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Foliar Nitrogen Uptake from Wet Deposition and the Relation with Leaf Wettability and Water Storage Capacity

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Abstract This study assessed the foliar uptake of ^{15}N-labelled nitrogen (N) originating from wet deposition along with leaf surface conditions, measured by wettability and water storage capacity. Foliar ^{15}N uptake was measured on saplings of silver birch, European beech, pedunculate oak and Scots pine and the effect of nitrogen form (NH_4^+ or NO_3^-), NH_4^+ to NO_3^- ratio and leaf phenology on this N uptake was assessed. Next to this, leaf wettability and water storage capacity were determined for each tree species and phenological stage, and the relationship with $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake was examined. Uptake rates were on average five times higher ($p<0.05$) for NH_4^+ than for NO_3^- and four times higher for deciduous species than for Scots pine. Developing leaves showed lower uptake than fully developed and senescent leaves, but this effect was tree species dependent. The applied NH_4^+ to NO_3^- ratio did only affect the amount of N uptake by senescent leaves. The negative correlation between measured leaf

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contact angles and foliar N uptake demonstrates that the observed effects of tree species and phenological stage are related to differences in leaf wettability and not to water storage capacity.

Keywords Foliar uptake · Nitrogen deposition · Wettability · Water storage capacity · ^{15}N labelling

1 Introduction

During the last decades, human activities have dramatically increased atmospheric concentrations of reactive nitrogen (N) oxides (NO_y) and ammonia (NH_3) (Vitousek et al. 1997). Semi-natural ecosystems such as forests are exposed to higher inputs of these atmospheric pollutants compared with open-field circumstances because of their high surface roughness, leaf area index and physiological leaf characteristics (Erisman and Draaijers 2003). This increased N deposition to forest ecosystems has significantly altered internal N cycling and other forest ecosystem processes with potentially both beneficial and adverse consequences (Vitousek et al. 1997). Adverse consequences such as N saturation, soil acidification and shifts in biodiversity have widely been recognised (Aber et al. 1998; Gilliam 2006; Gundersen et al. 2006; Bowman et al. 2008), as well as the initial positive effect on tree and forest growth (Laubhann et al. 2009; Solberg et al. 2009; Thomas et al. 2010). However, the size and duration of the effect of enhanced N deposition on carbon (C) sequestration, still remains to be discussed (Nadelhoffer et al. 1999; Magnani et al. 2007; De Vries et al. 2009).

Atmospheric N can be taken up either by above-ground tree parts or by the forest soil (Rennenberg and Gessler 1999), two pathways which have shown to be interrelated (Bruckner et al. 1993; Rennenberg et al. 1998; Vallano and Sparks 2008). Nitrogen incorporated through foliage is a direct addition to metabolism, while N additions to the soil surface may also be incorporated into microbial biomass, revolatilized or leached (Rennenberg and Gessler 1999; Sparks 2009). Recent studies show that canopy N retention can amount to up to 70% of N deposition, providing as much as one third of tree N requirements without any competition from soil microbial biomass (Gaige et al. 2007; Sievering et al. 2007). However, other canopy elements than leaves, such as branches, boles and lichens can also provide a substantial sink for N through physico-chemical processes rather than by N assimilation (Dail et al. 2009). Next to this, Dail et al. (2009) showed that the vegetation and not the soil is the most important short-term sink under increased N availability. This retained N can be rapidly assimilated by the canopy (Calanni et al. 1999). Therefore, it is possible that canopy uptake will favour the growth response of historically N-limited forests (Sparks 2009).

Nitrogen uptake can occur via dry deposition (e.g. NH_3 and NO_2) and/or wet deposition (NH_4^+, NO_3^-) by foliage via stomata and the cuticle (Rennenberg and Gessler 1999). Generally, gaseous uptake of N compounds is driven by stomatal characteristics and can be modelled well using a compensation model developed by Sutton et al. (1998). In contrast, foliar uptake processes from wet deposition are stipulated by the surface conditions of the leaves, i.e. surface roughness and wax chemistry, which can be characterised by leaf wettability and water storage capacity (WSC) (Boyce et al. 1991). Since the uptake of dissolved ions is assumed to occur mainly via the cuticle (Peuke et al. 1998), higher wettability could favour this process by enlarging the contact area between incident rain droplets and the cuticle (Sase et al. 2008). Similarly, a higher WSC would prolong the contact period between incident rain and leaves and as such the time for exchange processes. These two parameters differ between tree species (Holloway 1969; Hall and Burke 1974) but also between different phenological stadia within one species (Sase et al. 2008). Moreover, they are easy to measure and could give clear indications about the expected foliar uptake for a range of different tree species and through the growing season.

An accurate quantification of canopy N uptake from wet deposition is frequently obtained by applying ^{15}N-labelled sources at tracer levels. In contrast to throughfall measurements (Thimonier et al. 2005), it is possible with this technique to quantify direct foliar uptake and subsequent assimilation of N and to distinguish between N assimilated by living tissue or N adsorbed to canopy elements (Gaige et al. 2007; Dail et al. 2009). This ^{15}N pulse chase technique has been used to investigate effects of tree species, N load and N form on foliar uptake, both in field studies (Brumme et al. 1992; Eilers et al. 1992;

Lumme 1994; Boyce et al. 1996; Garten et al. 1998; Dail et al. 2009) and laboratory studies (Bowden et al. 1989; Garten and Hanson 1990; Lumme 1994; Lumme and Smolander 1996; Wilson and Tiley 1998; Chavez-Aguilar et al. 2006). However, most of these studies have used N loads far above ambient levels.

In this study, the ^{15}N pulse chase technique was used to quantify foliar N uptake from wet deposition under ambient N load levels for four ecologically different tree species. We hypothesise that foliar N uptake is influenced by tree species, N form, applied NH_4^+ to NO_3^- ratio and leaf phenological stage (the periods of leaf development, fully developed leaves and leaf senescence) and assessed the relative importance of these factors. Moreover, we test the hypothesis whether this N uptake is related to leaf wettability and leaf WSC.

2 Materials and Methods

2.1 Plant Material and Climate

This study was performed using living, leafed twigs of 3-year-old potted saplings of European beech (*Fagus sylvatica* L.), pedunculate oak (*Quercus robur* L.), silver birch (*Betula pendula* Roth) and Scots pine (*Pinus sylvestris* L.). These species are widespread in temperate regions but have a different ecological behaviour (i.e. pioneer vs climax species, deciduous vs coniferous species and sun leaves vs shade leaves) (Fitter and Peat 1994; http://www.ecoflora.co.uk) and a different canopy structure (i.e. leaves vs needles), which may have an influence on foliar nitrogen uptake. All saplings were grown in a tree nursery and were of known provenance. They were potted on 27th February 2008 in a mixture of peat and slow releasing fertiliser (8.6 $\mathrm{g\,N\,kg^{-1}}$ dry soil $\mathrm{year^{-1}}$) in order to eliminate the effect of varying soil nutrient availability on aboveground N uptake by leaves. The saplings were placed at a wind sheltered location in the close vicinity of the laboratory near Ghent (north of Belgium, approximately 60 km from the North Sea in the prevailing wind direction; 50°58 N, 3°49 E). Mean annual precipitation (1980–2008) at a nearby weather station operated by the Belgian Royal Meteorological Institute (www.kmi.be) is 784 mm and is distributed equally over the year, and mean annual temperature is 10.4°C. The region of Flanders is characterised by relatively high atmospheric N and sulphur (S) deposition. In the period 2002–2006, average annual total deposition fluxes to a mixed beech forest nearby amounted to 30 $\mathrm{kg\,N\,ha^{-1}\,year^{-1}}$ and 20 $\mathrm{kg\,S\,ha^{-1}\,year^{-1}}$ (Verstraeten et al. 2007).

2.2 Experimental Setup and Sample Analysis

2.2.1 ^{15}N Application

Two twigs with a length of approximately 30 cm were selected per tree (four replicated trees per treatment) and were sprayed with an artificial rainwater solution of inorganic salts in deionised water. It contained all major ions, with concentrations (in $\mathrm{mg\,L^{-1}}$) based on average precipitation data for this area in 2002–2006 (VMM 2007): 0.82 (Na^+), 0.09 (K^+), 0.44 (Ca^{2+}), 0.17 (Mg^{2+}), 0.67 (SO_4^{2-}-S) and 2.07 (Cl^-). The pH amounted to 5.5. One twig was sprayed with a solution containing ^{15}N-labelled (99 at.%) NH_4^+; the other was sprayed with ^{15}N-labelled (99 at.%) NO_3^-. Two types of solutions were applied as separate treatments, subsequently called the 'high NH_4^+ to NO_3^- ratio' and the 'low NH_4^+ to NO_3^- ratio' treatments. The concentration of NH_4^+–N was similar in both solutions (0.84 $\mathrm{mg\,L^{-1}}$). However, in the low NH_4^+ to NO_3^- ratio treatments, the concentration of NO_3^--N (1.26 $\mathrm{mg\,L^{-1}}$) and Cl^- (5.00 $\mathrm{mg\,L^{-1}}$) were three and 2.42 times higher than those in the high NH_4^+ to NO_3^- ratio treatments (0.42 mg NO_3^-–$\mathrm{N\,L^{-1}}$ and 2.07 mg Cl^- L^{-1}). As such, the NH_4^+ to NO_3^- ratio differed by a factor of three between the treatments (2:3 versus 2:1), hereby simulating increased future emissions of reactive N compounds by combustion processes.

A preliminary test revealed that the ^{15}N enrichment in sprayed leaves was similar 2 and 24 h after ^{15}N application. Therefore, branches were removed 2 h after the first application to ensure complete N uptake from plant surfaces while preventing meaningful transport of ^{15}N containing assimilation products to other tree parts. Each twig was sprayed two times with 100 ml of the rainwater solution with a 1-h interval to allow leaves to dry, and was cut 1 h after the second application. The volume sprayed was sufficient to start drainage from the twigs. All equipment was used in duplicate to avoid any cross-contamination between $^{15}NH_4^+$ and $^{15}NO_3^-$ treatments. The living twigs were sprayed in boxes to prevent ^{15}N contamination of other plant parts. To quantify the exact amount of solution applied to each twig, the initial and final weights of the sprayer were registered. The experiment was conducted on developing leaves (15 May 2008 for birch and pine and 28 May 2008 for beech and oak), fully developed leaves (19 Aug 2008 for all species) and senescent leaves (19 Sept 2008 for birch and pine and 16 Oct 2008 for beech and oak) to assess the effect of leaf phenology on N uptake. The spring and autumn experiments were done earlier for birch and pine than for beech and oak to account for the different phenology of the species. Since needle sprouting of pine occurred late in the growing season (June), no results are available for the developing stage of pine.

2.2.2 Measurement of Leaf Wettability and Visualisation of Leaf Surface Structure

Contact angles (CAs) of standardised water droplets with the leaf surface can be used as a proxy for leaf wettability (Brewer et al. 1991). Low angles indicate the tendency to form water films rather than droplets, high angles indicate water repellence. The CAs were measured, using the software ImageJ, from digital photographs of a water droplet (7 µl for the deciduous species; 3 µl for Scots pine) on the abaxial and adaxial leaf surfaces of non-treated branches (Canon EOS 5D digital camera and Sigma macro lens EX DG 105 mm, f 2.8). Ten replicates per species, phenological stage and leaf side were measured.

Next to this, the leaf surface structure was visualised by digital photographs at different magnifications. Firstly, four samples of fresh leaf material of all species were taken and kept in a 70% ethanol solution. Later, they were mounted on aluminium stubs using a double-sided adhesive tape and immediately dried in a critical point dryer (Balzers CPD-010 unit). Afterwards, they were sputtered with gold (30–40 nm thick) using a sputter coater (Cressington 108 auto) and transferred to the cold stage of a scanning electron microscope (Philips 515 SEM) by means of a transfer rod under high vacuum, after opening the transfer valve with an acceleration voltage of 20 kV.

2.2.3 Estimation of Leaf/Needle Biomass, Water Storage Capacity and Surface Area

Before ^{15}N application, a representative twig of each tree was sampled to measure leaf biomass, WSC and surface area. The WSC of leaves, defined as the difference between the wetted and dry biomass divided by leaf area, was determined to estimate interception during a normal rain event. In contrast to leaf wettability, WSC is also influenced by leaf orientation and could render a more realistic measurement under ambient conditions. For this purpose, a laboratory experiment adapted from Aston (1979) and Herwitz (1985) was conducted. The representative twig was severed at its base only seconds before the experiment in order to prevent wilting. Next, each twig was suspended in turn from a balance (precision of 0.001 g) at an angle to the vertical approximately the same as its former angle to the stem. The weight of the branch was then recorded under the following conditions: (a) non-wetted (fresh weight), (b) fully wetted by continuous spraying with a garden sprayer and (c) after dripping. Next, the leaves were removed from the twigs and the experiment was repeated. The WSC was calculated for fully wetted leaves and after dripping, representing respectively calm and windy conditions, as the difference between the WSC of fully leafed branches and the WSC of twigs only. After the experiment, the remaining detained water was allowed to evaporate, the leaves and branches were separated and the surface area was measured. Total one-sided leaf area of the deciduous species was measured with a LI-3000 Portable Area Meter (LICOR, Lincoln, Nebraska). Needle surface area was estimated assuming a half cylindrical shape and based on the number of needles per branch and the mean needle length and diameter of ten randomly selected needles.

2.2.4 Analysis of Plant Material

After harvesting, all twigs were washed thoroughly with deionised water in order to remove any ^{15}N adsorbed to the surface of leaves/needles or branches. Next, leaves, needles and branches were separated, dried in paper bags for 48 h at 50°C, weighed and ground to a fine powder (<250 µm) using a centrifugal mill (Retsch ZM1, Germany). Finally, samples were analysed for total N and ^{15}N in duplicate using an elemental analyzer (EA) interfaced to an isotope ratio mass spectrometer (IRMS) (20–20, SerCon, UK). Machine error ($n=10$) of this EA-IRMS system is 0.2‰ for $\delta^{15}N$. Cross-contamination was avoided by including method blanks and analytical quality was checked by the standard deviation of two replicate measurements.

2.2.5 Analysis of Plant Available Inorganic Nitrogen

From April to October 2008, soil was sampled monthly from three different control pots (without a tree) and frozen immediately. Plant available soil NH_4^+ was determined colorimetrically in a KCl extract (1 M, soil: solution ratio 1:2, 1 h) by the salycilate-nitroprusside method on an auto-analyzer (AA3, Bran and Luebbe, Germany). Nitrate was determined colorimetrically (AA3) after reduction in a Cu–Cd column followed by reaction of nitrite with *N*-1-napthylethylenediamine to produce a chromophore.

2.3 Data Processing

Uptake of ^{15}N per unit dry plant material ($\mu g N g^{-1}$ DM) was calculated by accounting for the natural abundance of ^{15}N in untreated branches (Harrison et al. 2000):

$$Y = X \cdot \frac{\text{at } \%_{treated} - \text{at } \%_{untreated}}{\text{at } \%_{solution} - \text{at } \%_{untreated}} \cdot 10^3$$

with Y being the ^{15}N uptake ($\mu g g^{-1}$ DM) and X is the total N concentration ($mg g^{-1}$ DM) in the treated samples.

Calculated N uptake for each combination of tree species, NH_4^+ to NO_3^- ratio, N form and leaf phenological stage was tested for significant difference from zero by means of a one sample t test. ANOVA on the measured ^{15}N uptake was not possible due to heteroscedasticity across tree species, leaf phenology, N form and NH_4^+ to NO_3^- ratio according to Levene's equality of variance test. However, by splitting up the data according to the N form, three-way ANOVA allowed us to assess the effect of the remaining factors on ^{15}N uptake. Three-factor interaction was excluded from the model because of insufficient degrees of freedom. Means were separated by Tukey's honestly significant difference test and p values were calculated for each factor. Afterwards, data were split up according to tree species, leaf phenology and NH_4^+ to NO_3^- ratio to assess two-factor interactions. Data of WSC and ^{15}N uptake were log-transformed to meet normality conditions. The effect of N form was then tested by means of two-tailed t tests per tree species, NH_4^+ to NO_3^- ratio and phenological stage. For pine, an additional univariate analysis was done including needle age as extra factor. Differences in WSC for fully wetted leaves and for leaves after dripping and CA on the abaxial and adaxial side were tested by analysis of variance (ANOVA) with tree species and phenological stage as factors. Correlations between WSC, CA, foliar N content and $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake were assessed by calculating Spearman's rank coefficients. All statistical analyses were performed using SPPS 15.0 with a minimum significance level of $p<0.05$.

3 Results

3.1 Foliar N Uptake

The foliar uptake of $^{15}NH_4^+$ ranged from 0.70 $\mu g g^{-1}$ DM for pine to 3.76 $\mu g g^{-1}$ DM for birch, while the uptake of $^{15}NO_3^-$ varied between 0.11 $\mu g g^{-1}$ for pine and 0.86 $\mu g g^{-1}$ DM for birch. This uptake was significantly higher than zero in most cases (Table 1). Generally, the uptake of $^{15}NH_4^+$ was significantly higher than the uptake of $^{15}NO_3^-$, except for the fully developed needles of pine under both the low and high NH_4^+ to NO_3^- ratio and senescent birch and developing beech leaves under high NH_4^+ to NO_3^- ratio. On average, the uptake of NH_4^+ was approximately seven and three times higher than the uptake of NO_3^- in the high (2:1) and low (2:3) NH_4^+ to NO_3^- ratios, respectively, but varied with tree species and phenological stage.

According to the univariate analysis, both the $^{15}NH_4^+$ and $^{15}NO_3^-$ uptake were significantly influenced, in decreasing order of importance, by tree species, leaf phenology and NH_4^+ to NO_3^- ratio and by the two-factor interactions of these three factors (Table 2). Under both the high and low NH_4^+ to NO_3^- ratio, the tree species effect on $^{15}NH_4^+$ uptake was pronounced by a higher uptake for deciduous species versus pine (Table 1). For $^{15}NO_3^-$, the uptake was significantly larger for birch and beech than for oak and pine. In general, $^{15}NH_4^+$ uptake was significantly higher for senescent leaves than for developing and

Table 1 Mean $^{15}N–NH_4^+$ and $^{15}N–NO_3^-$ uptake (μgg^{-1} DM) by the leaves of four tree species in three-leaf phenological stages after applying artificial rainwater with high (2:1) or low (2:3) NH_4^+ to NO_3^- ratio

Tree species	Leaf phenology	High NH_4^+ to NO_3^- ratio		Low NH_4^+ to NO_3^- ratio	
		$^{15}N–NH_4^+$	$^{15}N–NO_3^-$	$^{15}N–NH_4^+$	$^{15}N–NO_3^-$
Silver birch	Developing	2.78^{*a}	0.17^{b}	6.88^{*a}	1.40^{*b}
	Fully developed	2.33^{*a}	0.40^{*b}	1.96^{*a}	0.59^{*b}
	Senescence	4.47^{*a}	0.71^{*b}	2.66^{*a}	1.90^{*a}
European beech	Developing	1.33^{*a}	0.00^{b}	0.59^{*a}	0.62^{a}
	Fully developed	2.87^{*a}	0.70^{*b}	1.76^{*a}	0.34^{*b}
	Senescence	2.68^{*a}	0.30^{*b}	2.50^{*a}	0.54^{*b}
Pedunculate oak	Developing	0.57^{a}	-0.37^{*b}	2.55^{*a}	-0.06^{b}
	Fully developed	3.14^{*a}	0.69^{b}	2.00^{*a}	0.54^{*b}
	Senescence	3.21^{*a}	0.68^{*b}	2.22^{*a}	0.82^{*b}
Scots pine	Developing**	0.46^{*a}	-0.13^{*b}	0.44^{*a}	-0.02^{b}
	Fully developed	0.59^{a}	0.29^{*a}	0.21^{a}	0.12^{*a}
	Senescence	1.78^{*a}	0.21^{*b}	0.70^{*a}	0.31^{*b}

Negative values indicate higher ^{15}N values in control samples than in treated samples. Different small letters within a row indicate significant ($p<0.05$) differences between NH_4^+ and NO_3^- uptake for a specific tree species, phenological stage and NH_4^+ to NO_3^- ratio

*$p<0.05$, N uptake is significantly different from zero

**Only last year's needles

fully developed leaves ($p<0.0001$), but this effect was species dependent. For example, in the leaf developing stage birch showed the highest uptake rates while beech, oak and pine showed the lowest uptake rates. Nitrate uptake of all species was significantly lower for developing leaves compared with fully developed and senescent leaves. The effect of NH_4^+ to NO_3^- ratio is largely manifested on senescent leaves which showed a lower $^{15}NH_4^+$ uptake and a higher $^{15}NO_3^-$ uptake under the low NH_4^+ to NO_3^- ratio.

Univariate analysis of variance for pine reveals a significant effect of needle age on the observed $^{15}NO_3^-$ uptake ($p=0.029$). The higher $^{15}NO_3^-$ uptake by current year needles compared with last year needles was only visible during summer (Fig. 1). For the developing stage, however, no comparison could be made. Ammonium uptake was not found to be affected by needle age, which was probably due to the relatively large standard deviation of the $^{15}NH_4^+$ uptake. The effect of leaf phenology and the NH_4^+ to NO_3^- ratio was significant for both the $^{15}NH_4^+$ and NO_3^- uptake of current and last year needles and similar to the effects described in the univariate analysis for all species.

Table 2 Degrees of freedom (*df*) and *F* and *p* values of a three-way ANOVA: effect of tree species, leaf phenology and NH_4^+ to NO_3^- ratio on the observed foliar ^{15}N uptake of NH_4^+ and NO_3^-

Source of variation	$^{15}NH_4^+$ uptake			$^{15}NO_3^-$ uptake		
	df	*F*	*p*	*df*	*F*	*p*
Tree species	3	71.68	<0.001*	3	32.07	<0.001*
Leaf phenology	2	17.94	<0.001*	2	47.63	<0.001*
NH_4^+ to NO_3^- ratio	1	4.34	0.040*	1	27.07	<0.001*
Tree species × leaf phenology	6	9.05	<0.001*	6	12.53	<0.001*
Tree species × NH_4^+ to NO_3^- ratio	3	3.23	0.027*	3	7.61	<0.001*
Leaf phenology × NH_4^+ to NO_3^- ratio	2	16.21	<0.001*	2	17.91	<0.001*

*$p<0.05$, significant

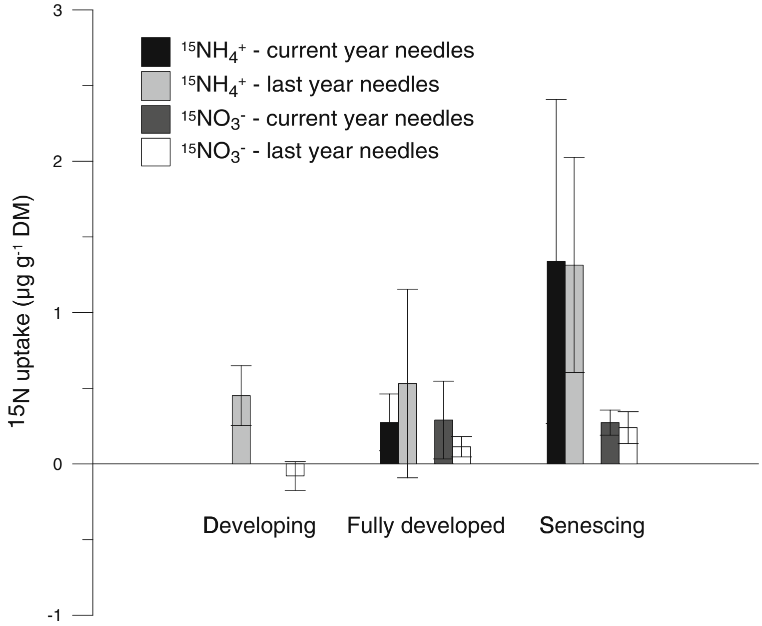


Fig. 1 ^{15}N uptake (µgg^{-1} DM) (±standard deviation) by current and last year needles of Scots pine per phenological stage

3.2 Leaf Characteristics

The foliar N content of the deciduous tree species decreased from spring (3.91%) to autumn (2.62%) whereas for current year and last year needles of pine foliar N increased slightly from 1.36% to 1.58% (Fig. 2).

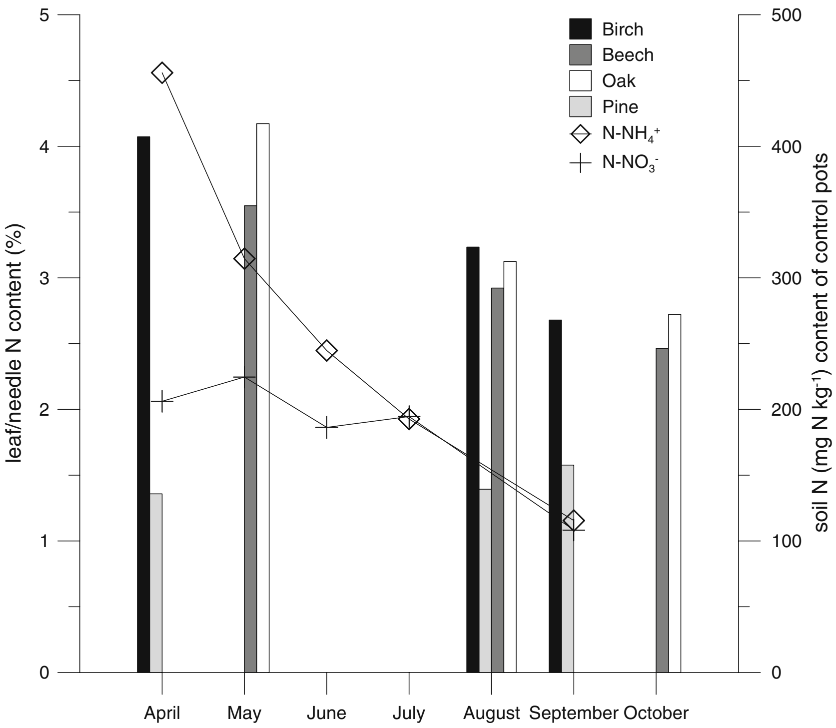
The CA of droplets (Table 3) on the abaxial side of leaves was significantly lower for birch than for the other species, while the highest CA was observed for oak, although differences were not significant for all species. At the adaxial side, birch also showed the lowest CA, but differences between species were less significant. Pine showed the highest adaxial CA in the stages with fully developed and senescent leaves. At the abaxial side, CA of oak and pine was lower at the developing stage than at the later stages. The same pattern was observed at the adaxial side for pine, but for birch and oak, a lower CA and thus higher wettability can be seen towards the end of the growing season (at senescence) and, for beech, at the stage of fully developed leaves.

Birch and beech show a smooth leaf surface structure, both on the adaxial and on the abaxial side, with no outgrowing hairs or trichomes visible (Fig. 3). In contrast, oak leaves have distinct crystalline epicuticular wax structures on the abaxial side, which also cover the stomatal guard cells and trichomes. On the adaxial side, the same crystalline wax structures are visible, but less abundant. Pines have a sinuate needle surface structure orientated perpendicular to the length axis of the needles, along which the stomata are situated. Pine stomata are raised above the surface, which is covered with a wax layer that has some crystalline structures.

The WSC of fully wetted leaves and WSC after dripping differed significantly between tree species, but the ranking depends on the phenological stage (Table 3). Nonetheless, at each phenological stage, we observed the lowest WSC values for pine or birch and the highest for beech or oak. WSC for fully wetted leaves and WSC after dripping were significantly correlated (correlation coefficient= 0.72), with the highest difference between both values for oak.

Relating the CA data of the adaxial leaf side to the N uptake rates found in this experiment yielded a correlation coefficient of −0.70 for $^{15}NH_4^+$ (p= 0.019) and −0.73 for $^{15}NO_3^-$ (p=0.011) (Fig. 4). Next to this, $^{15}NH_4^+$ uptake was positively correlated to the foliar N content ($p<0.0001$; r=0.391). However, on a species basis, we found a negative relationship between $^{15}NH_4^+$ uptake and foliar N content for beech ($p<0.001$; r=−0.72) and oak (p=

Fig. 2 Total N content (%) of birch, beech and oak leaves and pine needles in the different phenological stages and KCl-extractable soil NH_4^+ and NO_3^- content ($mg N kg^{-1}$ dry soil) in the control pots through the growing season of 2008



0.048; $r=-0.407$) and no significant relation ($p>0.05$; $r<0.418$) for birch and pine. The CA of the abaxial leaf side and WCS after dripping were not significantly correlated with $^{15}NH_4^+$ or $^{15}NO_3^-$ uptake ($p>0.05$; $r<0.57$).

Plant available inorganic N in the control pots decreased through the growing season (Fig. 2). The NH_4^+ availability, in particular, decreased from April (456 $mg N kg^{-1}$ soil) towards October, to the same level as the NO_3^- availability (±120 $mg N kg^{-1}$ soil).

Table 3 Contact angle (CA; °) of water droplets on the adaxial and abaxial leaf side and water storage capacity (WSC; $g m^{-2}$) of fully wetted leaves and leaves after dripping per phenological stage and tree species

Leaf phenology	Tree species	CA adaxial side	CA abaxial side	WSC fully wetted	WSC after dripping
Developing	Silver birch	54.8^{aB}	44.8^{a}	162.5^{bB}	86.2^{B}
	European beech	73.7^{bB}	75.7^{b}	156.6^{b}	88.7
	Pedunculate oak	74.8^{bB}	94.7^{cA}	180.7^{b}	76.4
	Scots pine*			104.2^{aA}	68.4
Fully developed	Silver birch	52.7^{aB}	49.7^{a}	117.5^{A}	58.8^{aA}
	European beech	55.0^{aA}	73.7^{b}	157.7	82.3^{b}
	Pedunculate oak	77.1^{bB}	120.8^{dB}	168.7	79.4^{ab}
	Scots pine	88.6^{c}	84.4^{c}	158.1^{B}	74.2^{ab}
Senescence	Silver birch	38.0^{aA}	46.0^{a}	125.3^{aA}	68.0^{aAB}
	European beech	69.4^{bB}	75.6^{b}	165.3^{aB}	88.8^{ab}
	Pedunculate oak	60.6^{bA}	106.8^{dA}	227.0^{b}	120.4^{b}
	Scots pine	84.2^{c}	85.0^{c}	136.9^{aAB}	59.4^{a}

Small letters within a column indicate significant ($p<0.05$) differences between tree species in a specific phenological stage; capital letters indicate significant ($p<0.05$) differences between phenological stages for a specific tree species

*Only last year's needles

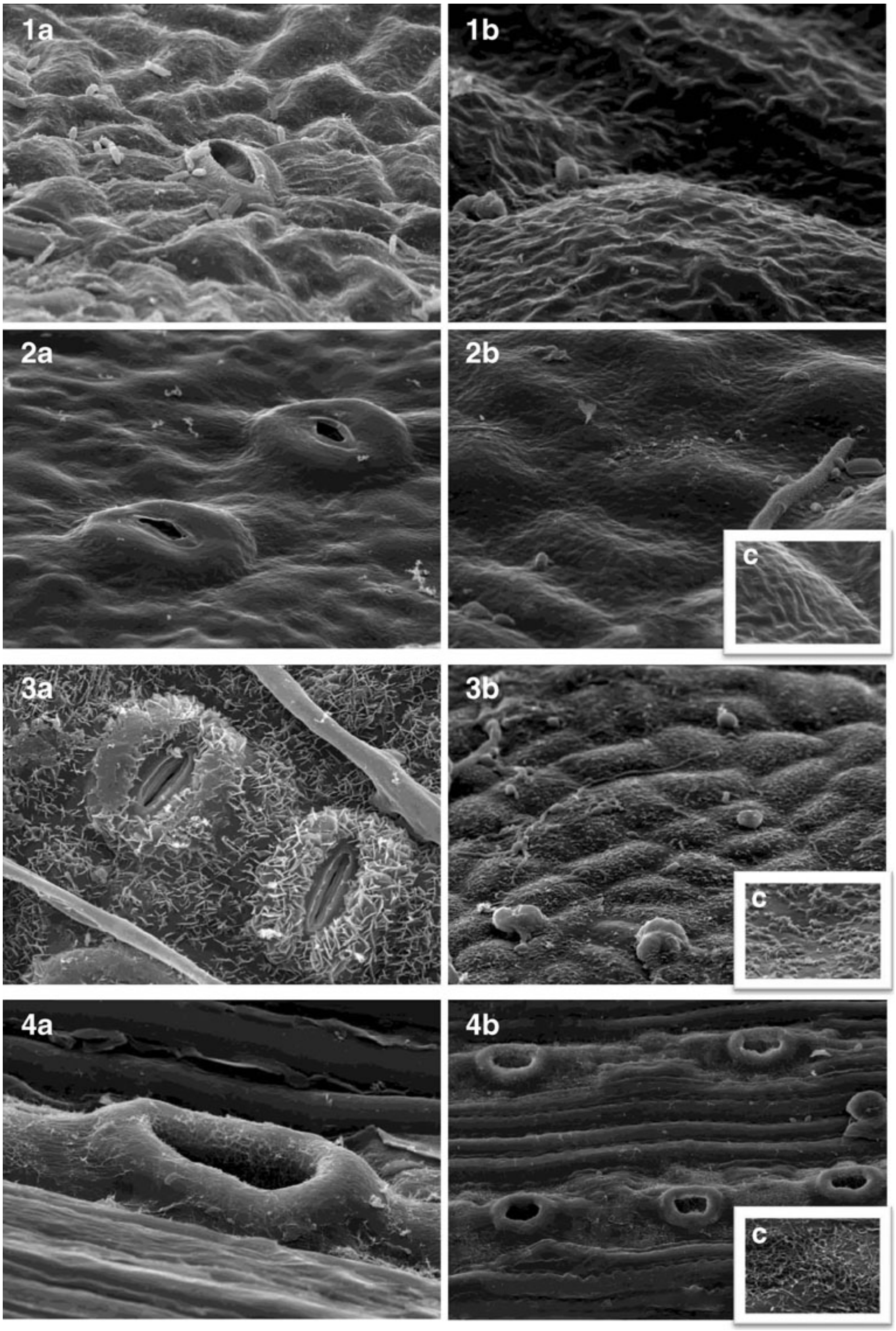


Fig. 3 Scanning electron microscope images taken from the (**a**) abaxial and (**b**–**c**) adaxial leaf side of respectively (1) birch (×1,200;×4,780), (2) beech (×2,620;×2,720;×10,900), (3) oak (×2,500;×1,010;×9,600) and (4) pine (×2,500;×625;×8,400)

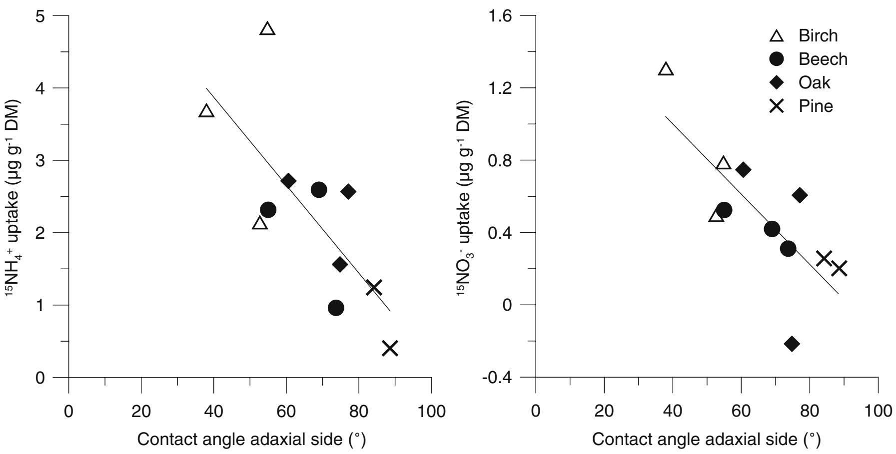


Fig. 4 Relation between the leaf contact angles (°) measured at the adaxial leaf side and the $^{15}NH_4^+$ (p=0.019; r=−0.70) and $^{15}NO_3^-$ uptake (p=0.011; r=−0.73; μg g^{-1} DM)

4 Discussion

4.1 Effect of N Form

In our study, NH_4^+ was more readily taken up than NO_3^-, even when the NO_3^- concentration in the solution was higher than that of NH_4^+. One reason may be that the transport of NO_3^- and NH_4^+ primarily occurs through cuticular diffusion (Peuke et al. 1998). Cations are transported much more readily through cuticles than anions because of the presence of hydrophilic pores within the cuticle. These small pores are lined with fixed negative charges (presumably mainly from polygalacturonic acids), with increasing density from the outside of the cuticle to the inside. Accordingly, permeation of cations along this gradient is enhanced whereas anions are repulsed from this region (Tyree et al. 1990). There is also evidence of a stomatal pathway for the uptake of polar and ionic solutes, which is located at the surface of the stomatal pores and is not affected at all by the charge of the N compound entering (Eichert and Goldbach 2008). However, the importance of this stomatal pathway is low in comparison with the cuticular uptake pathway.

The preferential N uptake of NH_4^+ compared with NO_3^- is consistent with previous studies for both deciduous (Garten and Hanson 1990; Brumme et al. 1992) and coniferous trees (Bowden et al. 1989; Eilers et al. 1992; Lumme 1994; Boyce et al. 1996; Lumme and Smolander 1996; Wilson and Tiley 1998). The ratio of $^{15}NH_4^+$ uptake to $^{15}NO_3^-$ uptake varied from 7.9 for pine under the high NH_4^+ to NO_3^- ratio treatment to 3.0 for oak under the low NH_4^+ to NO_3^- ratio treatment, which is concordant with most of these literature data (Bowden et al. 1989; Garten and Hanson 1990; Eilers et al. 1992; Boyce et al. 1996; Lumme and Smolander 1996; Wilson and Tiley 1998), but not with all (Garten and Hanson 1990; Brumme et al. 1992; Dail et al. 2009). All these studies applied a NH_4^+ to NO_3^- ratio of one.

Significant uptake of both NH_4^+ and NO_3^- occurs through leaves when rainwater is passing the canopy, and this uptake should be accounted for when calculating canopy uptake from throughfall measurements. It is also clear from this experiment that the proposed efficiency factor by de Vries et al. (2001) of NH_4^+ vs NO_3^- uptake with a value of 6 is not applicable for all tree species and throughout the year. In addition, it is necessary to account for retention by branches and boles as well (Dail et al. 2009) and for dry deposition processes to determine the value of this efficiency factor.

4.2 Tree Species Effect

Next to the analogous studies of Brumme et al. (1992) and Eilers et al. (1992) with European beech and Norway spruce, respectively, to our knowledge the present report is the only experimental comparison of foliar ^{15}N uptake by deciduous and coniferous species under similar conditions. We found that the mean NH_4^+ uptake rates of silver birch, European beech and oak were respectively six, three and four times higher than for pine. For NO_3^- uptake, this ratio was respectively six, three and three. Brumme et al. (1992) found that NH_4^+ uptake by beech leaves was 1.8 times higher than spruce needles, and NO_3^-

uptake 2.7 times. In that experiment, however, the applied N concentration was much higher than ambient levels, which could explain the different result.

Several explanations can be put forward for the observed tree species effect on N uptake. Firstly, the tree species showed significant differences in wettability, which relates to N uptake (see section 4.5), with higher wettability for birch and lower for pine. Secondly, although of less importance, broadleaf trees are found to differ in internal resistance for NO_3^- compared with conifers (Ammann et al. 1999). Thirdly, the N demand of coniferous tree species is thought to be relatively low, owing to lengthy needle retention, efficient internal N recycling and the ability of these species to produce large amounts of aboveground biomass per unit N assimilated (Dail et al. 2009). The latter is supported by the N content in the pine needles (1.4%), which is less than half of that in the leaves of the three deciduous species (3.2%) (Fig. 3). Fourthly, the cuticular pore density (see section 4.1) is higher in cell walls between guard cells of stomata and subsidiary cells (Maier-Maercker 1983). Positive relationships between the number or distribution of stomata and the intensity of mineral nutrient uptake from foliar sprays have been observed (Levy and Horesh 1984).

Despite this large difference in uptake rates, it is likely that the annual foliar N uptake of pine will be higher than for the deciduous species, considering the presence of needles throughout the year compared with only 6 months for deciduous species. Extrapolating the average results of the high NH_4^+ to NO_3^- ratio treatment from one branch to mature stands with a LAI of 1.4 for silver birch (Wuyts et al. 2008), 5.5 for beech (Mussche et al. 2001), 2.4 for oak and 2.9 for pine (Sampson et al. 2006) results in an annual foliar uptake of 0.08, 0.43, 0.24 and 2.23 $\mathrm{kg\,N\,ha^{-1}}$, respectively, from wet deposition under ambient levels. Leaf surface area of the treated branches, was calculated from their leaf biomass and the specific leaf area of the control branches, which was respectively 203, 302, 239 and 369 $\mathrm{cm^2\,g^{-1}}$ DM for birch, beech, oak and pine. Considering a wet N deposition of 10 $\mathrm{kg^{-1}\,N\,ha^{-1}\,year^{-1}}$ (N level as in the high NH_4^+ to NO_3^- ratio and a mean annual precipitation of 791 mm), the fraction of N taken up by foliage from wet deposition would equal 0.8% for birch, 4.3% for beech, 2.4% for pedunculate oak and 22.3% for pine. These annual uptake values suggest that the contribution of foliar uptake from wet deposition by deciduous species is of minor importance in the total canopy N uptake of 4–5 $\mathrm{kg\,N\,ha^{-1}\,year^{-1}}$ calculated from throughfall measurements (Thimonier et al. 2005; Staelens et al. 2008). However, on a canopy level, it is also important to consider the role of woody plant surfaces (see section 4.1), since these have been shown to contribute significantly to the total water storage capacity (Herwitz 1985; Andre et al. 2008), and the effect of varying rain intensity (Hansen et al. 1994).

4.3 Effect of Leaf Phenology

Generally, the highest uptake rates were observed for fully developed and senescent leaves. This is in line with observations from throughfall studies for both coniferous (Houle et al. 1999) and deciduous stands (Neary and Gizyn 1994; Houle et al. 1999). One reason that N uptake occurs at leaf senescence, is that it is probably linked to N retranslocation by trees (Houle et al. 1999; Hagen-Thorn et al. 2006). Species with early leaf fall seem to resorb nutrients at a higher rate, thereby exhibiting a trade-off between early leaf fall and efficient nutrient resorption (Niinemets and Tamm 2005). This also held true in the present study, where the N content of birch decreased by 17% from fully developed leaves towards senescent leaves whereas for beech and oak this was 16% and 13%, respectively.

However, there are some arguments that indicate that plant N status may not be the only driving factor for foliar N uptake. Firstly, for both the current and last year needles of pine a higher $^{15}NH_4^+$ uptake was observed in the senescence stage, which was not coupled with a decrease in needle N content. Current year needles are fully developed at the end of the growing season, and, as indicated by studies on other evergreen species, will serve as main N storage site during winter (Millard and Proe 1993), which can explain their higher uptake. For last year needles no clear mechanism can be put forward. Secondly, beech and oak showed an increased $^{15}NH_4^+$ uptake with decreasing N content, with the lowest uptake rates occurring during leaf emergence, whereas birch and pine did not show this pattern. The difference during leaf emergence could be due to the higher photosynthetic capacity of the sunlit leaves/needles of birch and pine compared with the shadow leaves of beech and oak (Hikosaka 2005). It is also possible that the higher N availability in the pots during leaf emergence had an influence the foliar N uptake, although it has been shown that leaf growth in spring is largely determined by the N stored in plant tissue during the previous year (Millard and Thompson 1989). So, although plant N status can partly explain the differences between the different phenological stadia, there is an interaction with leaf wettability and species-specific photosynthetic capacity.

4.4 Effect of NH_4^+ to NO_3^- Ratio

In the present study, no distinct effect of the applied NH_4^+ to NO_3^- ratio was found, except for senescent leaves, in contrast to previous reports for European beech (Brumme et al. 1992), sacred fir (*Abies religiosa*) (Chavez-Aguilar et al. 2006) and Norway spruce (Eilers et al. 1992; Lumme and Smolander 1996). In the experiments of Eilers et al. (1992) and Brumme et al. (1992), however, the low and high dose differed by a factor of seven, compared with the factor of three used in this experiment. In the other studies mentioned, NH_4^+ and NO_3^- were applied separately, thereby not accounting for preferential uptake.

The absence of a clear effect of the applied NO_3^- concentration on the measured ^{15}N uptake, and consequently of the NH_4^+ to NO_3^- ratio, indicates that the affinity of leaves and needles for NH_4^+ is much higher than for NO_3^- (see section 4.1), and that the ratios of NH_4^+ to NO_3^- used in this experiment are not sufficient to counter this balance. This implies that the potentially increasing future NO_2 emissions in industrialised regions (Carslaw et al. 2007) with simultaneously decreasing NH_3 emissions (Monks et al. 2009) will result in higher N deposition fluxes to the forest floor. So, although total N emissions may decrease in the near future, ecosystems will still be subject to N saturation and enhanced soil acidification and consequent changes in vitality and biodiversity.

4.5 Leaf Surface Characteristics and N Uptake

Leaf wettability is species dependent, since the physico-chemical characteristics of the epicuticular wax deposit and epidermal cell morphology differ between species (Holloway 1969; Hall and Burke 1974; Haines et al. 1985). Therefore, the SEM images (Fig. 3) partly explain the observed differences in wettability through visible differences in surface structure: the smooth surface of birch and beech causes water droplets to spread, while the crystalline epicuticular wax structures for oak and pine retain droplets as a sphere. Although the WSC of leaves is also generally indicative of wettability, we found no relationship between the CA and laboratory estimates of WSC after dripping, which is in contrast to the negative relationship observed by Haines et al. (1985). We hypothesise that this lack of relation can be attributed to the use of other tree species in this study and the difference in leaf orientation of the deciduous species. Birch leaves have a lower WSC than oak leaves because they tend to hang down vertically compared with the horizontally orientated oak leaves, which causes water to drip off faster. The vertical orientation of pine needles causes WSC to be low. With regard to the foliar N uptake, adaxial leaf wettability appears to be a more determinant factor than WSC. This could be explained by the fact that permeability through cuticular pores increases with leaf surface humidity. Therefore, a higher leaf surface covered by water, as in the case of low CAs, causes a higher permeability for inorganic ions (Schreiber et al. 2001). The WSC is a static parameter, measuring only the water amount that remains on the leaf surface after a rain event, while during this event more water makes contact with a leaf but then drips off. This suggests that the process of leaf N uptake occurs very fast, since the WSC, and as such the contact time, is less for birch than for oak leaves though N uptake is higher for birch than for oak.

To our knowledge, this is the first study relating foliar uptake of N originating from wet deposition to leaf surface properties. Our finding is concordant with the work of Sase et al. (2008) indicating a positive correlation between the net fluxes of NH_4^+ and NO_3^- from throughfall water and the leaf CA for Japanese cedar through the growing season. In their case, however, N uptake may have occurred through wet and dry deposition. It should be mentioned that possibly a similar pattern exists for dissolved N uptake by branches, which remains to be investigated. Also, within one species, leaf wettability can vary according to canopy height (Boyce et al. 1991) and stand characteristics (Hall et al. 1965) as an adaption to different microclimatic conditions such as light availability and relative humidity, and to the degree of leaf surface contamination (Barthlott and Neinhuis 1997). Since in this study the relation between N uptake and wettability was only observed for saplings growing in the same climatic conditions, it is important to further assess this relation within one species under varying conditions.

5 Conclusions

This study demonstrates that N assimilation of wet deposited NH_4^+ and NO_3^- occurs through foliage of deciduous and coniferous trees and that it is significantly influenced by N form, tree species, leaf phenology and NH_4^+ to NO_3^- ratio. Uptake of NH_4^+ and NO_3^- is strongly related to leaf wettability, and not to water storage capacity, a relationship which enables the indirect estimation of N uptake from this easy to measure parameter. However, there is still not much known about within-species dependence of N uptake on leaf wettability. In this study, the N uptake was small, so, it should be further examined to what extent N uptake from wet deposition contributes to total canopy N uptake. Furthermore, more research is needed to understand how this N uptake is related to throughfall deposition as easy and inexpensive technique for assessing total deposition fluxes.

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