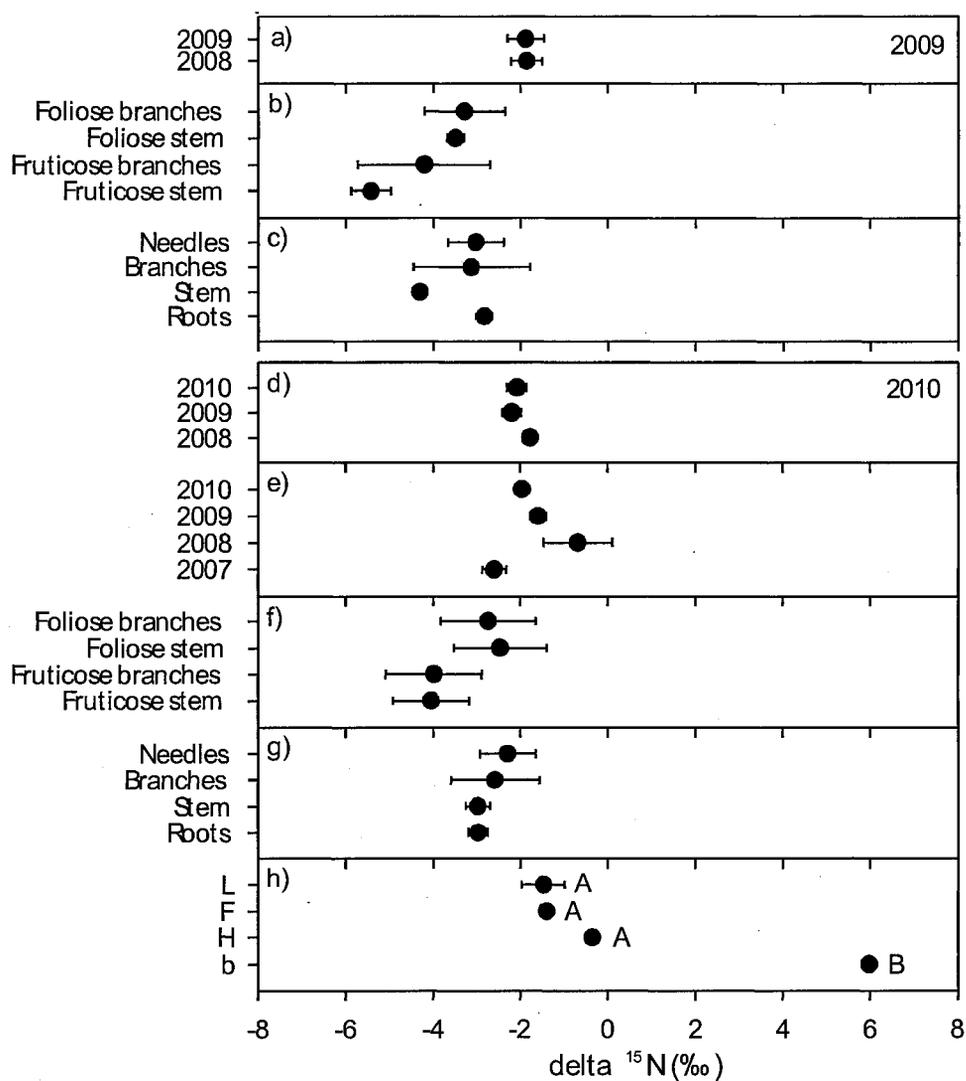
Figure 2. Natural abundance of needles (a, d) and twigs originating from different years (e), of foliose and fruticose lichens (b, f), of seedlings (c, g) and of soil (h) in control plots at the end of 2009 (a-to-c) and 2010 (d-to-h). Mean ± SE and differences between sub-compartments within each compartment are presented. Differences were considered significant at $P < 0.05$.

3.2. The effect of treatments on N content and $\delta^{15}N$

3.2.1. N content

Spraying the canopy with isotopically enriched $^{15}NH_4$ and $^{15}NO_3$-tracers did not have any significant effect on the N content of any of the tree or soil compartments analyzed. There were also no significant interactions between all factors tested in each compartment investigated ($P > 0.05$) (Appendix).

3.2.2. ^{15}N abundance

At the end of each season, except for $\delta^{15}N$ of twigs, seedlings and soils (F = 2.96; P = 0.0344, F = 13.29; $P < 0.0001$ and F = 15.96; $P < 0.0001$, respectively), there were no significant interactions between all factors tested in each compartment investigated.

After one year of treatment, except for needles and roots of seedlings, in the $^{15}NH_4^+$ and $^{15}NO_3^-$ labeled plots, all compartments sampled had significantly higher ^{15}N values than those of the same compartments in the controls. However, there were no differences between $^{15}NH_4^+$ and $^{15}NO_3^-$ in any of these compartments. As for $\delta^{15}N$ natural values, because seedlings' stem values in control plots included only two replicates they were not included in the statistical analysis.

After two years of treatment, except for the stem and roots of seedlings and soil F, H and B horizons, in the $^{15}NH_4^+$ and $^{15}NO_3^-$ labeled plots, all compartments sampled had significantly higher ^{15}N values relative to those of the same compartments in the untreated

plots. With the exception of the needles of mature trees where the values of the $^{15}NH_4^+$ plots were significantly higher than those of the $^{15}NO_3^-$ plots, there were no significant differences between the two forms of N.

In spring 2010, the $\delta^{15}N$ signature of all needle classes had decreased compared to that of autumn 2009 (Figure 3).
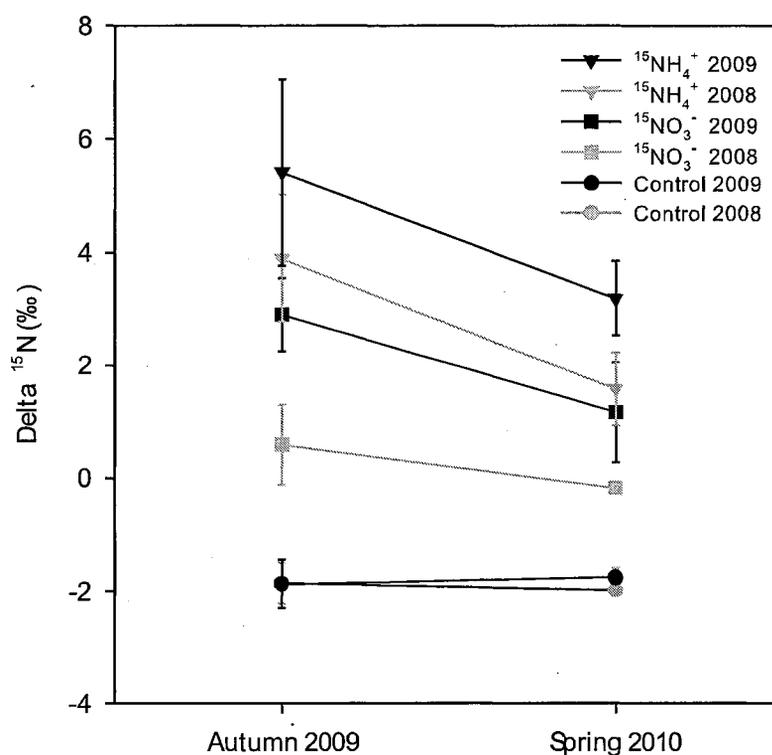
Figure 3. Delta ^{15}N (‰) values of needles originating from 2009 and 2008 in control, $^{15}NH4$ and $^{15}NO3$ plots at the end of the 2009 season (Autumn) and at the beginning of the 2010 season (Spring), just before bud break. Mean ± SE are presented.

3.3. ^{15}N enrichment

In the $^{15}NH_4^+$ plots sampled in 2009, lichens (7.16 to 10.71‰) were the most enriched compartment, followed by seedlings' branches (7.50‰), mature trees' needles (5.77 to 7.26‰), seedlings' needles and stem (3.00 to 3.83‰), organic soil layers (1.06 to 1.11‰) and seedlings' roots (0.97‰). In the $^{15}NO_3^-$ plots sampled in 2009, lichens on the branches of mature trees (9.76 to 10.70‰) and seedlings' branches (10.16‰) were the most enriched followed by lichens on the stem (4.28 to 5.71‰), mature trees' 1-year-old needles (4.75‰), soil litter (L) layer (3.79‰), seedlings' stem and needles (3.25 to 3.30‰), soil H horizon (2.97‰), mature trees' current-year needles (2.46‰), seedlings' roots (1.48‰) and soil F horizon (1.22‰). There were no statistical interactions between all factors tested in each compartment investigated or between results of each treatment. There were also no significant differences between sub-compartments of each compartment (Figure 4).

In the $^{15}NH_4^+$ plots sampled in 2010, mature trees' twigs (32.51 to 82.44‰) were the most enriched followed by foliose lichens on the branches (21.27‰) of mature trees, seedlings' branches (18.10‰), fruticose lichens on the branches and foliose lichens on the stem of mature trees (14.60 to 15.74‰), mature trees' needles (8.45 to 13.21‰), fruticose lichens on the stem (8.00‰) of mature trees, soil litter (L) layer (6.96‰), seedlings' needles and stem (4.10 to 6.65‰), seedlings' roots (1.21‰) and soil F and H organic horizons (0.24 to 0.92‰). In the $^{15}NO_3^-$ plots sampled in 2010, mature trees' twigs (21.70 to 144.02‰) were the most enriched followed by seedlings' branches (19.84‰), lichens on the branches (16.27 to 17.81‰) of mature trees, lichens on the stem of mature trees (10.52

to 10.69‰), mature trees' 2-year-old needles (8.13‰), soil litter (L) layer (7.56‰),

seedlings' needles (7.53‰), mature trees' current- and 1-year-old needles (3.72 to 3.95‰)

seedlings' stem (3.52‰), seedlings' roots (1.29‰) and soil F and H organic horizons (0.61

to 1.12‰). As for $\delta^{15}N$ values, there were no significant differences between the two forms

of N for twigs, seedlings, lichens and soils. There were also no significant interactions

between all factors tested in each compartment investigated. Significant differences were

found between needles (F = 5.75; P = 0.0283) and twigs (F = 14.95; P = 0.0002) from

different age classes and between seedling (F = 69.82; P < 0.0001) and soil (B excluded) (F

= 61.33; P < 0.0001) sub-compartments. $^{15}NO_3^-$ enrichment in the B horizon of the soil was

negative and hence not used for statistical analysis. For the stem and roots of seedlings as

well as F and H horizons of the soils, ^{15}N enrichment was calculated and included in the

statistical analysis although keeping in mind that the $\delta^{15}N$ values of those sub-

compartments in treated plots were not significantly different from those in control plots

(Figure 4).
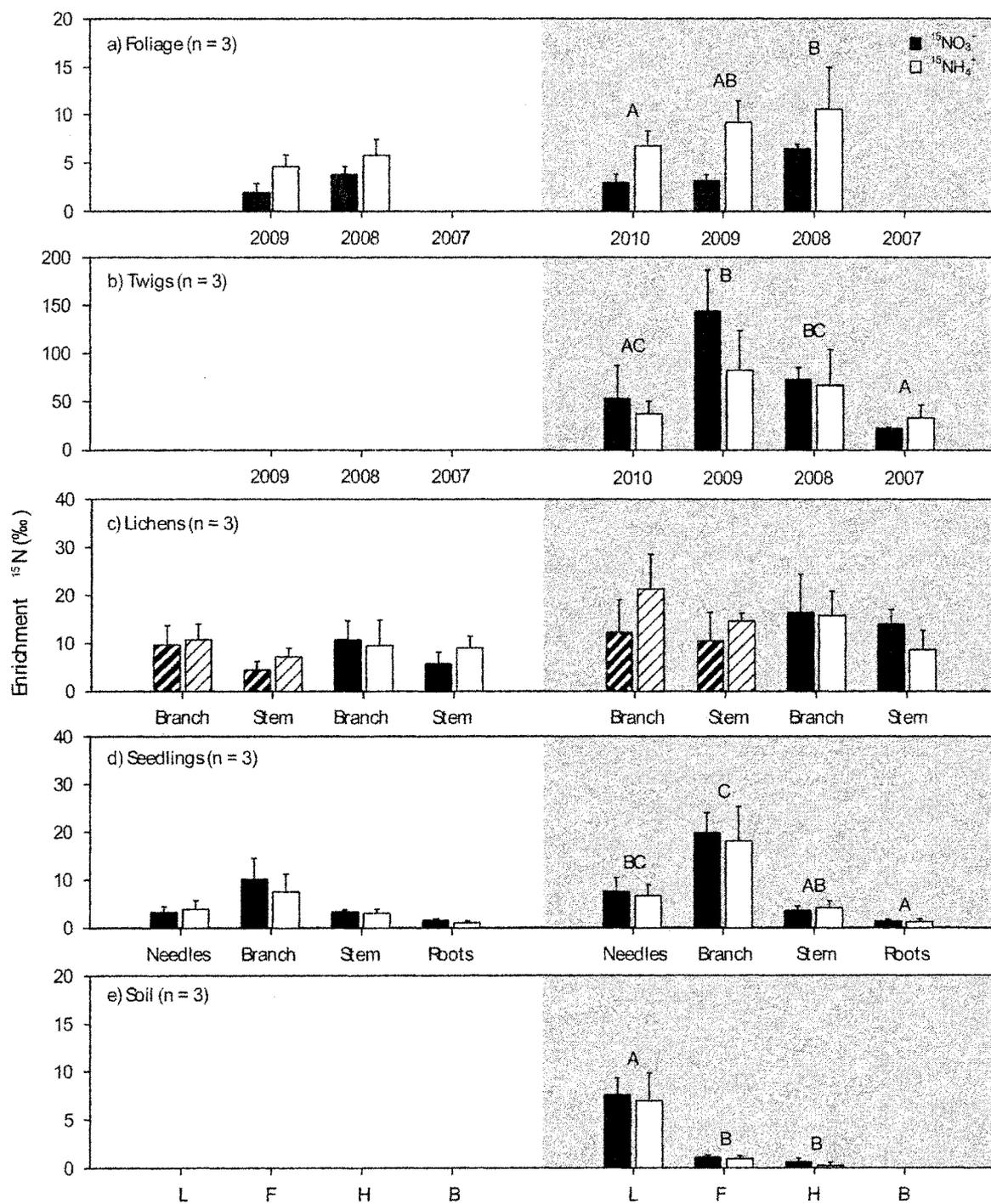
Figure 4. Enrichment (‰) of needles (a,) and twigs (b) originating from different years, of foliose (hatched) and fruticose (plain) lichens (c), of seedlings (d) and of soil (e) at the end of 2009 (white) and 2010 (gray) in $^{15}NH_4$ and $^{15}NO_3$ plots. Twigs and soil were not sampled in 2009. Similarly, needles originating from 2007 were not sampled in either 2009 or 2010. No enrichment was measured for the B horizon of the soil. Mean ± SE and differences between sub-compartments within each compartment are presented. Differences were considered significant at P < 0.05. Differences between both ions are not presented.

A decrease in N isotope enrichment of all age-class needles was observed between autumn 2009 and spring 2010. Current-year needles showed 36 and 42% lower ^{15}N values in the $^{15}NH_4^+$ and $^{15}NO_3^-$ labeled plots, respectively, whereas N isotope values of needles from the 1-year-old age class decreased by 34 and 28% in the same plots (data not shown).

3.4. ^{15}N recovery

Recoveries were calculated only for 2010. The B horizon was excluded because the enrichment was negative and not significant. For the stem and roots of seedlings, in addition to F and H horizons of the soils, the same remarks as for enrichment apply, i.e. keeping in mind that the $\delta^{15}N$ values of these sub-compartments in treated plots were not significantly different from those in control plots.

Taking into consideration that the effective surface sprayed was between 42 and 87 m^2, total tracer recovery was estimated at between 25.8 and 53.2% for $^{15}NH_4^+$ and 39.6 and 81.5% for $^{15}NO_3^-$ (Table 1).

Table 1. Recovery of total experimental ^{15}N at the end of 2010 in $^{15}NH_4$ and $^{15}NO_3$ plots on different scales. Mean ± SE are presented.

Compartments	$^{15}NH_4+$ (%)		$^{15}NO_3$ (%)	
	42 m^2	86 m^2	42 m^2	86 m^2
Needles				
Cr-yr	0.84 (0.16)	1.74 (0.33)	0.71 (0.27)	1.46 (0.55)
1-yr	1.20 (0.19)	2.47 (0.39)	0.64 (0.03)	1.32 (0.06)
2-yr	1.23 (0.40)	2.53 (0.83)	1.21 (0.21)	2.49 (0.43)
	3.27 (0.75)	**6.73 (1.54)**	**2.56 (0.51)**	**5.27 (1.05)**
Twigs				
Cr-yr	2.10 (0.65)	4.33 (1.35)	4.66 (3.14)	9.59 (6.47)
1-yr	3.40 (1.63)	7.01 (3.35)	10.58 (3.58)	21.79 (7.37)
2-yr	4.88 (2.44)	10.05 (5.02)	7.52 (2.94)	15.48 (6.06)
3-yr	2.49 (0.94)	5.14 (1.94)	2.18 (0.51)	4.50 (1.06)
	12.88 (5.66)	**26.53 (11.66)**	**24.93 (10.17)**	**51.36 (20.95)**
Lichens				
Foliose branches	0.20 (0.04)	0.42 (0.08)	0.20 (0.14)	0.42 (0.29)
Fruticose branches	0.14 (0.03)	0.29 (0.07)	0.14 (0.06)	0.29 (0.13)
Foliose stem	0.03 (0.00)	0.06 (0.00)	0.02 (0.01)	0.04 (0.02)
Fruticose stem	0.02 (0.01)	0.04 (0.03)	0.02 (0.01)	0.04 (0.02)
	0.39 (0.09)	**0.81 (0.18)**	**0.38 (0.22)**	**0.79 (0.45)**
Seedlings				
Needles	0.02 (0.01)	0.04 (0.01)	0.02 (0.01)	0.05 (0.01)
Branches	0.03 (0.01)	0.07 (0.03)	0.04 (0.01)	0.07 (0.02)
Stem	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)	0.01 (0.00)
Roots	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	0.06 (0.02)	**0.13 (0.04)**	**0.07 (0.02)**	**0.14 (0.03)**
Vegetation	**16.6 (6.51)**	**34.2 (13.42)**	**27.94 (10.91)**	**57.56 (22.48)**
Soil				
L	4.25 (1.72)	8.78 (3.54)	4.47 (0.82)	9.23 (1.70)
F	3.24 (0.88)	6.70 (1.82)	3.32 (0.25)	6.86 (0.52)
H	1.72 (2.08)	3.56 (4.30)	3.82 (1.59)	7.88 (3.28)
B	-	-	-	-
	9.22 (4.68)	**19.04 (9.67)**	**11.61 (2.66)**	**23.97 (5.50)**
TOTAL	**25.82 (11.20)**	**53.23 (23.09)**	**39.55 (13.58)**	**81.54 (27.98)**

When tracer recovery was calculated only for the amount of ^{15}N recovered within the experimental trees' surface (13 m^2), including the seedlings and soils below the radius of

the tree canopy, about 12.6 and 49.8% of ^{15}N recovered in the ^{15}NH$_4^+$ plots was retained in the foliage and twigs of mature firs, respectively. In those plots, 1.5 and 0.2% of recovered ^{15}N was also retained in the lichens and seedlings, reaching a total of 64.3% for the entire vegetation pool. Finally, 16.5% of recovered ^{15}N was retained in the litter (L) layer and 19.2% in the organic horizons (FH), reaching a total of 35.7% for the entire soil pool. In the ^{15}NO$_3^-$ plots, about 6.5 and 63.0% of recovered ^{15}N was retained in the foliage and twigs of mature firs, respectively. In those plots, 1.0 and 0.2% of recovered ^{15}N was also retained in the lichens and seedlings, reaching a total of 70.7% for the entire vegetation pool. Finally, 11.3% of recovered ^{15}N was retained in the litter (L) layer and 18.1% in the organic horizons (FH), reaching a total of 29.4% for the entire soil pool (Table 2). There were no statistical interactions between all factors tested in each compartment investigated or between results of each treatment. There were only significant differences between twigs from different age classes (F = 14.95; P = 0.0002) and between seedlings' sub-compartments (F = 61.33; P < 0.0001).

Table 2. Relative recovery of experimental ^{15}N at the end of 2010 in the $^{15}NH_4$ and $^{15}NO_3$ plots. Mean is presented.

Compartments	$^{15}NH_4+$ (%)	$^{15}NO_3$ (%)
Needles		
Cr-yr	3.27	1.79
1-yr	4.64	1.62
2-yr	4.75	3.06
	12.66	**6.47**
Twigs		
Cr-yr	8.14	11.77
1-yr	13.17	26.75
2-yr	18.90	19.00
3-yr	9.66	5.52
	49.88	**63.04**
Lichens		
Foliose branches	0.79	0.52
Fruticose branches	0.54	0.35
Foliose stem	0.12	0.04
Fruticose stem	0.07	0.05
	1.52	**0.97**
Seedlings		
Needles	0.08	0.06
Branches?	0.13	0.09
Stem	0.02	0.01
Roots	0.00	0.00
	0.24	**0.17**
Vegetation	**64.29**	**70.65**
Soil		
L	16.46	11.31
F	12.56	8.40
H	6.68	9.65
B	-	-
	35.71	**29.35**
TOTAL	**100.00**	**100.00**

3.5. Needles' macro-nutrient contents (P, K, Ca, Mg) in control plots

In 2009, in the control plots, except for foliar Ca (F = 93.45; P = 0.0105), where the content was higher in older needles, there were no significant differences in P (F = 17.77; P

= 0.0519), K (F = 3.57; P = 0.1994) or Mg (F = 0.48; P = 0.5596) contents between needles from different age classes. In 2010, consistently with the results from the preliminary survey of the first year, significant differences in Ca content were observed between needles from different age classes (F = 18.78; P = 0.0093). Significant differences in P (F = 243.96; P < 0.0001), K (F = 30.77; P = 0.0037) and Mg contents (F = 28.38; P = 0.0043) that were not present after the first year of the study were also observed after the second year. Contents of P and K were higher in current-year needles than in 1- and 2-year-old needles. The Mg content was also higher in younger needles (current- and 1-year-old) compared to the oldest age-class needles (3-year-old needles). Ca content, however, increased with needle age. No significant differences were observed between needles from different age classes for Mn (F = 6.72; P = 0.0526) or Zn (F = 2.91; P = 0.1660) contents, two elements that were not analyzed in the first year of the experiment.

3.6. Effect of treatments on needles' macro-nutrient contents (P, K, Ca, Mg)

No effects were observed on P, K, Ca and Mg content of mature trees' foliage after the first or second year of treatment.

4. DISCUSSION

4.1. Fertilization and rain effect

The results obtained are similar to the values and patterns described in the literature for balsam-fir seedlings and mature trees (Czapowskyj et al. 1980; Lang et al. 1982; Sprugel 1984; Sprugel and Bormann 1981; Young and Carpenter 1967), green-algae lichens (Dahlman et al. 2003; Dahlman et al. 2004; Lang et al. 1980; Palmqvist et al. 2002) and soil (Houle and Carignan 1992; Marty et al. 2011). Factors controlling nutrient concentrations in each pool will not be reviewed in detail here.

A decrease in the physiological activity of needles with age (Warren 2006) may explain why N, P and K differed among the different age classes (Wyttenbach and Tobler 1988). On the other hand, accumulation of Ca in the needles with age might be explained by the relative immobility of this element (Wyttenbach and Tobler 1988). For twigs, Augusto et al. (2008) suggested that nutrient concentration in living branches is more related to dimension than physiological activity associated with age. The bark has higher N content compared to the wood of the branches (Young and Guinn 1966) and the proportion of bark decreases with increasing branch diameter (Augusto et al. 2008). Higher N content in needles compared to twigs is associated to increasing ratio of metabolic versus structural tissues in the latter (Couto-Vazquez and Gonzalez-Prieto 2010).

After treatments, the chemistry of needles was not modified. Similarly, Sheppard et al. (1999) did not find any significant differences in N, P, K and Ca content between their no-spray control and low N treatment.

4.2. Natural ^{15}N abundance

General trends in ^{15}N natural abundances were in agreement with observations from previous studies conducted in forest ecosystems of different regions. Negative values near atmospheric N_2 standard ($\delta^{15}N = 0$‰) for most compartments are common in N-limited forests with relatively closed N cycles (Hogberg 1990; Martinelli et al. 1999) i.e. with highly efficient internal recycling and low N losses (Rennenberg et al. 2009).

Nitrogen isotope fractionation associated with microbial mineralization, nitrification (Nadelhoffer and Fry 1988; Templer et al. 2007) and denitrification (Mariotti et al. 1981) in the soil are usually invoked to explain the lower $\delta^{15}N$ values of this pool relative to plant tissues. As the ^{15}N-depleted products of fractionation are absorbed by the plant roots, the residual substrates become more enriched. ^{15}N-depleted products may also be lost by leaching with water or by gaseous volatilization and contribute to the higher ^{15}N values in the soil. In northern latitudes coniferous forests where N uptake from the soil by plants is strongly mediated by ectomycorrhizal fungi, fractionation during the creation and transfer of N compounds (amino acids) by fungi to plants may further exacerbate the difference between soil and plants $\delta^{15}N$ signature (Hobbie and Colpaert 2003; Hobbie et al. 2000).

Isotope fractionation during organic matter decomposition is also given as an explanation for the relative enrichment with increasing soil depth that is common in boreal forests (Marty et al. 2011). Accumulation of fresh litter (^{15}N-depleted) on the soil surface initially controls its δ^{15}N signature (Nadelhoffer and Fry 1988; Weber et al. 2008). Litter then becomes more and more enriched as it decomposes and mixes with deeper soil layers (for a complete synthesis see Hobbie and Ouimette (2009)).

Lichen was the most ^{15}N-depleted compartment in the study site, suggesting dependence upon less processed N in comparison with mature trees and seedlings. This observation is supported by evidence suggesting atmospheric deposition (depleted relative to atmospheric N_2 standard) as a major source of N for epiphytic lichens (Nadelhoffer et al. 1999a; Nadelhoffer et al. 1999b; Tozer et al. 2005; Wania et al. 2002). In comparison, N from the soil, principal source of N for plants (Chavez-Aguilar et al. 2006), tends to be closer to or more enriched than atmospheric N_2 standard (δ^{15}N = 0‰) depending on the depth from which it is derived. This may explain the higher δ^{15}N values of seedlings' and mature trees' needles and twigs in comparison to those of lichens. Similarly, differences between trees and seedlings may be explained by their different rooting depths. Seedlings are usually rooted in the upper part of the organic layer (Pothier and Prevost 2008) where the soil presents the lowest δ^{15}N values whereas the root system of mature trees reaches deeper layers. Olesinki et al. (2011) showed that more than 60% of the roots of mature balsam firs are distributed below the superficial organic soil. In the present study, an important shift towards more enriched δ^{15}N values occurred beyond that point.

Comparisons between each compartment have not been tested statistically and are presented in an exploratory way only.

4.3. Comparisons of ^{15}N enrichment in the different compartments

4.3.1. Canopy compartments: needles, twigs and lichens

Following additions of ^{15}NH$_4$ and ^{15}NO$_3$-tracers, twigs were most enriched followed by lichens and needles. After experimental application, most coniferous species tend to show higher δ^{15}N needles values for ^{15}NH$_4^+$ than for ^{15}NO$_3^-$ (Dail et al. (2009); mature White pine and Northern cedar, Adriaenssens et al. (2011); 3-year-old potted Scots pine (*Pinus sylvestris* L.) saplings, Wilson and Tiley (1998); 5-year-old Norway spruce (*Picea abies* (L.) Karst.) (fertilized with P and K), Bowden et al. (1989); 2-year-old Red spruce seedlings (*Picea rubens* Sarg.), Eilers et al. (1992); 10-year-old Norway spruce, Lumme (1994) and Lumme and Smolander (1996); 3-year-old Norway spruce). Discrepancies between foliar NH$_4^+$ and NO$_3^-$ retention are usually explained by the net negative charge of the cuticle surfaces, which tends to repel anions such as NO$_3^-$ and attract cations such as NH$_4^+$ (Schönherr (1982) in Wilson and Tiley (1998)). In addition, the transport of ions primarily occurs by diffusion through the cuticle (Macklon and Armstrong 1994; Peuke et al. 1998) in which cations are transported much more readily than anions (Tyree et al. 1990). Macklon et al. (1996) suggested that transport across the cuticle is the rate limiting step for NH$_4^+$, whereas for NO$_3^-$ the limiting step is most likely the entry into the leaf cell by diffusion through the plasmalemma, which has to be done by diffusion against the electrochemical gradient. It was also suggested that the cuticle of the needles may involve

specific membrane carriers which favor cations (Hayne, 1986 in Wilson and Tiley (1998)) or that absorption rate of nitrate may be limited by the constitutive capacity of needles to metabolize NO_3^- to NH_4^+ (Norby et al. 1989; Wilson and Skeffington 1994). Foliar N uptake has been discussed in detail by Sparks (2009). Similar values for both N forms were also observed for other coniferous species in different studies (Johansson et al. (2010); mature Norway spruce, Macklon et al. (1996); cloned Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedling grafts, Chavez-Aguilar et al. (2006); 3-year-old *Abies religiosa* (H. B. K.) Schl. et Cham.). Dail et al. (2009) with mature Eastern hemlock and Red spruce, and Wilson and Tiley (1998) when analyzing unfertilized and fertilized (PK) 5-year-old clonal Norway spruce together, even found higher values for NO_3^-.

In this study, although not statistically significant due to the wide variability found in the results, mean enrichment ^{15}N values in $^{15}NO_3^-$ plots were higher than those in $^{15}NH_4^+$ plots for current- and one-year-old twigs. Most studies presented higher, although not always statistically significant, values for NH_4^+ (Bowden et al. 1989; Boyce et al. 1996; Eilers et al. 1992; Wilson and Tiley 1998) or similar values for both N forms (Dail et al. 2009; Macklon et al. 1996). It has been suggested that ions uptake in twigs may occur via simple diffusion through the bark in the region of radial rays which does not discriminate against NO_3^- (Klemm, 1989 in Wilson and Tiley (1998)).

Higher retention of added ^{15}N by twigs or branches than by needles is generally observed when applying ^{15}N on tops of seedlings (Bowden et al. 1989; Wilson and Tiley

1998), isolated branches of mature trees (Boyce et al. 1996) or mature tree canopy (Dail et al. 2009). However, in the present study very high enrichment ratios of twigs:needles were observed for $^{15}NH_4^+$ (5.6) and particularly for $^{15}NO_3^-$ (17.8). Bowden et al. (1989) with 2-year-old Red spruce seedlings and after 5 consecutive daily applications of 10 h and a total of 100 mg of N as either $^{15}NH_4Cl$ or $K^{15}NO_3$ at 99 at.% excess found enrichment ratios for stem (including twigs):needles of 9.3 and 8.6 for $^{15}NH_4^+$ and $^{15}NO_3$. Wilson and Tiley (1998) with 5-year-old Norway spruce after a single event of 0.5 h and a total of 3.75 mg of N as either 99 at.% $(^{15}NH_4)_2SO_4$ or $HNO_3/Na^{15}NO_3$, found higher ^{15}N retention for both forms of N by twigs (ratio twigs:needles of ≈ 4.0 and ≈ 3.0). Only Eilers et al. (1992) with 10-year-old Norway spruce found higher retention by needles than twigs (ratio twigs:needles of ≈ 0.5) after 5 months of treatment once to three times per week and N dose varying between 4 to 30 mg N/l as either 10 at.% $^{15}NH_4^{15}NO3$ or $^{15}NH_4NO_3$. Boyce et al. (1996) working with mature Red spruce after five weekly applications to a single branch of each treated tree and a total of 190 mg of N as either 5 at.% $^{15}NH_4NO_3$ or $NH_4^{15}NO_3$ found ratios of twigs:needles enrichment of 2.0 and 1.2 in $^{15}NH_4^+$ plots and $^{15}NO_3^-$ plots respectively. In the study of Dail et al. (2009), which was realised at the stand level with mature trees (Eastern hemlock and Red spruce) enrichment ratios of twigs:needles of 2.0 and 1.4 in $^{15}NH_4^+$ plots and $^{15}NO_3^-$ plots respectively were observed after three years of treatment and a total of 19.8 kg·ha^{-1}·yr^{-1} of N added as either 10 at.% $(^{15}N-NH_4)_2SO_4$ or $Na^{15}N-NO_3$.

Most of the above-mentioned studies used elevated N amounts that were applied in a few applications. The method in the present study, of N addition at low inorganic N concentrations and with a high number of sprayed applications, probably reproduced natural deposition conditions in a more realistic way than previous studies. In this context, the very high retention capacity of twigs was potentially underestimated in previous studies.

The greater wettability and lower resistance to liquid phase diffusion make bark more permeable to ion movements than needle cuticles (Schaefer and Reiners (1990) in Wilson and Tiley (1998)). In addition, precipitations may remain on twig surfaces for a longer period of time than on foliage (Boyce and McCune 1992). Although Macklon et al. (1996) suggested that high levels of ^{15}N in the bark of the trees in their study was mostly due to transport from the needles, Dail et al. (2009) suggested that with mature trees, physicochemical interactions with plant surfaces rather than physiological uptake could be the dominant pathway for canopy N retention. They also suggested that epiphytic micro-organisms may increase the retention by bark and branches. In the present study, as sampling was restricted to relatively young twigs, the latter may have less effect than on larger and older branches that are generally more colonised. High recovery by branches is one of the main differences between soil and foliage application. In soil application, more ^{15}N is recovered in foliage than in branches (Buchmann et al. 1995; Nave and Curtis 2011; Preston and Mead 1994). Twigs and branches show the most striking comparison between studies of N application at the soil or canopy level. Nitrogen isotope enrichment in twigs

and branches is low in the former whereas it is much higher in the latter. A comparison of the two application methods in the same experiment yielded similar results (Boyce et al. 1996).

The tendency to higher recovery by older needles found in the present study is contrary to the majority of results found in the literature (Adriaenssens et al. 2011; Bowden et al. 1989; Boyce et al. 1996; Dail et al. 2009; Eilers et al. 1992). Wilson and Tiley's (1998) study is the only one where recovery was higher or equal in older needles. They suggested that for Norway spruce, the ionic permeability of the cuticle is independent of the stage of maturation of the needles. Higher physiological activity of fast-growing young foliage might explain high recovery rates. On the other hand, although young leaves are hydrophobic and are wetted with difficulty in comparison to older or more mature ones (Schier, 1987 in Tukey 1970), their incomplete cuticle formation is supposed to result in higher transcuticular diffusion and maintenance of higher ionic gradients between rainwater and intercellular free spaces which may counterbalance this (Boyce et al. 1991; Lakhani and Miller 1980 in Sayre and Fahey 1999).

Lichens are known to be very efficient at scavenging ions like NH_4^+ and NO_3^- from atmospheric sources and are often suspected of being responsible for a part of the canopy retention in canopy budget studies when they are present. They do not have a vascular system but the lack of waxy cuticle and large surface area enable them to uptake ions over their entire surface (reviewed in Nash 2008). Although green algae lichens can show

preference for NH_4^+ uptake relative to NO_3^- (Crittenden 1996; Dahlman et al. 2004; Lang et al. 1976; Palmqvist and Dahlman 2006), in the present study lichens enrichment was the same for both forms of N. Both functional groups of lichens can also show similar preference for NH_4^+ (Dahlman et al. 2004; Gaio-Oliveira et al. 2005; Lang et al. 1976), but in the present study enrichments of both functional groups were also the same for both forms of N. Under field conditions, Johansson et al. (2010) added ^{15}N at ecologically relevant concentrations and doses for three years on whole trees and found no differences in uptake of the two N forms. Similarly, under natural conditions in the Antarctic, lichens showed high efficiency for simultaneous uptake of NH_4^+ and NO_3^- (Crittenden 1998). Johansson et al. (2010) and Hauck (2010) suggested dose-dependent responses to explain this. Lichens show preferential retention for NH_4^+ when they are exposed to high doses of N but do not at low doses.

4.3.2. Understory seedlings

Needle enrichment was of the same order as that of mature trees and even higher in some cases. Although not as high as in mature trees' twigs, seedlings' branches enrichment was still the dominant sink in this compartment. Seedlings' branches even have higher enrichment than mature trees' needles. However, contrary to mature trees, needles and branches of seedlings do not show any difference between N forms. Eilers et al. (1992) suggested that higher recovery in needles and branches and lower recovery in the stem may be explained by the smaller surface area of stem compared to needles and branches. The results presented in this study, as well as those from Eilers et al. (1992), are in contrast to

the results obtained by Bowden et al. (1989), where N uptake was higher in stem compared to needles. Bowden et al. (1989) also found higher uptake for NH_4^+ than NO_3^- for all compartments they analyzed, except roots. In the present study, enrichments for roots and stem were not significant.

4.3.3. Soils

As for the needles, the organic layer of the soil was not sifted prior to analysis making it impossible to distinguish between N retained directly by soil particles or by fine roots and ectomycorrhizal fungi (either alive or dead). For B horizon, $\delta^{15}N$ values were not significantly different compared to the control, similarly to Dail et al. (2009), and calculation of enrichment gave negative values. On the contrary, slightly higher enrichment in $^{15}NH_4^+$ plots was found for litter. The organic horizons in both studies showed similar enrichment for both ions although in the present study the $\delta^{15}N$ was not significantly different from the control.

4.4. Recovery of ^{15}N

Total ^{15}N recovery in the present study was estimated at between 25.8 and 53.2% for $^{15}NH_4^+$ and 39.6 and 81.5% for $^{15}NO_3^-$. This is below the values obtained by Gaige et al. (2007) (57 to 75% and 73 to 83% of the N added for NO_3^- and NH_4^+, respectively), but closer to the amount recovered by Dail et al. (2009) (recovery of 38 and 67% of ^{15}N added as $^{15}NH_4^+$ and $^{15}NO_3^-$, respectively), after a three-year ^{15}N addition in a mature forest. The high dose of N used in those studies may have increased recovery as higher recoveries have

been associated with increased N doses (Boyce et al. 1996; Chavez-Aguilar et al. 2006; Lumme and Smolander 1996; Vose and Swank 1990).

A part of incomplete recovery may be attributed to exportation by ectomycorrhiza (He et al. 2009; Høgh-Jensen 2006) or to losses through gaseous volatilization from the soil (Dail et al. 2009). Rainstorms and precipitation events following the N addition would favor a deeper penetration of the added N within the soil profile and may have exported some ^{15}N below the B horizon (Moldan and Wright 1998). This seems unlikely however since the B horizon beneath the treated trees was not significantly enriched as compared to the control. Moreover, Houle and Moore (2008), using much higher doses of N in soil application at the same site as the present study, estimated losses of inorganic N at below 5% of the N added. Some N losses may also have occurred during snowmelt (Piatek et al. 2005; Jones and Pomeroy, 2001) between the first and second year of the experiment. Moreover, Nason et al. (1988) found substantial losses of N during warm and dry weather by volatilization as surface water evaporated.

On the other hand, another part of the incomplete recovery could also be due to the fact that all the potential sinks were not sampled due to the need for sampling the tree compartments in a non-destructive way with low impacts on the canopy biomass and photosynthesis. For trees, other possible sinks for N include: roots, wood and bark of stem and large branches (Augusto et al. 2011; Dail et al. 2009), as well as reproductive organs (McDowell et al. 2000). However, a massive translocation of the tracer retained in the

canopy parts to the roots, stem or large branches appears unlikely given the relatively short duration of our experiment. In their experiment, Dail et al. (2009) suggested that much of the added ^{15}N was not internalized but just retained at the surface of the trees as wood recovery was as low as that of foliage (1.5%). However, some N is undoubtedly absorbed physiologically and assimilated by needles as shown in experiments with a vigorous plant-washing procedure (Bowden et al. 1989). These two processes can take less than one day, as has been shown using labeled amino acids (Calanni et al. 1999). Redistribution pattern of this N is still unknown. Senescent and dead needles and branches also have the capacity to retain some N even if they are non-active (Adriaenssens et al. 2011). The treated trees will be destructively sampled at the end of the experiment, which will allow a thorough assessment of the ^{15}N repartition.

Nitrogen enrichment for lichens is second in importance to twigs. Nevertheless, when considering their small biomass, their retention capacity is very low as is impact on the N cycle (Friedland et al. 1991; Klopatek et al. 2006; Lang et al. 1980; Sievering et al. 2007; Tomaszewski et al. 2003). The difference between lichens on the stem and lichens on the canopy that becomes apparent when taking their respective biomass into account is not surprising and reflects solely that the majority of lichens, based on the values presented by Lang et al. (1980), are located near the canopy.

Similarly, when taking into account the N biomass of seedlings, their capacity to retain the added ^{15}N was low. The ^{15}N recovery levels were usually below what is found in the

literature but this discrepancy may be explained by the fact that those N applications were done directly on seedlings or young trees, whereas in our study the added ^{15}N had to pass through the mature tree canopy before reaching the seedlings. N dose may also have an effect on the recovery as it is usually higher with higher concentration of N in artificial solutions.

Soil recovery in the present study was low, in agreement with results obtained by Dail et al. (2009). However those results are far from those obtained by experiments adding N directly on the soil in forests receiving low N deposition, where soil presents high capacity to trap inorganic N in the short and long-term (Feng et al. 2008; Houle and Moore 2008). For example, in a review on the ability of forest ecosystems to efficiently retain added ^{15}N after soil application, Feng et al. (2008) reported that in forests that received relatively low N input (< 20 kg·ha^{-1}·yr^{-1}), 3-44%, 38-87% and $> 6\%$ of N input is retained in plants, in soil or is leached out, respectively. In contact with the soil, N is rapidly immobilized and chemically bound to soil organic matter (Houle and Moore 2008; Magill and Aber 2000; Nave and Curtis 2011; Wright and Tietema 1995) and thus becomes and remains inaccessible to trees for days to decades or even centuries (Compton and Boone 2002; Currie et al. 2004; Preston and Mead 1994). Nave and Curtis (2011) showed that organic matter content explains up to 80% of the variation in soil retention. Interestingly, when the tracer is directly injected into the soil i.e. below the feather moss layer and the top soil horizons, instead of applied directly on the soil, N recovery by trees is higher (Yano et al. 2010). Those results suggest that soil application might underestimate the contribution of

canopy uptake of N deposition on ecosystem N cycle and overestimate the effect of the soil. Similar results have also been reached in a meta-analysis based on natural δ^{15}N and δ^{13}C (Guerrieri et al. 2011).

5. CONCLUSION

In this study, artificial precipitation with isotopically labeled $NH_4{}^{15}N$-NO_3 or ^{15}N-NH_4NO_3 (98 at.%) at ambient rain concentration were sprayed on the crown of individual mature balsam fir trees *in situ* for two years. This method permits the natural fate of low wet deposition of N to be better estimated in comparison to the other study available on the subject, where tracer was applied by helicopter. The results of $\delta^{15}N$ and ^{15}N recovery of tree, seedling and soil compartments highlight the capacity of the aboveground compartments to intercept the downward flux of reactive N from wet atmospheric deposition and suggest that this capacity has been largely underestimated in the past. More specifically, the results show that aboveground biomass may capture N in amounts similar to or higher than the soil, comparatively to experiments considering only soil applications, and confirmed the high N retention capacity of twigs as compared to other forest compartments. Further experiments are necessary to elucidate the proportion of N retained by physicochemical interactions with plant surfaces from that retained by physiological uptake and the remobilization of the retained N within the tree parts with time. The N retention capacity of aboveground biomass should be considered in order to make a realistic quantification of the fate of atmospherically deposited N in similar ecosystems.

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APPENDIXES

Appendix A: Data

Table 1. $\delta^{15}N$ of needles of different age classes at different times and N content at the end of each year. Needles of 2009 (from summer 2009 to spring 2010) and needles of 2010 (in summer and autumn 2010). Mean ± SE are presented

	2009				2010		
	Summer-09	Autumn-09		Spring-10	Summer-10	Autumn-10	
	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	N (g·kg^{-1})	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	N (g·kg^{-1})
Cr-year							
Control	n.d.	-1.8737 (0.4207)	12.7 (1.0)	-1.7580 (0.1605)	-1.8727 (0.5863)	-2.0817 (0.2215)	12.6 (1.0)
$^{15}NH_4$	3.7037 (1.8323)	3.8953 (1.1199)	12.9 (0.4)	1.5710 (0.6535)	3.6717 (1.1487)	6.3677 (1.7261)	12.0 (0.2)
$^{15}NO_3$	-0.9317 (0.4213)	0.5820 (0.7077)	13.1 (0.1)	-0.1823 (0.0699)	-0.3253 (0.5026)	1.6417 (0.7908)	12.7 (0.9)
1-year							
Control	n.d.	-1.8567 (0.3623)	12.5 (0.5)	-1.9823 (0.0696)	-2.4513 (0.3111)	-2.1930 (0.2134)	11.3 (0.5)
$^{15}NH_4$	3.9603 (1.5782)	5.4064 (1.6390)	13.3 (0.3)	3.1830 (0.6627)	5.6957 (1.6737)	9.2973 (2.5686)	12.8 (0.4)
$^{15}NO_3$	0.1567 (0.9957)	2.8933 (0.6443)	12.1 (0.1)	1.1550 (0.8937)	0.2897 (0.7606)	1.7520 (0.5467)	12.4 (0.2)
2-year							
Control	n.d.	n.d.	n.d.	n.d.	n.d.	-1.7847 (0.1631)	10.6 (0.3)
$^{15}NH_4$	n.d.	n.d.	n.d.	n.d.	n.d.	11.4250 (5.3233)	11.7 (0.6)
$^{15}NO_3$	n.d.	n.d.	n.d.	n.d.	n.d.	6.3447 (0.4094)	10.8 (0.2)

n.d. : not determined

Table 2. $\delta^{15}N$ of twigs of different age class at different times and N content at the end of 2010. Needles of 2009 (in spring 2009) and needles of 2010 (in summer and autumn 2010). Mean ± SE are presented

	2009				2010	
	Autumn-09		Spring-10	Summer-10	Autumn-10	
	$\delta^{15}N$ (‰)	N (g·kg^{-1})	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	$\delta^{15}N$ (‰)	N (g·kg^{-1})
Cr-year						
Control	n.d.	n.d.	-1.3447 (0.9329)	-3.0730 (0.1886)	-1.9647 (0.0469)	11.2 (0.4)
$^{15}NH_4$	n.d.	n.d.	23.2663 (6.8887)	18.2583 (5.9565)	35.2587 (12.9757)	11.3 (0.3)
$^{15}NO_3$	n.d.	n.d.	26.8880 (6.8640)	4.1663 (2.1368)	51.0397 (33.8815)	10.2 (0.9)
1-year						
Control	n.d.	n.d.	-0.1450 (2.4123)	-2.4653 (0.3754)	-1.5963 (0.1869)	8.1 (0.2)
$^{15}NH_4$	n.d.	n.d.	35.9530 (10.2022)	64.7567 (29.2584)	80.8397 (41.0706)	8.2 (0.1)
$^{15}NO_3$	n.d.	n.d.	55.1343 (8.6787)	39.7853 (18.3625)	142.4280 (42.8236)	8.8 (0.7)
2-year						
Control	n.d.	n.d.	n.d.	n.d.	-0.6807 (0.7903)	6.3 (0.4)
$^{15}NH_4$	n.d.	n.d.	n.d.	n.d.	65.8377 (36.8110)	7.6 (0.3)
$^{15}NO_3$	n.d.	n.d.	n.d.	n.d.	72.5670 (11.435)	5.2 (1.2)
≥ 3-year						
Control	n.d.	n.d.	n.d.	n.d.	-2.5930 (0.2822)	5.0 (0.3)
$^{15}NH_4$	n.d.	n.d.	n.d.	n.d.	29.9137 (13.4809)	5.1 (0.2)
$^{15}NO_3$	n.d.	n.d.	n.d.	n.d.	19.1023 (1.6718)	3.9 (0.2)

n.d. : not determined

Table 3. Lichens $\delta^{15}N$ and N content under natural condition and experimental treatments. Mean ± SE are presented

	2009		2010	
	$\delta^{15}N$ (‰)	N (g·kg^{-1})	$\delta^{15}N$ (‰)	N (g·kg^{-1})
Foliose				
Branches				
Control	-3.2802 (0.9284)	n.d.	-2.7397 (1.0866)	8.9 (0.5)
$^{15}NH_4$	7.4288 (2.3001)	n.d.	18.5262 (6.2752)	7.8 (0.6)
$^{15}NO_3$	6.4840 (2.9695)	n.d.	15.0747 (12.2993)	8.1 (0.8)
Stem				
Control	-3.4910 (0.1986)	n.d.	-2.4635 (1.0652)	7.1 (0.5)
$^{15}NH_4$	3.6663 (1.6541)	n.d.	12.1368 (0.5186)	7.2 (0.2)
$^{15}NO_3$	0.7927 (1.6244)	n.d.	8.0552 (4.7963)	6.2 (0.9)
Fruticose				
Branches				
Control	-4.2065 (1.5231)	n.d.	-3.9828 (1.1046)	7.9 (0.5)
$^{15}NH_4$	5.2380 (3.7782)	n.d.	11.7582 (3.9360)	6.9 (0.4)
$^{15}NO_3$	6.4928 (2.4816)	n.d.	12.2885 (7.0125)[a]	6.5 (0.1)
Stem				
Control	-5.4205 (0.4594)	n.d.	-4.0515 (0.8715)	6.7 (0.2)
$^{15}NH_4$	3.5985 (1.9421)	n.d.	3.9343 (5.0398)[a]	7.0 (0.5)
$^{15}NO_3$	0.2913 (1.9982)	n.d.	6.6422 (4.8448)	6.9 (0.8)

a : repetition lower than expected
n.d. : not determined

Table 4. Seedlings $\delta^{15}N$ and N content under natural condition and experimental treatments. Mean ± SE are presented

	2009		2010	
	$\delta^{15}N$ (‰)	N (g·kg^{-1})	$\delta^{15}N$ (‰)	N (g·kg^{-1})
Needles				
Control	-3.0220 (0.6412)[b]	n.d.	-2.2865 (0.6365)[a]	11.7 (0.3)[a]
$^{15}NH_4$	0.8053 (1.1929)	n.d.	4.3609 (1.7312)[a]	12.2 (0.3)[a]
$^{15}NO_3$	0.2266 (0.5670)	n.d.	5.2466 (2.2600)[a]	11.7 (0.1)[a]
Branches				
Control	-3.1163 (1.3390)[b]	n.d.	-2.5793 (1.0213)[a]	6.3 (0.3)[a]
$^{15}NH_4$	4.3854 (2.2902)[a]	n.d.	15.5189 (6.2200)[a]	6.3 (0.3)[a]
$^{15}NO_3$	7.0451 (3.0404)[a]	n.d.	17.2657 (3.1474)[a]	6.4 (0.5)[a]
Stem				
Control	-4.3150 (0.1070)[bc]	n.d.	-2.9641 (0.2899)[a]	3.0 (0.5)[a]
$^{15}NH_4$	-1.3180 (0.6576)[a]	n.d.	1.1315 (1.1726)[a]	3.6 (0.1)[a]
$^{15}NO_3$	-1.0180 (0.2935)[a]	n.d.	0.5604 (0.7107)[a]	3.3 (0.4)[a]
Roots				
Control	-2.8232 (0.1898)[b]	n.d.	-2.9624 (0.2206)[a]	3.2 (0.3)[a]
$^{15}NH_4$	-1.8533 (0.1772)[a]	n.d.	-1.7538 (0.3144)[a]	3.9 (0.2)[a]
$^{15}NO_3$	-1.3448 (0.2434)[a]	n.d.	-1.6742 (0.1644)[a]	3.9 (0.4)[a]

a : repetition lower than expected
b : only one seedling by tree
c : only two replicates
n.d. : not determined

Table 5. Soil $\delta^{15}N$ and N content under natural condition and experimental treatments. Mean ± SE are presented

		2009		2010	
		$\delta^{15}N$ (‰)	N (g·kg⁻¹)	$\delta^{15}N$ (‰)	N (g·kg⁻¹)
L					
	Control	n.d.	n.d.	-1.4682 (0.4937)	15.8 (0.7)
	$^{15}NH_4$	-0.3601 (0.4264)	n.d.	5.4904 (2.3752)	14.3 (1.0)
	$^{15}NO_3$	2.3188 (2.0745)[a]	n.d.	6.0903 (1.2837)	14.4 (0.3)
F					
	Control	n.d.	n.d.	-1.3958 (0.0967)	16.5 (0.5)
	$^{15}NH_4$	-0.3327 (0.3450)	n.d.	-0.4719 (0.2485)	18.1 (0.2)
	$^{15}NO_3$	-0.1781 (0.1418)	n.d.	-0.2771 (0.1308)	15.6 (1.0)
H					
	Control	n.d.	n.d.	-0.3484 (0.0796)	17.1 (0.3)
	$^{15}NH_4$	2.3831 (0.4645)	n.d.	-0.1078 (0.2963)	18.4 (1.2)
	$^{15}NO_3$	2.6178 (0.3327)	n.d.	0.2661 (0.2539)	17.4 (0.6)
B					
	Control	n.d.	n.d.	5.9738 (0826)	1.97 (0.2)
	$^{15}NH_4$	n.d.	n.d.	4.9941 (0.3548)	1.87 (0.2)
	$^{15}NO_3$	n.d.	n.d.	5.3638 (0.0838)	1.87 (0.2)

a : repetition lower than expected
n.d. : not determined

Table 6. P, K, Ca and Mg content of needles of different ages at the end of 2009 and 2010. Mean ± SE are presented

	P (g·kg⁻¹)		K (g·kg⁻¹)		Ca (g·kg⁻¹)		Mg (g·kg⁻¹)	
	2009	2010	2009	2010	2009	2010	2009	2010
Cr-year								
Control	2.12 (0.21)	1.95 (0.01)	5.8 (0.82)	4.9 (0.20)	4.06 (0.21)	4.50 (0.49)	0.89 (0.15)	0.98 (0.03)
^{15}NH$_4$	2.18 (0.18)	1.98 (0.05)	6.9 (0.94)	6.3 (0.24)	4.28 (0.67)	3.70 (0.76)	0.79 (0.08)	0.83 (0.08)
^{15}NO$_3$	2.02 (0.03)	2.07 (0.03)	5.4 (0.41)	6.1 (0.61)	4.15 (0.45)	3.63 (1.21)	0.84 (0.04)	0.88 (0.05)
1-year								
Control	1.64 (0.17)	1.22 (0.06)	5.2 (0.59)	3.9 (0.37)	6.37 (0.19)	5.84 (0.66)	0.86 (0.16)	0.67 (0.03)
^{15}NH$_4$	1.64 (0.07)	1.55 (0.08)	6.6 (0.60)	5.9 (0.39)	6.70 (1.21)	5.71 (0.89)	0.67 (0.10)	0.62 (0.09)
^{15}NO$_3$	1.37 (0.03)	1.38 (0.03)	5.5 (0.55)	5.2 (0.90)	6.96 (1.17)	5.27 (1.29)	0.65 (0.06)	0.62 (0.10)
2-year								
Control	n.d.	1.11 (0.05)	n.d.	3.6 (0.35)	n.d.	6.84 (0.52)	n.d.	0.56 (0.09)
^{15}NH$_4$	n.d.	1.21 (0.10)	n.d.	4.9 (0.47)	n.d.	6.76 (0.83)	n.d.	0.46 (0.09)
^{15}NO$_3$	n.d.	1.12 (0.10)	n.d.	4.3 (0.68)	n.d.	6.39 (0.77)	n.d.	0.40 (0.12)

n.d. : not determined

Appendix B: Chemical content figures
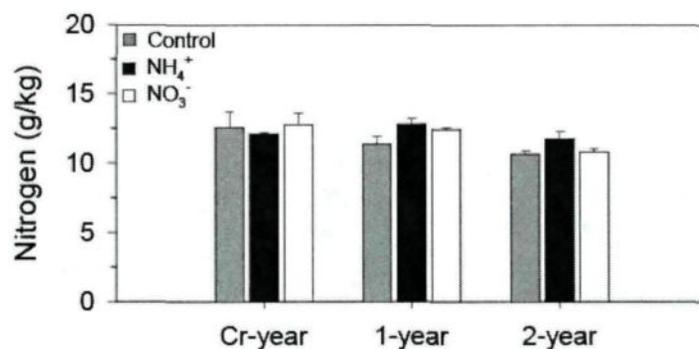
Figure 1. Nitrogen (N) content of needles of different age classes at the end of 2010. Mean ± SE are presented.
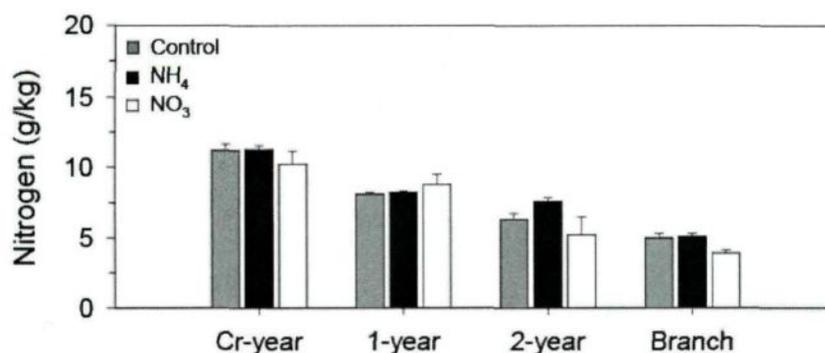
Figure 2. Nitrogen (N) content of twigs of different age classes at the end of 2010. Mean ± SE are presented.
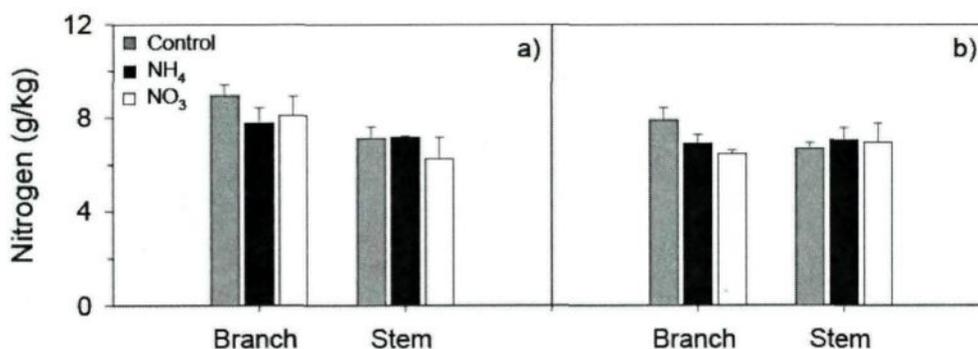
Figure 3. Nitrogen (N) content of foliose (a) and fruticose (b) lichens of the branch and stem at the end of 2010. Mean ± SE are presented.
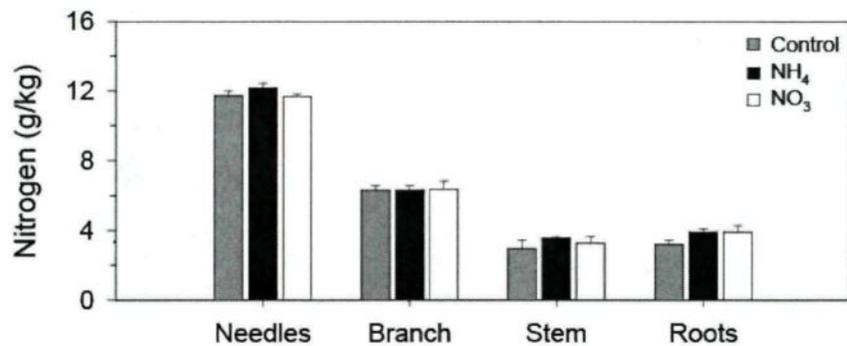
Figure 4. Nitrogen (N) content of the different seedling parts at the end of 2010. Mean ± SE are presented.
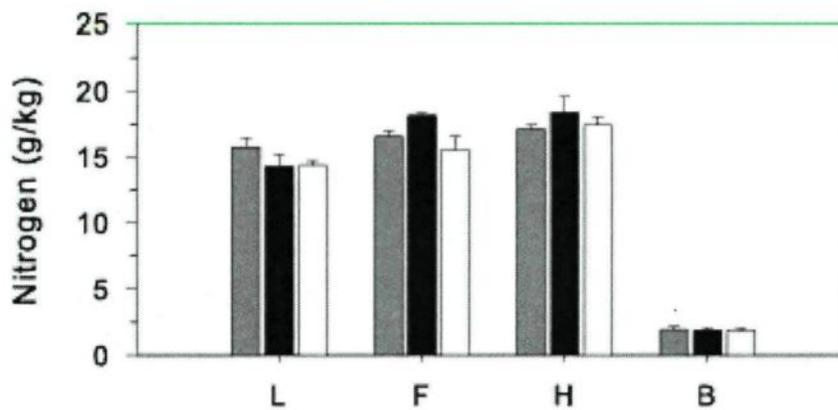
Figure 5. Nitrogen content of the different organic soil layers at the end of 2010. Mean ± SE are presented.

Appendix C: Statistical analysis

Table 1. ANOVA results of $\delta^{15}N$ and N content for each compartment (2009; control only)

Source of variation	$\delta15N$			N		
	df	F	p	df	F	p
Needles						
Tree	2	---	---	2	---	---
Age class	1	0.02	0.8993	1	0.07	0.8163
Residual	2			2		
Total	5			5		
Lichens						
Tree	2	---	---	n.d.	---	---
Position	1	7.22	0.1151	n.d.	n.d.	n.d.
Tree*Po	2	---	---	n.d.	---	---
Functional group	1	0.55	0.5354	n.d.	n.d.	n.d.
Tree*Fg	2	---	---	n.d.	---	---
Po*Fg	1	2.1	0.284	n.d.	n.d.	n.d.
Residual	2			n.d.		
Total	11			n.d.		
Seedlings						
Tree	2	---	---	n.d.	---	----
Sub-compartment	2	1.44	0.3292	n.d.	n.d.	n.d.
Residual	4			n.d.		
Total	8			n.d.		

n.d. : not determined

Table 2. ANOVA results of $\delta^{15}N$ and N content for each compartment (2010; control only)

Source of variation	$\delta15N$			N		
	df	F	p	df	F	p
Needles						
Tree	2	---	---	2	---	---
Age class	2	2.93	0.1648	2	3.47	0.1336
Residual	4			4		
Total	8			6		
Twigs						
Tree	2	---	---	2	---	---
Age class	3	3.65	0.083	3	73.21	< 0.0001
Residual	6			6		
Total	11			11		
Lichens						
Tree	2	---	---	2	---	---
Position	1	72.5	0.0135	1	8.52	0.1001
Tree*Po	2	---	---	2	---	---
Functional group	1	0.06	0.8333	1	7.86	0.1072
Tree*Fg	2	---	---	2	---	---
Po*Fg	1	0.11	0.7759	1	0.56	0.5318
Residual	2			2		
Total	11			11		
Seedlings						
Tree	2	---	---	2	---	---
Sub-compartment	3	0.72	0.577	3	218.08	< 0.0001
Residual	6			6		
Total	11			11		
Soil						
Tree	2	---	---	2	---	---
Sub-compartment	3	69.99	< 0.0001	3	212.47	< 0.0001
Residual	6			6		
Total	11			11		

Table 3. ANOVA results of $\delta^{15}N$ and N content for each compartment (2009)

Source of variation	$\delta15N$ df	F	P	N df	F	p
Needles						
Treatment	2	26.53	0.0010	2	0.50	0.6313
Tree(treatment)	6	---	---	6	---	---
Age class	1	6.19	0.0473	1	1.03	0.3486
Tr*Ag	2	2.09	0.2049	2	2.52	0.1604
Residual	6			6		
Total	17			17		
Lichens						
Treatment	2	17.62	0.0031	n.d.	n.d.	n.d.
Tree(treatment)	6	---	---	n.d.	---	---
Position	1	5.76	0.0534	n.d.	n.d.	n.d.
Tr*Po	2	1.80	0.2445	n.d.	n.d.	n.d.
Tree(treatment)*Po	6	---	---	n.d.	---	---
Functional group	1	5.24	0.0621	n.d.	n.d.	n.d.
Tr*Fg	2	1.14	0.3802	n.d.	n.d.	n.d.
Tree(treatment)*Fg	6	---	---	n.d.	---	---
Po*Fg	1	0.03	0.8629	n.d.	n.d.	n.d.
Tr*Po*Fg	2	0.72	0.5228	n.d.	n.d.	n.d.
Residual	6			n.d.		
Total	35			n.d.		
Seedlings						
Treatment	2	10.34	0.0114	n.d.	n.d.	n.d.
Tree(treatment)	6	---	---	n.d.	---	---
Sub-compartment	2	2.87	0.0960	n.d.	n.d.	n.d.
Tr*Sc	6	4.18	0.0238	n.d.	n.d.	n.d.
Residual	12			n.d.		
Total	26			n.d.		

n.d. : not determined

Table 4. ANOVA results of $\delta^{15}N$ and N content for each compartment (2010)

Source of variation	$\delta15N$			N		
	df	F	p	df	F	p
Needles						
Treatment	2	41.99	0.0003	2	0.62	0.5696
Tree(treatment)	6	---	---	6	---	---
Age class	2	8.72	0.0046	2	8.51	0.0050
Tr*Ag	4	2.27	0.1224	4	1.75	0.2047
Residual	12			12		
Total	26			26		
Twigs						
Treatment	2	33.40	0.0006	2	2.04	0.2112
Tree(treatment)	6	---	---.	6	---	---
Age class	3	18.39	< 0.0001	3	98.68	< 0.0001
Tr*Ag	6	2.96	0.0344	6	1.90	0.1354
Residual	18			18		
Total	35			35		
Lichens						
Treatment	2	10.32	0.0114	2	1.74	0.2536
Tree(treatment)	6	---	---	6	---	---
Position	1	8.47	0.0270	1	6.02	0.0495
Tr*Po	2	2.08	0.2054	2	0.12	0.8912
Tree(treatment)*Po	6	---	---	6	---	---
Functional group	1	0.95	0.3680	1	7.80	0.0315
Tr*Fg	2	0.37	0.7072	2	1.69	0.2617
Tree(treatment)*Fg	6	---	---	6	---	---
Po*Fg	1	1.10	0.3353	1	1.99	0.2078
Tr*Po*Fg	2	0.29	0.7553	2	0.40	0.6887
Residual	6			6		
Total	35			35		
Seedlings						
reatment	2	17.21	0.0033	2	0.88	0.4615
Tree(treatment)	6	---	---	6	---	---
Sub-compartment	3	57.02	< 0.0001	3	735.46	< 0.0001
Tr*Sc	6	13.29	< 0.0001	6	0.71	0.6450
Residual	18			18		
Total	35			35		
Soil						
Treatment	2	10.34	0.0114	2	1.05	0.4057
Tree(treatment)	6	---	---	6	---	---
Sub-compartment	3	84.86	< 0.0001	3	578.88	< 0.0001
Tr*Sc	6	15.96	<.0.0001	6	2.46	0.0650
Residual	18			18		
Total	35			35		

n.d. : not determined

Table 5. ANOVA results for P, K, Ca, Mg, Mn, Zn content of each compartment

Source of variation	2009			2010		
	df	F	p	df	F	p
P						
Treatment	2	0.85	0.4741	2	2.95	0.1282
Tree(treatment)	6	---	---	6	---	---
Age class	1	95.11	< 0.0001	2	193.08	< 0.0001
Tr*Ag	2	0.74	0.5156	4	2.58	0.0916
Residual	6			12		
Total	17			26		
K						
Treatment	2	1.29	0.3423	2	2.59	0.1549
Tree(treatment)	6	---	---	6	---	---
Age class	1	3.5	0.1104	2	49.14	< 0.0001
Tr*Ag	2	1.38	0.3215	4	1.28	0.3324
Residual	6			12		
Total	17			26		
Ca						
Treatment	2	0.07	0.9344	2	0.16	0.8594
Tree(treatment)	6	---	---	6	---	---
Age class	1	44.9	0.0005	2	38.05	< 0.0001
Tr*Ag	2	0.15	0.8609	4	0.28	0.888
Residual	6			12		
Total	17			26		
Mg						
Treatment	2	0.59	0.5833	2	0.62	0.5678
Tree(treatment)	6	---	---	6	---	---
Age class	1	17.89	0.0055	2	94.87	< 0.0001
Tr*Ag	2	3.09	0.1197	4	0.99	0.4482
Residual	6			12		
Total	17			26		
Mn						
Treatment	n.d.	n.d.	n.d.	2	0.69	0.5382
Tree(treatment)	n.d.	---	---	6	---	---
Age class	n.d.	n.d.	n.d.	2	19.41	0.0002
Tr*Ag	n.d.	n.d.	n.d.	4	0.08	0.9857
Residual	n.d.			12		
Total	n.d.			26		
Zn						
Treatment	n.d.	n.d.	n.d.	2	2.09	0.2043
Tree(treatment)	n.d.	---	---	6	---	---
Age class	n.d.	n.d.	n.d.	2	7.05	0.0094
Tr*Ag	n.d.	n.d.	n.d.	4	0.58	0.6836
Residual	n.d.			12		
Total	n.d.			26		

n.d. : not determined

Appendix D: Glossary

branch. Axis of lesser stature to that on which it is located (Moffett 2000).

canopy. The aboveground plant organs within a community. *Plants have summits or crowns and plant communities have canopies* (Moffett 2000; Sauvageau 1995).

cover. Percentage of sky obscured or of ground area obscured by vegetation (Moffett 2000).

crown. Aboveground parts of a tree or shrub, and particularly its topmost leaves and limbs. *Plants have summits or crowns and plant communities have canopies* (Moffett 2000; Sauvageau 1995).

epiphyte. Organism living on any aboveground plant surface and sustained entirely by nutrient and water received nonparasitically from within the canopy in which it resides (Moffett 2000).

host. Any plant on or in which another species resides, either for extended periods or briefly (Moffett 2000).

nitrogen input. Nitrogen inputs < 20 kg ha-1 yr-1 are considered low while nitrogen inputs > 20 kg ha-1 yr-1 are considered high (Feng et al. 2008). Nitrogen inputs > 40 kg N ha-1 yr-1 are associated with harmful effect on vegetation (Mohren, 1986 in Sievering et al. 2000).

nitrogen saturation. Long-term removal of N limitations on biotic activity, accompanied by a decrease in N retention capacity. Degree of N saturation of a forest stand is determined by the balance input of available N (e.g., from mineralization and atmospheric deposition) and the N retention capacity of the plant-soil-microbial system (Fenn et al. 1998). For saturation stages in terrestrial ecosystem see Aber et al. (1998). For saturation stages in watershed see Stoddard (1994).

nitrogen cascade. See Galloway et al. (2003).

overstory. The stratum of trees that have out-grown the other vegetation in a forest to have their uppermost crown foliage largely or fully in direct sunlight, usually as a relatively continuous layer (excluding gaps) (Moffett 2000).

sapling. A general term for a young tree no longer a seedling but not yet a pole, about 1-2 m high and 2-4 cm in diameter at breast height, typically growing vigorously and without dead bark or more than an occasional dead branch. Also, a young tree having a diameter at breast height greater than 1 cm but less than the smallest merchantable diameter (Sauvageau 1995).

seedling. A young tree, grown from seed, from the time of germination to the sapling stage, having a diameter at breast height of no more than 1 cm and a height of no more than 1.5 m (Sauvageau 1995).

stemflow. Water from mist or rain flowing to the ground along the outside of stems (Moffett 2000).

throughfall. Water from mist or rain dripping from foliage to the ground (Moffett 2000).

understory. Stratum of trees that (barring gaps) lies in the shade immediately below the overstory (Moffett 2000).